ผลของสารสกัดหยาบที่ได้จากเม่าสร้อยต่อภาวะเครียดออกซิเดชั่นในแมวป่วยด้วยโรคไตวายเรื้อรังโดยธรรมชาติ

นางสาวน้ำฟ้า เฟื่องบุญ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Effects of *Antidesma acidum* Crude Extract on Oxidative Stress in Cats with Spontaneous Chronic Renal Failure

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A Thesis Submitted in Partial Fulfillment of the Requirement
for the Degree of Master of Science Program in Veterinary Medicine
Department of Veterinary Medicine
Faculty of Veterinary Science
Chulalongkorn University
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Thesis Title	Effects of *Antidesma acidum* Crude Extract on Oxidative Stress in Cats with Spontaneous Chronic Renal Failure.
By	Miss Numfa Fungbun
Field of Study	Veterinary Medicine
Thesis Advisor	Associate Professor Dr.Rosama Pusoonthornthum
Thesis Co-Advisor	Associate Professor Dr.Sumphan Wongseripipatana

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Accepted by the Faculty of Veterinary Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

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น้ำฟ้า เฟื่องบุญ : ผลของสารสกัดหยาบที่ได้จากเม่าสร้อยต่อภาวะเครียดออกซิเดชั่นในแมวป่วยด้วยโรคไตวายเรื้อรังโดยธรรมชาติ (Effects of *Antidesma acidum* Crude Extract on Oxidative Stress in Cats with Spontaneous Chronic Renal Failure) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.สพ.ญ.ดร.รสมา ภู่สุนทรธรรม, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.ดร.สัมพันธ์ วงศ์เสรีพิพัฒนา, 95หน้า.

ทำการศึกษาผลของสารสกัดหยาบที่ได้จากเม่าสร้อยในแมวป่วยด้วยโรคไตวายเรื้อรังโดยธรรมชาติ ในแมวจำนวน 30 ตัว โดยแบ่งเป็น 3 กลุ่ม ตามข้อกำหนดของสมาคมโรคไตนานาชาติ โดยแบ่งตามระดับครีเอตินีนในเลือด คือแมวปกติจำนวน 10 ตัว ซึ่งมีระดับครีเอตินีนน้อยกว่า 1.6 มิลลิกรัมต่อเดซิลิตร แมวป่วยด้วยโรคไตในระยะที่สอง ซึ่งมีระดับครีเอตินีนระหว่าง 1.6-2.8 มิลลิกรัมต่อเดซิลิตร และแมวป่วยด้วยโรคไตในระยะที่สาม ซึ่งมีระดับครีเอตินีนระหว่าง 2.9-5.0 มิลลิกรัมต่อเดซิลิตร แมวแต่ละกลุ่มถูกแบ่งโดยการสุ่มออกเป็นสองกลุ่มย่อยที่เท่ากัน กลุ่มย่อยแรกเป็นแมวที่ได้รับยาหลอก กลุ่มย่อยที่สองได้รับสารสกัดหยาบที่ได้จากเม่าสร้อยในขนาด 120 มิลลิกรัมต่อกิโลกรัม วันละครั้ง เป็นเวลา 56 วัน ทำการเก็บตัวอย่างเลือดในแมวทุกกลุ่ม ทุก 2 สัปดาห์ ติดต่อกันเป็นเวลา 10 สัปดาห์ และติดตามผลหลังหยุดยาไปอีก 14 วันต่อมา เพื่อตรวจค่าโลหิตวิทยา และค่าเคมีคลินิก ประกอบด้วยค่า ยูเรียไนโตรเจน ครีเอตินีน เอนไซม์อะลานีนอะมิโนทรานเฟอเรส และ อัลคาไลน์ฟอสฟาเตส ทำการตรวจวิเคราะห์ตัวชี้วัดภาวะเครียดออกซิเดชั่นคือ รีดิวส์กลูตาไธโอน ออกซิไดซ์กลูตาไธโอน และการทำงานของเอนไซม์กลูตาไธโอนเปอร์ออกซิเดสในเม็ดโลหิตแดง ผลการศึกษาพบว่าแมวป่วยด้วยโรคไตในระยะที่สามที่ได้รับสารสกัดหยาบที่ได้จากเม่าสร้อยมีค่าเอนไซม์อะลานีนอมิโนทรานเฟอเรสลดลงในวันที่ 42, 56 และ 70 ของการศึกษา ค่าเอนไซม์อัลคาไลน์ฟอสฟาเตส ลดลงอย่างมีนัยสำคัญทางสถิติในวันที่ 56 และ 70 ของการศึกษา และมีค่าครีเอตินีน ลดลงในวันที่ 28 และ 42 ของการศึกษา แมวที่ได้รับสารสกัดหยาบที่ได้จากเม่าสร้อยซึ่งป่วยโรคไตในระยะที่สองและสาม มีแนวโน้มการเพิ่มขึ้นของสารกลูตาไธโอนในวันที่ 56 ของการศึกษา สรุปได้ว่าสารสกัดหยาบที่ได้จากเม่าสร้อยมีผลในการลดค่าเอนไซม์อะลานีนอะมิโนทรานเฟอเรส อัลคาไลน์ฟอสฟาเตส ครีเอตินีน และมีผลต่อยับยั้งการลดลงของสารกลูตาไธโอน อย่างไรก็ดีควรมีการศึกษากลไกการทำงานของสารสกัดหยาบที่ได้จากเม่าสร้อยต่อภาวะเครียดออกซิเดชั่นในแมวป่วยด้วยโรคไตต่อไปในอนาคต

ภาควิชา ..อายุรศาสตร์..	ลายมือชื่อนิสิต ..น้ำฟ้า เฟื่องบุญ..
สาขาวิชา ..อายุรศาสตร์สัตวแพทย์..	ลายมือชื่ออ. ที่ปรึกษาวิทยานิพนธ์หลัก: ..
ปีการศึกษา ..2552..	ลายมือชื่ออ. ที่ปรึกษาวิทยานิพนธ์ร่วม ..

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KEYWORDS: *Antidesma acidum* chronic renal failure oxidative stress cats

NUMFA FUNGBUN; EFFECTS OF ANTIDESMA ACIDUM CRUDE EXTRACT ON OXIDATIVE STRESS IN CATS WITH SPONTANEOUS CHRONIC RENAL FAILURE. THESIS ADVISOR: ASSOCIATE PROFESSOR ROSAMA PUSOONTHORNTHUM, Ph.D., THESIS CO-ADVISOR: ASSOCIATE PROFESSOR SUMPHAN WONGSERIPIPATANA, Ph.D., 95pp.

The effects of crude extract of *Antidesma acidum* in cats with spontaneous chronic renal failure were performed. Thirty cats were assigned into 3 groups according to the International Renal Interest Society, 10 cats in the clinically normal group (creatinine levels < 1.6 mg/dl), 10 cats in the mild azotemia group (creatinine levels 1.6-2.8 mg/dl) and 10 cats in the moderate azotemia group (creatinine levels 2.9-5.0 mg/dl). Each group was randomly divided into 2 subgroups: subgroup 1 received placebo and subgroup 2 received the crude extract of *Antidesma acidum* 120 mg/kg per oral, once daily for 56 days. Blood collection was performed every 2 weeks for 10 consecutive weeks. Blood hematology was determined. The blood chemistries were analyzed for blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The oxidative stress markers were tested using blood reduced glutathione (GSH), blood oxidizing glutathione (GSSG) and the erythrocyte glutathione peroxidase (Gpx) activity. The results demonstrated that the moderate azotemia cats received the crude extract of *Antidesma acidum* had significantly decrease in ALT levels (day 42, 56 and 70), ALP levels (day 56 and 70) and creatinine levels (day 28 and 42). The mild and moderate azotemia cats received the crude extract of *Antidesma acidum* had the trend to increase in GSH levels (day 56). In conclusion, the crude extract of *Antidesma acidum* significantly decreased ALT, ALP, creatinine levels and inhibited the glutathione depletion. The exact mechanisms of how the crude extract of *Antidesma acidum* effects on the oxidative stress in cats with moderate azotemia remains to be investigated.

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Academic Year 2009 Co-advisor's Signature S. Wongseripipatana

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CHAPTER I

INTRODUCTION

Chronic renal failure (CRF) is one of the most common disease in old cats (Brown, 2004; Yu and Paetau-Robinson, 2006). It is defined as a primary renal failure that has persisted for an extend period, usually months to years (Polzin et al., 2000). It occurs when two-third to three-quarter of the kidney nephrons are not functioning. When irreversible renal abnormalities impair 75% of renal cells, it leads to a decrease in the glomerular filtration rate and the secondary effects including uremia, hypertension, hypoalbuminaemia, electrolyte metabolism disorder, hormonal disturbance and anemia occur (Goldschmeding, 2004).

Renal cells are known to maintain high level of mitochondrial oxidative phosphorylation and arterial blood flow which produce reactive oxygen metabolites. Normal cells have various defense mechanisms to protect this deleterious effect of reactive oxygen metabolites by producing various enzymes (eg. superoxide dismutase, catalase and glutathione peroxidase) and non enzymatic antioxidants (eg. vitamin E, C, A and glutathione) (McMichael, 2007; Brown, 2008). Reactive oxygen species (ROS) are normally generated by cellular metabolism. Accumulation of ROS in the body can attributed to the excessive capacity of renal antioxidant defense mechanism to scavenge. This leads to renal oxidative stress and tissue damage of the kidney (Brown, 2008). These phenomenon cause oxidative stress to occur as the result of an excessive accumulation of the reactive oxygen species while the antioxidant defense mechanism of the body is impaired. Several studies have reported that the degree of oxidative stress is significantly correlated with the degree of severity of renal failure (Mimic-Oka et al., 1999; Allen and Yu, 2004). Another studies also indicated an increased in the generation of oxidants occur in chronic renal failure and the antioxidant activity are enhanced following those high oxidative status causing incomplete recovery of the cellular homeostasis (Ceballos-Picot et al., 1996; Zwolinska et al., 2006).

Antioxidant therapy such as Vitamin E, carotenoids, flavonoids and phenolic compounds has been given to patients as the factors to limit oxidative injury to chronic renal failure (Allen and Yu, 2004). The studies of new sources from natural products with antioxidants properties are of interested worldwide. Many natural plants have been shown to contain the chemical compounds that exhibit antioxidant property.

Antidesma acidum (Mao soi) has been studied for its chemical compound compositions such as flavonoids, phenolic compounds, tannins, B-sitosterol and stigmasterol and tested for antioxidant property. These flavonoids and phenolic compounds of natural plants have several beneficial properties as antioxidant, antiviral, anti-tumor, and anti-inflammation. From the previous study in normal cats, given crude extract of *Antidesma acidum* (Mao soi) 120 mg/kgBW by orally for 90 days, have shown to increase in total white blood cells (leucocytosis) and lymphocytosis. It also decreased aspartated aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine levels (Sonkami et al., 2007). Previous study of the acute toxicity in rats

received the crude extract of *Antidesma acidum* in the amount of 5,000 mg/kg BW did not cause the mortality, no changes in behavior, and no pathological lesions in any internal organ of the rats (Thamaree et al., 2003).

Key words: *Antidesma acidum*, chronic renal failure, oxidative stress, cats

Objective

1. To compare the antioxidant activity of *Antidesma acidum* crude extract with quercetin hydrate and butylhydroxytoluene (3,5-di-tert-butyl-4-hydroxytol; BHT).
2. To study the effects of *Antidesma acidum* crude extract on oxidative stress in cats with spontaneous chronic renal failure.
3. To study the effects of *Antidesma acidum* crude extract on hematology, blood chemistry including alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine levels in the clinically normal cats and the cats with chronic renal failure.

Hypothesis

Antidesma acidum crude extract can decrease oxidative stress in cats with spontaneous chronic renal failure.

CHAPTER II

LITERATURE REVIEW

Feline chronic renal failure

Chronic renal failure (CRF) is an irreversible structural renal lesion of a certain duration, regardless of the extent and of the initial cause of nephron loss. Chronic renal failure is the most common abnormality occurs in the old cats (Brown, 2005). Studies in the USA indicated the prevalence of CRF in all ages of cats were 2.2%. About 31% of old cats that develop CRF are 10-15 years old and 32% are older than 15 years old (Chew et al., 2004). Allen et. al., (2000) have reported the prevalence of renal failure to be about 3% in cats of all ages examined at the University of Minnesota Veterinary Teaching Hospital, and nearly one-third of cats older than 15 years presented to that hospital had renal failure. The overall prevalence of renal disease in cats has been estimated to be 0.5-2% in the general pet population (Brown, 2005). The degree of renal injury and the clinical significance can be judged by glomerular filtration rate (GFR), and serum creatinine concentration. This high serum creatinine concentration reflects the decrease in the glomerular filtration rate (GFR) (Brown, 2005). Renal dysfunction is presented by the accumulation of nitrogenous waste product, disordered electrolyte metabolism, hydration disturbance and a decrease in erythropoietin production (Polzin et al., 2000). The International Renal Interest Society (IRIS) has developed a classification system to standardize the diagnostic approach of cats with CRF into four stages base on the serum creatinine level and two major determinants of progressive renal injury which are proteinuria and systemic hypertension (Table 1).

Table 1. IRIS staging system for cats with chronic renal disease base on the serum creatinine levels.

Main stages	Creatinine level (mg/dl)
I. non azotemic	< 1.6
II. mild azotemia	1.6-2.8
III. moderate azotemia	2.9-5.0
IV. severe azotemia	> 5.0
Substages; base on proteinuria	UPC
NP non-proteinuric	< 0.2
BP borderline proteinuric	0.2-0.4
P proteinuric	> 0.4
Substages; base on hypertension	SBP (mmHg)
NH non hypertensive	< 150
BH borderline hypertensive	150-179 (no extrarenal of HT)
HC hypertensive with complications	> 150 (extrarenal of HT)
HNC hypertensive without complications	>180 (no extrarenal of HT)
HND hypertension not determined	not measured

Note UPC: urine protein to creatinine ratio; SBP: systolic blood pressure; HT: hypertension (Brown, 2005).

The diagnosis of chronic renal failure is based on the history taking, completed physical examination, and the laboratory finding. Primary renal disease is defined as the abnormality of kidney that has persisted for two weeks to months. Chronic renal failure can be categorized into four stages base on the creatinine levels (Brown, 2004).

Stage I. Non azotemic stage

This stage is defined as the initial stage that renal tissue is damaged by primary disease with 75% destruction of renal mass but the compensatory renal responses are hidden the injury and the animal shows no clinical signs. The primary causes of renal disease that damage the kidney including bacterial pyelonephritis, heredity nephropathy, neoplasia, and hypertensive nephropathy. To diagnosted the primary cause of renal injury, renal biopsy should be performed to characterize the stage of disease and perform serial measurement of serum creatinine concentration at 1-6 months interval to assess the status of the animal (Brown, 2005).

Stage II. Mild renal azotemic stage

This stage occurs when the renal tissue is loss and the presentation of mild azotemia is without clinical signs. Plasma creatinine should be measured for renal dysfunction and to monitor the renal function.

Stage III. Moderate renal azotemic stage

The third stage is moderate azotemia or transitional stage that animals show clinical signs which are often uremia. Routinely evaluation are essential for diagnostic and to focus on the progression of the disease.

Stage IV. Severe renal azotemic stage

Cats in this stage are often exhibit abnormality of fluid and electrolyte imbalance. Cats in this group often have anorexia, nausea and vomiting which are the indicators of the uremic syndrome. Most patients exhibit negative energy balance and loss of the lean body mass.

Oxidative stress

Oxidative stress is defined as an imbalance between the formation of reactive oxygen species (ROS) and the anti-oxidative defense mechanisms. Oxidative stress can result from an excess of ROS, a reduction in antioxidants, or both (Galle, 2001).

Reactive oxygen species

Reactive oxygen species (ROS) or oxygen-derived molecules are products of partial reduction of oxygen in aerobic metabolism by normal cell during mitochondrial oxidative respiration or oxidative burst including activated phargocytes and can be generated by enzyme and non-enzymatic reactions within cells (Manderker, 2008). Cellular enzymes including mitochondrial oxidase, lipoxygenase, cycloxygenase, myeloperoxidase, NADPH oxidase, xanthine oxidase and P-450 monooxgenase have been shown to play the important role in the ROS formation (Galle, 2001; Manderker, 2008).

Major ROS are found in various all such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and hypochlorous acid (HOCl) (Gwinner and Grone, 2000; Galle, 2001)

Major reactive oxygen species

Reduction of molecular oxygen

$O_2 + e^- \rightarrow {}^{\bullet}O_2^-$ (superoxide anion)

Superoxide anion is formed by autoxidation reaction or by the mitochondrial electron transport chain. When ${}^{\bullet}O_2^-$ are reacted with nitric oxide (NO) or metal iron, it is reactive and has the beneficial role and damaging effect to tissues.

Dismutation of ${}^{\bullet}O_2^-$

${}^{\bullet}O_2^- + {}^{\bullet}O_2^- + 2\,H^+ \rightarrow H_2O_2$ (hydrogen peroxide)

In the presence of superoxide dismutase (SOD), dismutation of ${}^{\bullet}O_2^-$ convert to H_2O_2. It has paired electrons and considerated as a nonradical form.

Fenton reaction

$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$ (hydroxyl radical)

H_2O_2 can degrade heme protein to release iron and H_2O_2 which has react with metal (Fe^{2+}). It can generate hydroxyl radical.

Haber-Weiss reaction

$H_2O_2 + {}^{\bullet}O_2^- \rightarrow OH^- + O_2 + {}^{\bullet}OH$ (hydroxyl radical)

H_2O_2 can react with ${}^{\bullet}O_2^-$, giving an Fe^{3+} assisted Fenton reaction and can generate hydroxyl radical.

Myeloperoxidase reaction

$H_2O_2 + Cl^- + H \leftrightarrow H_2O + HOCL$ (hypochlorous acid)

Hypochlorous acid is formed H_2O_2, Cl^-, H and myeloperoxidase enzyme. It is highly reactive and can damage biomolecule. (Gwinner and Grone, 2000)

The major ROS formations are leakage from the electron transport chains. It is estimated that 90-95% of the oxygen are converted to water, the remaining 5-10% are reduced and formed ROS (Bulteau et al., 2006). Several defense mechanisms that play the important role in decrease the concentration of ROS are shown (Figure 1).

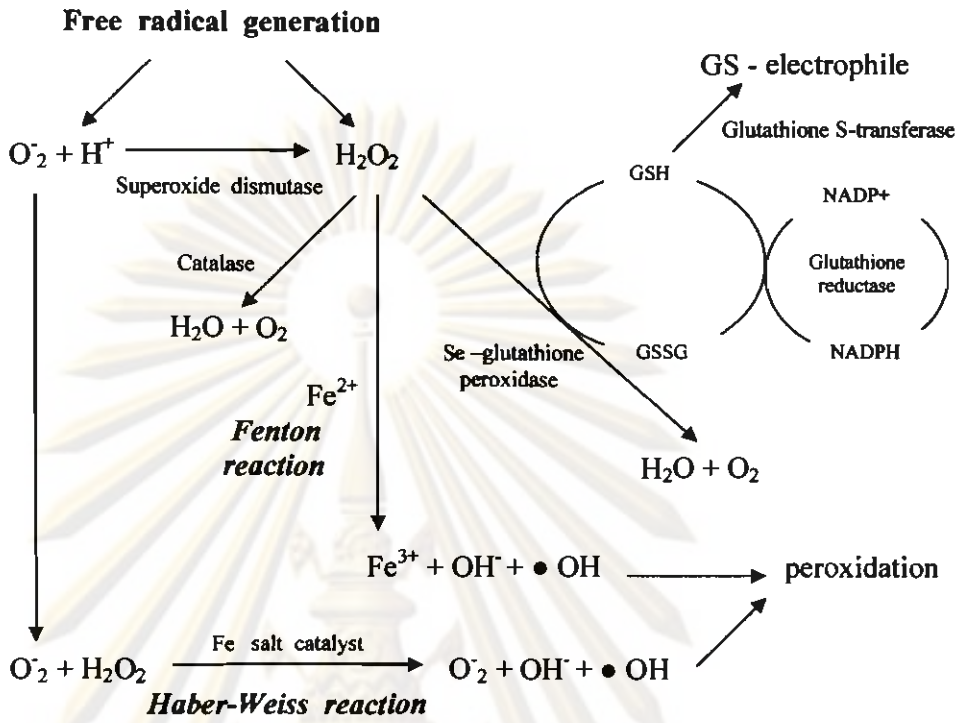


Figure 1. Formation of the reactive oxygen species (applied from Storey, 1996).

Superoxide dismutase (SOD) catalyze the dismutation of superoxide anion to hydrogen peroxide. Hydrogen peroxide will be converted to harmless H_2O and O_2 by catalase and glutathione peroxidase. However, the excess SOD activity and the presence of metal ions (eg. Fe^{2+}, Cu^{2+}) can give rise to the formation of reactive hydroxyl radical (Fenton reaction). When there is too little SOD activity, hydroxyl radical can be produced from O^{2-} (Haber Weiss reaction). The metal-binding proteins such as ferritin, transferin, caureloplasmin and metallothionein are presented to reduce the generation of hydroxyl radical. The complementary antioxidant such as vitamin A, C and E and bilirubin which act as the scavengers of ROS (Bast et al., 1991; Gwinner and Grone, 2000).

Moderate ROS levels play an important regulatory mediators in many cellular signaling processes. ROS are essential for several biologic functions such as in the synthesis thyroxine. Thyroid gland need hydrogen peroxide (H_2O_2) for attachment of iodine atom to thyroglobulin (Nakamura and Ohtaki, 1990). In phagocytosis process, activated macrophages and neutrophils must generate ROS to kill certain type of bacteria. Neutrophils also kill pathogens to control infection by using enzyme myeloperoxidase which catalyzes the reaction of hydrogen peroxide (H_2O_2) (Mandelker, 2008). Many of these ROS act to reduce infection but the higher level of ROS leading to tissue damage by several mechanism. It can damage structure and function of lipid, protein, DNA and carbohydrates (McMicheal, 2007). Important generators of ROS can

initiate and amplify many deleterious processes such as inflammation, oncogenesis, and degenerative disease (Mandelker, 2008).

Oxidative stress and chronic renal disease

Renal oxidative stress is defined as the tissue damage resulting from ROS accumulation which attribute to an imbalance of ROS exceeds the capacity of renal antioxidant defense mechanisms to scavenge (Brown, 2008). The kidney maintain high levels of mitochondrial oxidative phosphorylation and arterial blood flow causing ROS formation (Brown, 2008). Animal experiments have provided evidence that ROS play a major role in many glomerular disease (Gwinner and Grone, 2000). Local increases in the concentrations of ROS may lead to tissue damage by several mechanism:

i. Oxidation of lipid generates lipid radical ($L^{\bullet}$) which can initiate and self-sustain lipid peroxidation, cell and basement membranes may be deranged, a process that could be important to cause glomerulonephritis.

ii. Oxidative modification of protein residues can promote the loss of the scaffolding property of structural protein, can inactivate enzymes, degradation of enzyme and clearance of protein.

iii. Oxidation of purines, pyrimidine can give rise to cross-link or fragmentation of nucleic acid, leading to the alteration of gene expression.

The detection of products of lipid peroxidation in renal tissues have shown (Figure 2).

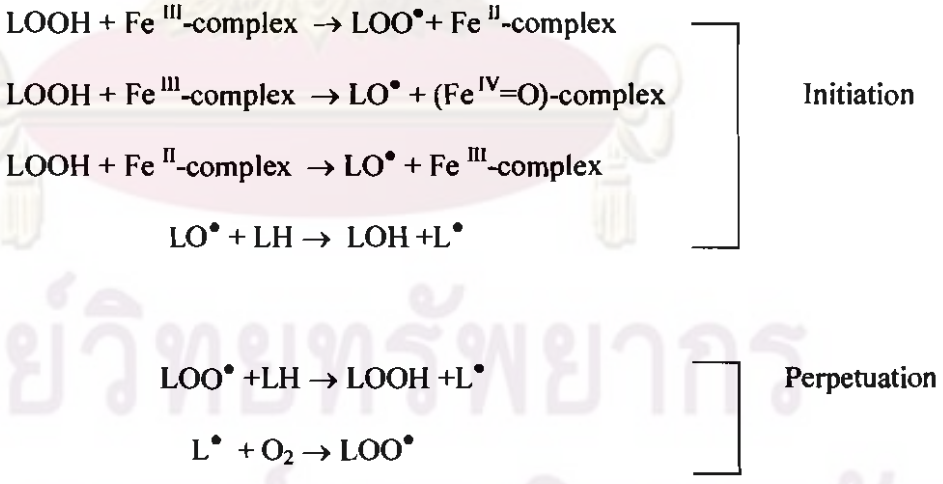


Figure 2. Initiation and perpetuation of lipid oxidation.
LH, unsaturated lipid; LOOH, lipid hydroperoxide; LO, lipid alkyl radical; $LOOH^{\bullet}$, lipid peroxy radical; $L^{\bullet}$, lipid radical (Gwinner and Grone, 2000).

Besides the ROS direct oxidative damage to cellular component, the increase in ROS may act as the transcellular and intracellular signals that activated the redox sensitive protein kinases or phosphatases resulting in alteration phospholylation of receptors and transcription factors. Finally this can lead to the critical change in the expression of cytokines, adhesion molecules, and proteins involved in proliferation, apoptosis, and affect the inflammation and cell death (Gwinner and Grone, 2000). In chronic renal failure, the surviving renal tissue becomes adaptatively hyperfunctional, leading to an increase in pro-oxidant activity and urinary markers of oxidative damage and presumably in cats (Brown, 2008). Animal with chronic renal failure often have concurrent conditions that increase the rate of generation of ROS including advanced age, activation of the rennin-angiotensin system, chronic renal and systemic inflammation. The rennin angiotensin system plays a role in the development of the renal lesion and the progressive of the kidney dysfunction (Lefebvre and Toutain, 2004). Angiotensin II is important promoter of renal oxidative stress and directly increases ROS production by glomerular and tubular cells. Angiotensin II constricts the efferent arteriole which leads to the glomerular hypertension and hyperfiltration (Figure 3 and 4).

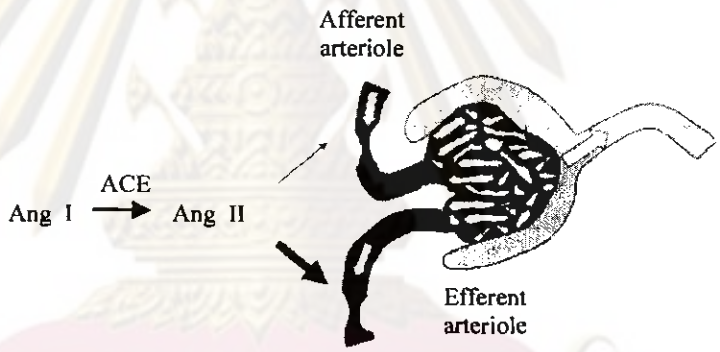


Figure 3. Angiotensin II causes the afferent and efferent vasoconstriction (applied from Lefebvre and Toutain, 2004).

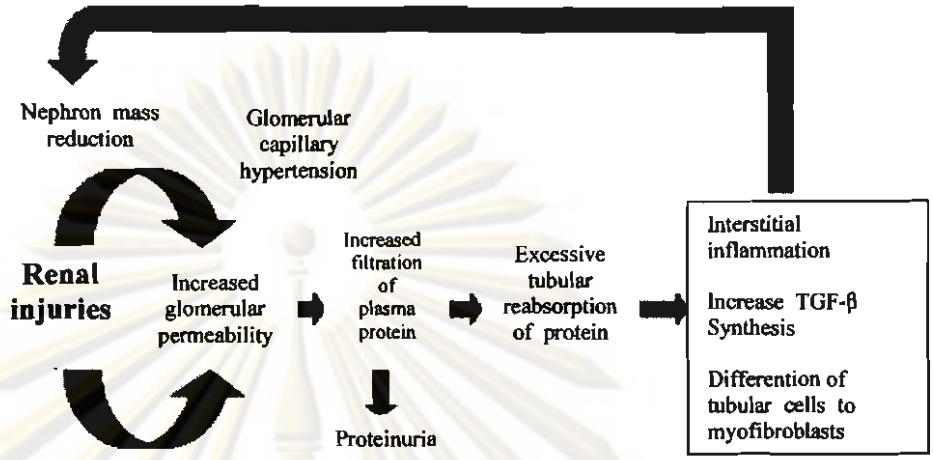


Figure 4. Pathophysiology consequence of glomerular hyperfiltration (applied from Lefebvre and Toutain, 2004).

The increase in the glomerular permeability and transglomerular protein filtration, forces tubular epithelial cells to reabsorb filtrated solutes excessively. Proteinuria, particularly albumin (if oxidized), is taken up at the tubular luminal membrane, stimulating the production of proinflamatory cytokines and chemokines in proximal tubules cells. This is partially attributable to the activation of nuclear factor-κβ, which is an important regulator of the proinflammatory genes. Transforming growth factor- β (TGF-β) also leads to interstitial inflammation, fibrosis and increased inflammation. All of these effects contribute to oxidative stress in the nephron (Lefebvre and Toutain 2004; Singh et al., 2006). The efferent arteriole constriction limits the flow to peritubular capillaries and produce relative hypoxia, stimulates fibroblastic collagen production leads to the increase in ROS generation (Rossert et al., 2002). Systemic hypertension is often increase the glomerular filtration rate and contributes to renal hyperfunction (Brown, 2004). This is a positive feedback loop because of the elevation of systemic blood pressure contributed to ROS. Factor that enhanced ROS in the chronic renal failure as the result of complex interaction of factors favoring the generation of ROS have been shown (Figure 5).

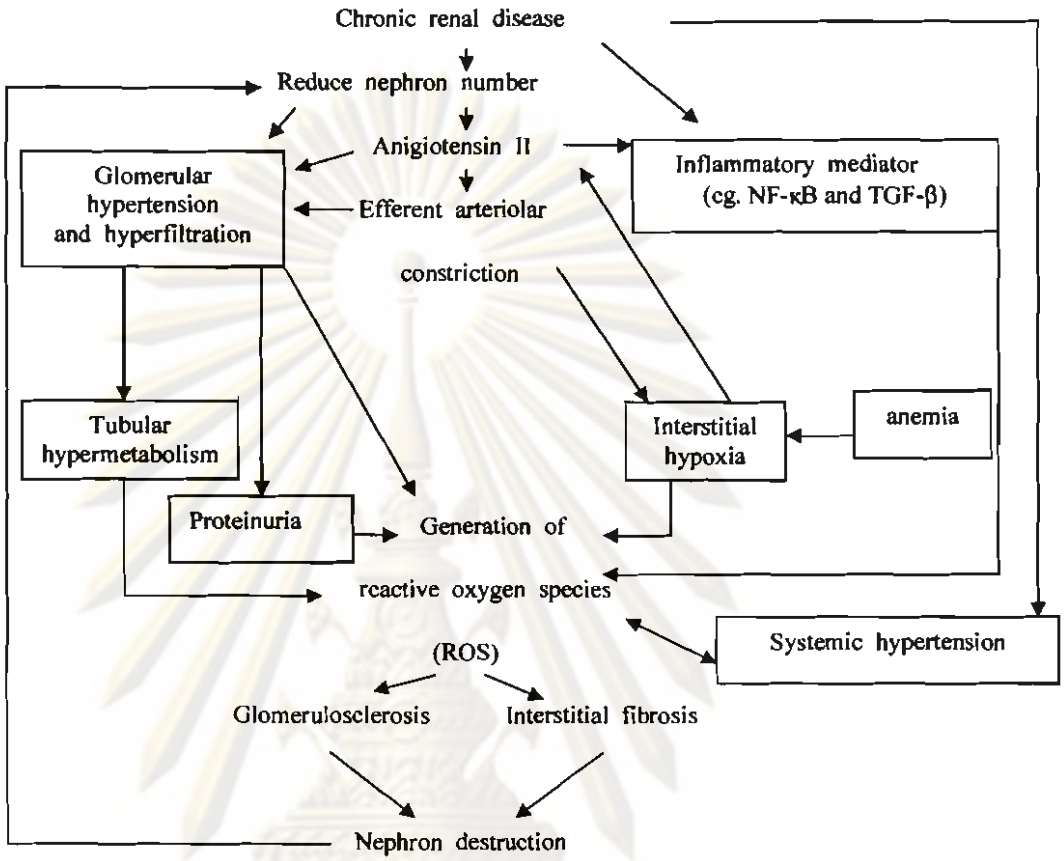


Figure 5. The generation of ROS in chronic renal disease (applied from Brown, 2008).

The hypothesis in dogs and cats with chronic renal disease suggests that renal oxidative stress contributes to renal interstitial fibrosis, glomerulosclerosis, glomerular and systemic hypertension, systemic and renal inflammation and progressive decline in kidney function (Brown, 2008).

Endogenous defense against antioxidants

ROS play an important regulatory roles in the cellular function. The homeostatic balance of ROS is modulated by endogenous defense mechanism against antioxidants. In general, there are 3 categories of endogenous defense: antioxidant proteins, enzymatic antioxidants, and small molecule antioxidants.

Antioxidant proteins, such as albumin, haptoglobin, ferritin, and ceruloplasmin, are abundant in plasma. Enzymatic antioxidants are expressed in most mammalian cells including superoxide dismutase (SOD), catalase, and glutathione peroxidase. Small molecule antioxidants are divided into water-soluble and lipid-soluble categories. Water-soluble antioxidants include ascorbic acid (vitamin C), uric acid, bilirubin, glutathione, zinc, and selenium. Lipid-soluble

antioxidants include tocopherol (eg. α-tocopherol: vitamin E), β-carotene, ubiquinol-10 (coenzyme Q 10) and licopene (Halliwell, 1999).

Albumin acts as an antioxidant via a free thiol group that is readily oxidized by ROS (McMicheal, 2007). Enzymatic antioxidants typically work synergistically and require cofactors. Superoxide is the major ROS produced during the physiological and pathophysiological states. It is scavenged by superoxide dismutase (SOD). SOD is found in the cytosol, mitochondria and extracellular surface. The reaction of SOD and superoxide anion require copper and zinc in the cytosol and extracellular surface, and required manganese in the mitochondria. Hydrogen peroxide is created by these reaction and then is detoxified by glutathione peroxidase and catalase. Catalase use iron to convert hydrogen peroxide to created oxygen and water in the cytosol or peroxisomes. In mitochondria, hydrogen peroxide is scavenged by glutathione peroixdase that reduce hydrogen peroxide to water and oxygen by the use of glutathione as the substrate (Bulteau et al., 2006). Glutathione peroxidase is enzyme which provide the protective against lipid peroxides, protect cell membranes. It is effective at low concentrations of hydrogen peroxide (Eaton, 2006). Vitamin E is located in the lipophilic interior of cell membrane, it is a scavenger of lipid peroxide. Vitamin C can directly scavenge ROS or help in the regeneration of vitamin E (McMicheal, 2007).

Glutathione

Glutathione (L-g-glutamyl-L-cysteinylglycine) is a ubiquitous non-protein thiol (low molecular weight sulfur containing compound) which plays a central role in cell biology (Jefferies et al., 2003; Pastore et al., 2003), involved in the antioxidant cellular defence mechanism. It is a tripeptide composed of cysteine, glutamic acid and glycine. Its active group is represented by the thiol (–SH) of cysteine residue (Figure 6).

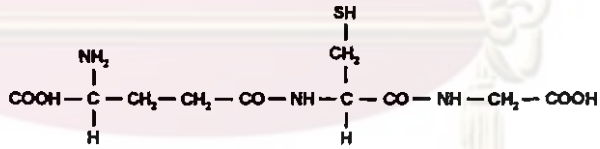


Figure 6. Structure of glutathione (Jefferies et al., 2003).

Glutathione is a ubiquitous molecule that is produced in all organs, especially in the liver. It is presented in all mammalian tissues. Intracellularly, it is found in millimolar concentrations. The plasma and urine contain at lower total GSH levels (Pastore et al., 2003). Reduced glutathione (GSH) are easily oxidized and can be regenerated very rapidly. These characteristics allow them to play an essential role in many biochemical and pharmacological reactions. It is the main intracellular antioxidant. L-cysteine is the factor for GSH synthesis, derives from the diet, from proteolysis or alternatively is synthesized from methionine (Sastre et al., 2005).

In cells, total glutathione can be free or bound to proteins. Free glutathione is present mainly in the reduced form which can be converted to the oxidized form during oxidative stress and can be reverted to the reduced form by the action of the enzyme glutathione reductase. The redox status depends on the relative amounts of the reduced and oxidized forms of glutathione (GSH/GSSG) (Pastore et al., 2003).

Reduced glutathione (GSH) is synthesized from its constituent amino acid in the cytosol of all mammalian cells by the sequential actions of g-glutamylcysteine synthetase (GCS) and glutathione synthetase (GS) in a series of six-enzyme-catalyzed reactions, which have been termed as the g-glutamyl cycle (Figure 7).

L-glutamate + L-cysteine + ATP ⟶ γ- glutamyl-L-cystein + ADP + Pi

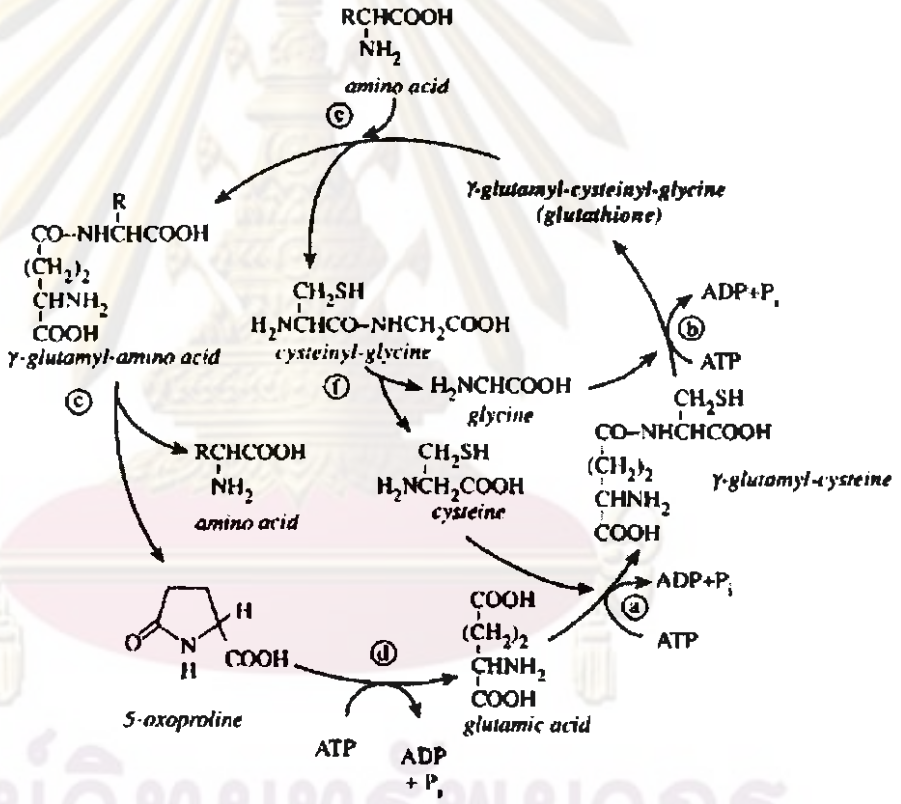


Figure 7. Glutathione synthesis and metabolism. (a) Glutamylcysteine synthetase; (b) glutathione synthetase; (c) g-glutamyl cyclotransferase; (d) oxoprolinase; (e) g-glutamyl transpeptidase; (f) peptidase (Pastore et al., 2003).

Glutathione is exported by g-glutamyl transpeptidase, an enzyme bound to the external surfaces of certain cells. The action of g-glutamyl transpeptidase leads to the formation of g-glutamyl amino acids and cysteinylglycine. The latter is cleaved by dipeptidase to form cysteine and glycine. g-Glutamyl amino

acids, formed in the transpeptidation reaction, are substrates of the intracellular enzyme g-glutamyl cyclotransferase, which converts g-glutamyl amino acids into the corresponding free amino acids and 5-oxoproline. 5-Oxoproline is decyclized to yield glutamate. The equilibrium between 5-oxoproline and glutamate, at pH values near neutrality, markedly favours cyclization. (Meister and Bukenberger, 1962; Pastore et al., 2003).

g-Glutamyl transpeptidase is found in the epithelia of tissues that are involved in transport including: the nephron, choroids plexus, jejunum, and ciliary body (Meister et al., 1976). It is localized on the outer surface of the cell membrane, whereas glutathione is found predominantly intracellularly (Meister, 1978). The transport of glutathione is a discrete step in the g-glutamyl cycle and it is a general cellular phenomenon (Figure 8). The normal cellular export of glutathione functions to provide a supply of thiols to the cell membrane and to its immediate environment.

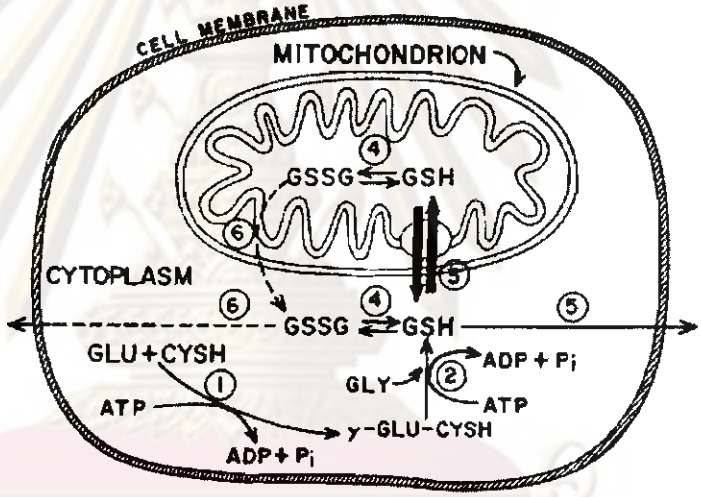


Figure 8. Scheme for synthesis and transport of GSH in mitochondria and cytoplasm (Griffith and Meister, 1985).

GSH is synthesized only in the cytoplasm (Reactions 1 and 2). GSH is transported into mitochondria by a system characterized by slow net transport and more rapid exchange transport (Reaction 3). Reversible oxidation of GSH to oxidized glutathione (GSSG) occurs in both cytoplasm and mitochondria (Reaction 4). GSH is exported across the membranes of cells of the liver and other tissues (Reaction 5). Under conditions of oxidative stress, GSSG is transported out of the mitochondria and across cell membranes (Reaction 6) (Figure 8).

Most of the plasma glutathione is supplied by the liver, which also exports some glutathione to the bile. Glutathione is removed from plasma by the action of transpeptidase, much of which is located in the kidney. Kidney uses glutathione that is transported from renal cells (intraorgan cycle), as well as

glutathione present in the plasma. The transport of glutathione from renal cells to the renal tubule in the rat accounts for about 80% of the tubular glutathione; the remainder comes from the plasma via the glomerular filtration (Griffith and Meister, 1979)

GSH, synthesized in the liver, is secreted in both bile and plasma. The plasma is the predominant route for GSH secretion. About 60% of circulating glutathione is hydrolyzed by the kidney and the remaining 40% is metabolized in extrarenal tissues. Some part of the GSH excreted in the bile is degraded within the bile tree, while the remaining part is hydrolyzed on the outer surface of the small intestine. All these epithelial tissues form interorgan and intraorgan cycles for glutathione synthesis, transport, and degradation (Figure 9).

The experimental using buthionine sulfoximine, a specific inhibitor of GSH synthesis, revealed that the half-life of GSH in liver and kidney of normal rats is 3.0 and 0.5 hr, respectively (Griffice, 1981). This indicates that the cycle for intracellular synthesis, secretion, and extracellular degradation of GSH might occur 4 and 24 times per day in the liver and the kidney, respectively (Inoue et al., 1987).

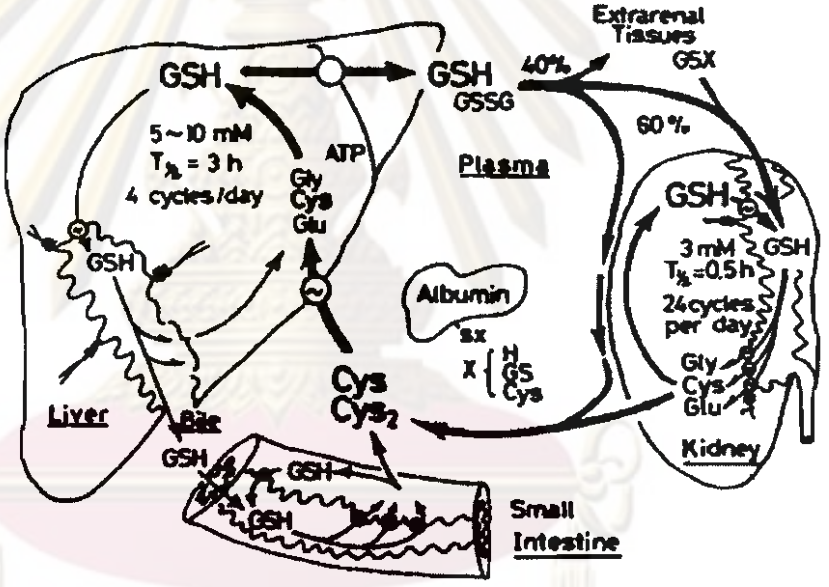


Figure 9. Interorgan and intraorgan metabolism and transport of GSH and related compounds (Inoue et al., 1987)

A role for antioxidant therapy in chronic renal failure.

The CRF patients have been shown to have the significantly reduce in plasma level of vitamin A and vitamin C (Zwolinska et al., 2006). Supplementation of antioxidants such as vitamins E and C and β-carotene significantly reduced oxidative DNA damage in both healthy cats and cats with renal insufficiency. Reduction of oxidative damage to DNA by antioxidant supplements may protect cells and organs from oxidative damage and the

progression of renal failure might be reduce (Yu and Paetau-Robinson, 2006). One study demonstrated a protective effect of hisperidine, a citrus bioflavonoid in CCl_4 induced oxidative stress in rat liver and kidney. This protective effect of hisperidine correlated to its direct antioxidant effect in the rat kidney (Tirkey et al., 2005).

Antidesma acidum

Antidesma acidum (Euphobiaceae family), is locally known as Mao Soi (Figure 10). *Antidesma acidum* is a native plant of north and northeast Thailand. *Antidesma acidum* is a shrub or treelet, up to 6-10 meter tall.

The leaf blade obovate to elliptic-oblong, (2-)5-10(-21) × (1.5-)2.5-4(-9) cm, papery, glabrous or rarely pilose adaxially, pubescent and rarely glabrous abaxially, dull, drying yellowish green, base acute to obtuse, rarely attenuate, apex rounded to acute or acuminate, sometimes mucronate; domatia present; midvein flat to impressed adaxially, lateral veins 4-9 pairs, tertiary veins reticulate. Inflorescences terminal to axillary, axes glabrous to pubescent, simple to branched twice at base.

The male flowers: pedicels 1-1.5 mm; calyx ca. 0.5 mm, cupular to globose, (3 or)4-lobed, divided for ca. 1/3, glabrous outside, pubescent inside with hairs often exceeding beyond calyx, margin erose, apex mainly rounded; disk cushion-shaped or annular and lobed between stamens, pubescent; stamens (1 or)2(or 3), 1.5-2 mm; rudimentary ovary terete or absent. The female flowers: pedicels 0.2-1.5 mm, 1.5-4 mm in fruit; calyx ca. 1 mm, cupular to nearly urceolate, 4- or 5-lobed, otherwise as in male; disk glabrous outside, glabrous to pilose inside; ovary glabrous; stigmas 3 or 4. Drupes ellipsoid, nearly terete to laterally compressed, 4-6 × 3-4 mm, glabrous; style terminal to slightly subterminal (Cha, 2008).

Figure 10. *Antidesma acidum*

Trakoontivakorn and Saksipitak (2000) had evaluated the antioxidant activity in 84 species of Thai indigenous vegetable by β-carotene bleaching method. This study found that *Antidesma acidum* had a very high potency of antioxidant activity which contained substance >100 mg of butylated hydroxyanisole (BHA) equally in 100 g fresh vegetable. The chemical compounds of methoxy flavone, phenolic compounds (Smittinan, 1980) and other chemical compound such as anthrocyanin, β-isbesterol, tannins, stigmasterol, triterypenes (Sanansieng, 1998).

Antidesma acidum had been studied for its activities including anti-HIV, antibacterial, antifungal or immunomodulating activity. Toxicity test was performed according to the Organization for Economic Co-0peration and Development (OECD) guideline 1981 and mutagenicity was test by Rec assay. The dose of extract given to albino rat were up to 5,000 mg/kg. That study had shown no acute toxicity of *Antidesma acidum* in rats and were not mutagenic. The LD_{50} was 90 g/kg body weight in rats (Thamaree et al., 2003).

CHAPTER III

MATERIALS AND METHODS

I. Preparation of the crude extract of *Antidesma acidum*

Plant material

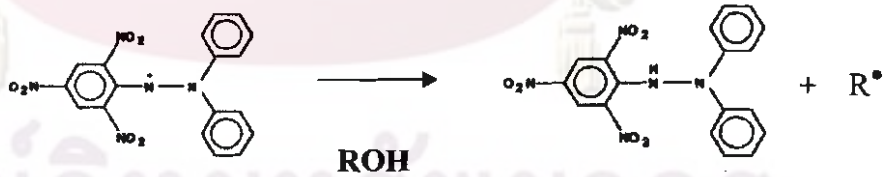
Antidesma acidum were harvested at different vegetation phase during May-September 2007. The freshly cut plants were sorted out, selected stem and bark parts, dried in the drying room at ambient temperature, packed in bags and stored before use.

Plant extraction

Selected stem and bark parts were dried in the oven at 50 °C for 5 days. The coarsely powdered stem and bark parts (3.3 kg) were macerated with 95% ethyl alcohol for seven days, three times and filtrated. The combine filtrate was evaporated under reduced pressure to yield crude extract (1.45 g). Before used in animals, the crude extract of *Antidesma acidum* was prepared in tablets form (120 mg/ tablet).

Evaluation of the antioxidant activity by DPPH radical scavenging assay

The radical scavenging activity of plant extracts against stable DPPH$^{\bullet}$ (2,2-diphenyl-1-picryhydrazyl hydrate, Sigma-Aldrich Germany) were determined spectrophotometrically. When DPPH$^{\bullet}$ reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (violet to light yellow) were measured at 515 nm on light spectrophotometer (MILTON ROY Spectronic 1001 plus, USA).



violet light yellow

The radical scavenging activity of crude extract were compared with standard antioxidant including: quercetin hydrate and butylhydroxytoluene (3,5-di-tert-butyl-4-hydroxytol; BHT, Fluka). DPPH solution at concentration of $6x10^{-5}$ M in absoluted ethanol, dissolved 2 mg of DPPH (394.32 MV) in 100 ml. absoluted ethanol. Extract solutions were prepared at the differences concentration of 100, 50, 25, 12.5, 6.25, 3.125 and 1.562 µg/ml in absolute ethanol. Standard antioxidant (quercetin hydrate and BHT) were prepared by the same procedure as the plant extract (Denrungruang, 2007).

Determination of radical scavenging activity

The absorbance of control sample containing absolute ethanol 1 ml. and DPPH solution 1 ml. was measured at 515 nm. One milliliter of DPPH solution was added to 1 ml. of the crude extract solution at the different concentration 100, 50, 25, 12.5, 6.25, 3.125 and 1.562 µg/ml. The sample were kept in the dark for 15 minutes at room temperature. The absorption was measured at 515 nm. The quercetin hydrate and BHT solution were determined for the radical scavenging with the same procedure. The experiment was carried out in triplicate. Comparing the results of radical scavenging activity between standard antioxidant (quercetin hydrate and BHT) and the crude extract was performed. Radical scavenging (%) was calculated by the following quation:

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

A_a= absorption of the crude extract and DPPH
A_b= absorption of DPPH

The effective concentration (EC_{50}) of the substance is defined as the concentration of a substance which can decreased 50 percent of DPPH concentration were calculated.

Statistical analysis

Data are presented as mean±S.E.M. of each triplicate test.

II. Animals

The experiment was performed in accordant with institutional guideline and conformed to the Faculty of Veterinary Science, Chulalongkorn University. Cats with aging between 3-15 years old were studied. Cats were included without gender and breed preference. Thirty cats were assigned into 3 groups according to the International Renal Interest Society (IRIS). The clinically normal group with serum creatinine levels < 1.6 mg/dl (n=10) and chronic renal failure (CRF) group. The CRF group were divided into 2 groups; mild azotemic cats with serum creatinine levels 1.6-2.8 mg/dl (n=10) and moderated azotemic cats with serum creatinine levels 2.9-5.0 mg/dl (n=10). During the study period, cats were not allow to received other medication.

The clinically normal group

Ten healthy client-owned cats presented to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University and the private veterinary hospitals aging between 3-15 years old were included. Cats were included without gender and breed preference. A history taking, physical

examination and blood collection for hematology and blood chemistry were all in the normal range.

The chronic renal failure (CRF) groups

Cats presented to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University and the private veterinary hospitals with clinical signs such as anorexia, weight loss, polyuria (PU), vomiting, oral ulceration and/or stomatitis were studied. Only cats between 3-15 years old were included. Cats were included without gender and breed preference. Cats were divided into 2 groups, according to the creatinine levels.

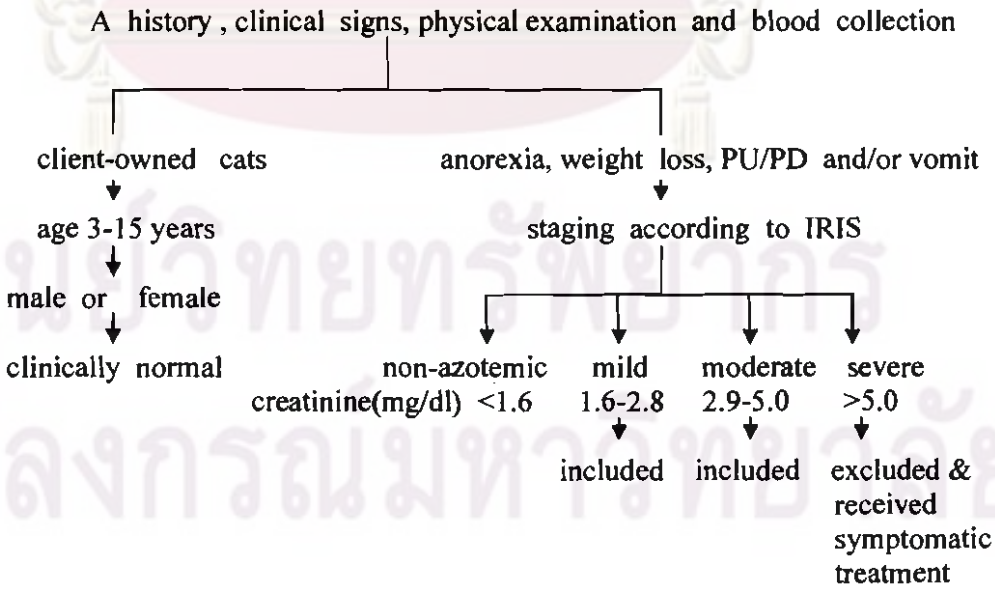
Mild azotemic cats: creatinine levels of 1.6-2.8 mg/dl. (n=10)

Moderated azotemic cats: creatinine levels of 2.9-5.0 mg/dl. (n=10)

Note: All owners were asked to signed a consent form before allowance their cats into the study. The cats in all groups received water ad libitum. During study cats were not receive other medications. All cats were assessed for the hydration status and correction of the dehydration with fluid replacement (crystalloid; Acetar®) 50-100 ml. SC every 24 hours. The cats with severe azotemic stage with the clinical signs such as severe weight loss, uremia, severe anemia were received the supportive or symptomatic treatment and excluded from the study.

Experimental Design

Cats presented to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University and the private veterinary hospitals.



Data: Blood collections for CBC, BUN, creatinine, ALT, ALP, reduced glutathione (GSH), oxidizing glutathione (GSSG) and glutathione peroxidase (GPx) were performed every 2 weeks for 10 consecutive weeks.

III. Methods

Animals preparation

A history and physical examination, clinical signs of chronic renal failure such as anorexia, depression, weight loss, PU/PD, vomiting, oral ulceration and/or stomatitis were recorded.

A. Blood was collected into heparinized tube for blood chemistry determination. It was divided according to experimental groups.

Clinically normal group (n=10), healthy cats with normal levels of BUN and creatinine.

CRF groups divided into 2 group; mild azotemic cats creatinine levels 1.6-2.8 mg/dl (n=10) and moderate azotemic cats: creatinine levels 2.9-5.0 mg/dl (n=10).

B. Both clinically normal group and CRF groups were divided into two subgroups, the first subgroup consisted of five cats received the crude extract of *Antidesma acidum* 120 mg/kg PO for 56 days and the second subgroup consisted of five cats received placebo PO for the same period.

C. Blood collection for complete blood count (CBC), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, reduce glutathione (GSH), oxidized glutathione (GSSG) and glutathione peroxidase (Gpx) were performed every 2 weeks test for 10 consecutive weeks.

Analytical methods

Collection of blood samples

Blood samples were collected, into tube containing 2 ml. of EDTA and 1 ml. of lithium heparin. Blood samples were placed immediately on crushed ice in EDTA tube, and were divided into two portions, one of which was used for measurements of pack cell volume, red blood cell counts, and white blood cell counts. The second part was used to measure hematocrit, reduce glutathione (GSH) and oxidized glutathione (GSSG). Blood in heparinized tube was centrifuged (2500 X g, 4 °C, 10 minutes) and the plasma was stored at -80 °C used to measure blood urea nitrogen, creatinine, ALT and ALP. The buffy coat was removed and the red blood cell were washed two times in an excess of chilled physiological saline solution. The washed red blood cells were hemolyzed in distilled water (1:4 v/v), freeze and thaw. The hemolyzed was centrifuged (22,000 X g, 4 °C, 60 minutes), used for measurement of glutathione peroxidase (Gpx) activity (Czuczejko et al., 2003).

Complete blood count (CBC)

Blood samples were collected into EDTA tube. The PCV, RBC WBC, and different WBC count were obtained by use of manual blood count.

Determination of serum chemistry

The plasma BUN was determined according to the method of Patton and Crouch (1977). The plasma creatinine was determined by Alkaline Picrate-End Point Reaction method. The plasma ALT was determined according to a method of Reitman and Frankel (1957). The plasma ALP was determined according to a method of Bessey et al., 1946.

Determination of oxidative stress markers

Blood reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined according to a modified method of Akerboom and Sies (1981). Glutathione peroxidase (Gpx) was determined according to a modified method of Beutler (1971).

Determination of reduce glutathione by enzymatic glyoxalase method

Principle

Reduced glutathione is spectifically converted by glyoxalase I according to the reaction

$$\text{Methylglyoxal} + \text{GSH} \xrightarrow{\text{glyoxalase I}} \text{S- Lactoylglutathione}$$

Procedure

0.4 ml. of whole blood sample was mixed with 1.6 ml distilled water and then precipitated with 3 ml of precipitating solution (metaphosphoric acid) kept in room temperature for 5 minutes. Then the solution was centrifuged and prepared according to the following reagents into the tube,

	Blank	Test
phosphate buffer (ml) pH 7.4	2.2	2.0
Filtrate (μL)	-	200
Glyoxalase I (1,000 U/ml)(μL)	40	40
Mixed		
110 mM Methylglycoxal (μL)	40	40

The absorbance was measured at 240 nm. The amount of GSH was determined and calculated from the standard curve.

Determination of oxidized glutathione

Samples for GSSG assay was performed by using 0.5 ml of whole blood, added 0.5 ml of a mixture containing 10 mmol of N-ethylmaleimide (NEM), 1.75 mmol of disodium EDTA, and 100 mmol of potassium phosphate per liter (pH 6.5). Mixed and centrifuged the sample and then mixed 800 µL of the NEM-plasma solution with 0.1 ml of a 200 g/L trichloroacetic acid solution. Remove excess NEM by extraction (five times) with diethyl ether. GSSG was measured according to a modified method of Akerboom and Sies (1981).

Principle: GSSG is determined specifically with glutathione reductase according to the reduction:

$$GSSG + NADPH + H^{+} \longrightarrow 2\,GSH + NADP^{+}$$

Procedure

Pipet into cuvett: 2.0 ml of 0.1 M phosphate buffer, 200 µl of sample, 20 µl of 100 mM EDTA and 20 µl of 1 mM NADPH. When the temperature equilibration (25 °C) is complete, the reaction is started by the addition of 10 µl glutathione reductase (20unit/ml).

Determination glutathione peroxidase

Glutathione peroxidase activity was measured according to a modified method of Beutler (1971). Which uses an antioxidation reaction of reduced glutathione was used by glutathione peroxidase coupled to the disappearance of NADPH by glutathione reductase using tert-butyl hydroperoxide as the substrate.

Reagent mixture preparation

	A1	A2
Tris-HCl 1M, EDTA 5mM pH 8.0 (µL)	100	100
GSH, 1.0 M (µL)	20	20
Glutathione reductase, 10 U/ml (µL)	100	100
NADPH, 2mM (µL)	100	100
1:20 hemolyzate (µL)	10	10
H_2O (µL)	670	660
* Incubate 37 °C 5 min.		
t- Butyl hydroperoxide, 7 mM (µL)	-	10
* Approximately 1:1000 dilution		

The absorbance was read at 340 nm (A1), after 1 minute read absorbance (A2)

Calculated $\Delta A = A1-A2$

Calculated Gpx from the equivalent;

Glutathione peroxidase activity (nmol/min/mL)

$$= \frac{\Delta A/min \times 161 \times Z}{X}$$

ΔA = the absorbance at 340 nm at t1 and t2

X = sample volume (ml)

Z = the reaction volume (ml)

Statistical analysis

All data were expressed as mean $\pm$ SEM. The GSH, GSSG, GSH/GSSG and Gpx levels were compared between pre-treatment and post-treatment by using paired t- test. The different groups were calculated by ANOVA test. Statistical differences were considered significant at $p<0.05$.

CHAPTER IV

RESULTS

Part I. Radical scavenging activity and the effective concentration

The crude extract of *Antidesma acidum* was 3.17% of total stem and bark parts (total stem and bark parts were 11.4 kg and the final crude extract yielded 423.61 g).

The results of $DPPH^{\bullet}$ activity on the percent radical scavenging (%) at different concentration of extract, quercetin hydrate and BHT were demonstrate (Table 2 and Figure 11). The effective concentration (EC_{50}) were presented (Table 3).

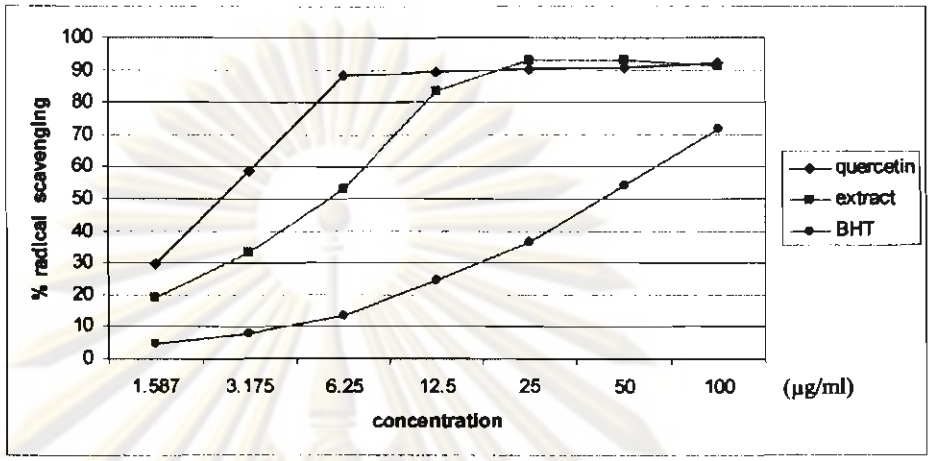
The crude extract of *Antidesma acidum* were as nearly as effective on DPPH radical scavengers as quercetin hydrate. At the concentration of 100 µg/ml, per cent radical scavenging activity of quercetin, the crude extract of *Antidesma acidum* and BHT were 91.90, 91.36 and 71.79, respectively. The percentages radical scavenging of both standard antioxidant (quercetin hydrate and BHT) depended on the concentration. At the lower concentration of quercetin hydrate and BHT, there were the decrease in the percentages of radical scavenging. But for the crude extract of *Antidesma acidum*, the percentages radical scavenging were effective at the concentration of 25 and 50 µg/ml. In the concentration of 25-100 µg/ml., both quercetin hydrate and the crude extract of *Antidesma acidum* showed strong property of the radical scavenger.

Table 2. Percent (%) radical scavenging of BHT, quercetin hydrate and the crude extract of *Antidesma acidum*.

	Concentration (µg/ml)						
	100	50	25	12.5	6.25	3.125	1.562
BHT	71.79±1.36	54.04±3.30	36.74±3.05	24.47±0.33	13.27±1.22	7.75±0.85	4.53±1.41
Quercetin	91.90±0.23	90.81±0.51	90.06±0.52	89.31±0.48	88.31±0.48	58.79±0.70	29.69±0.53
Extract	91.36±0.35	92.99±0.44	93.27±0.31	83.48±0.60	53.30±1.13	33.53±1.61	19.17±1.29

BHT = 3,5-di-tert-butyl-4-hydroxytol or Butylhydroxytoluene
Quercetin = quercetin hydrate
Extract = the crude extract of *Antidesma acidum*

Figure 11. Percent (%) radical scavenging concentration of BHT, quercetin hydrate, the crude extract of *Antidesma acidum.*



BHT = 3,5-di-tert-butyl-4-hydroxytol or Butylhydroxytoluene
quercetin = quercetin hydrate
Extract = the crude extract of *Antidesma acidum*

The effective concentration (EC_{50}) of substance which can decrease 50 percents of DPPH concentration. EC_{50} of quercetin hydrate, the crude extract and BHT were 3.13, 5.74 and 44.12 µg/ml, respectively. Quercetin hydrate and the crude extract were more effective than BHT at approximately 14 and 8 times, respectively. The effective concentration (EC_{50}) were demonstrated (Table 3).

Table 3. Mean±SEM of the effective concentration (EC_{50}) of BHT, quercetin hydrate and the crude extract of *Antidesma acidum.*

Type of sample	EC_{50}* (µg/ml)
BHT	44.12±2.25
Quercetin hydrate	3.13±0.77
Crude extract	5.47±0.02

EC_{50}* = Effective concentration of substance which can decreased 50 percents of DPPH concentration.
BHT = 3,5-di-tert-butyl-4-hydroxytol or Butylhydroxytoluene
Extract = the crude extract of *Antidesma acidum*

Part II. Signalment

Thirty cats (14 males, 13 sterile females and 3 females cats) were studied. In the clinically normal cats, there were six males, two females and two female neutered. The CRF cats were consisted of eight males, eleven females neutered and one intact female. Twelve cats were from the Small Animal Hospital Chulalongkorn University and eighteen cats were from private animal hospitals. All cats were divided into three groups according to the creatinine levels. Mean±SEM of age in the clinically normal, mild azotemia and moderate azotemia cats were 4.3±0.37, 10.8±0.88 and 11±0.45 years old, respectively (Figure 12). Mean ±SEM of weight in the clinically normal cats, mild azotemia cats and moderate azotemia cats were 3.76±0.35, 4.23±0.36 and 3.84±1.45 kilograms, respectively on day 0 of the study. Mean±SEM of weight in the clinically normal cats, mild azotemia cats and moderate azotemia cats were 4.2±0.52, 4.45±0.61 and 4.52±0.55 kilograms, respectively on day 56 of the study (Figure 13). All of the chronic renal failure cats were in the stable condition with no severe clinical signs.

Figure 12. Mean±SEM of age (years) in the clinically normal, mild azotemia and moderated azotemia cats.

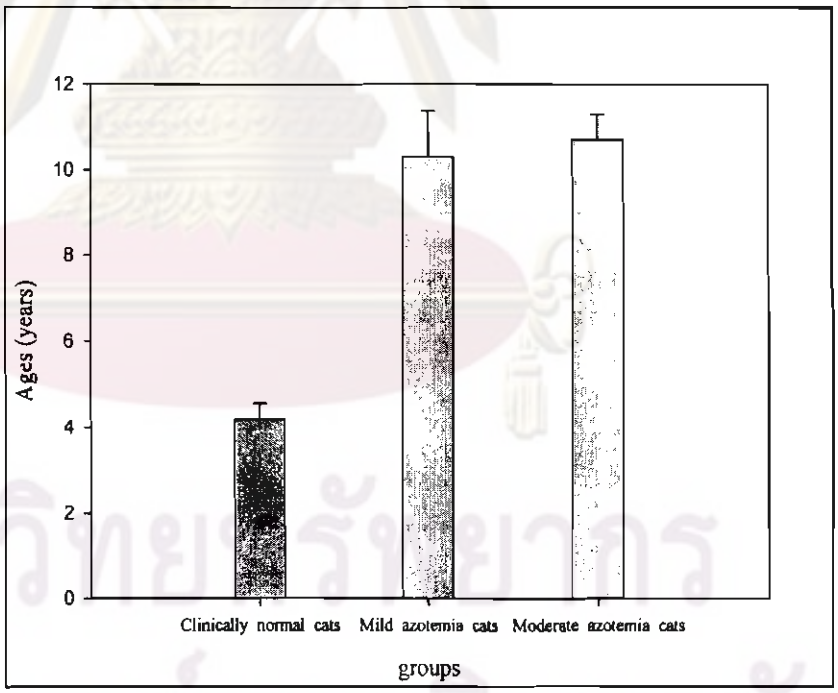
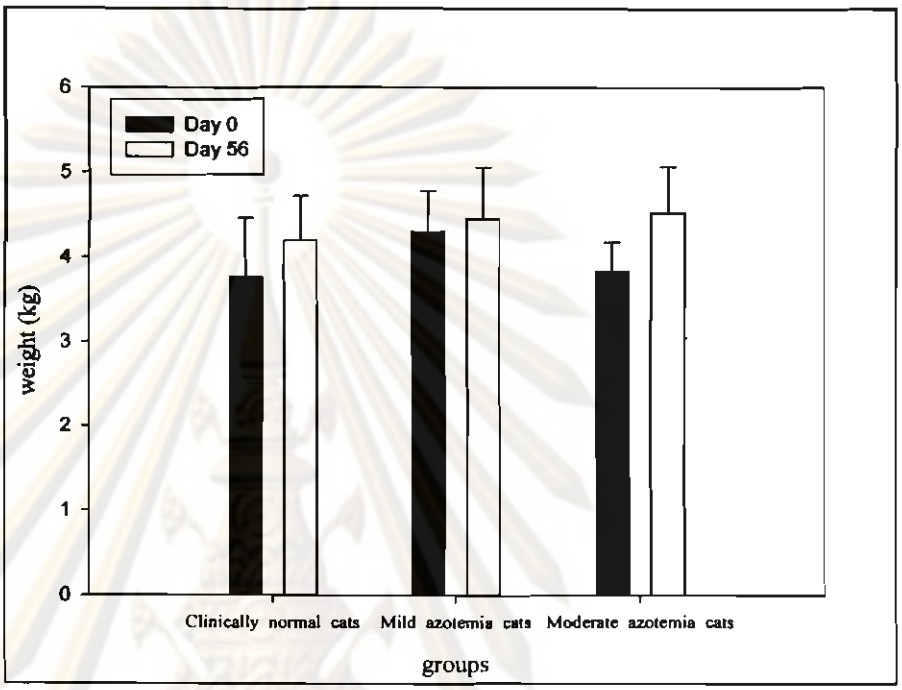


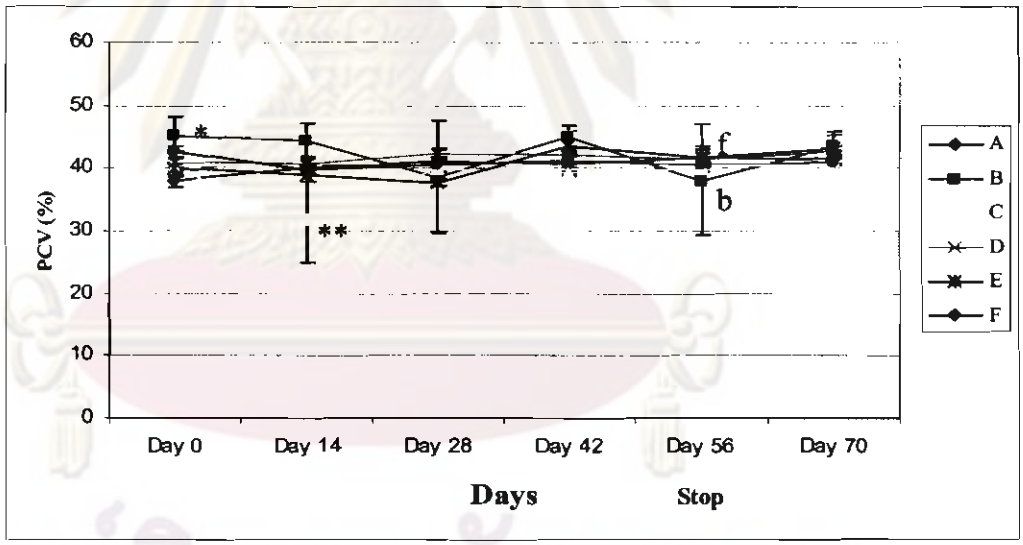
Figure 13. Mean±SEM of weight (kg) in the clinically normal, mild azotemia and moderated azotemia cats on day 0 and day 56 of the study.



Part III. Hematology

Pack cell volume (%) in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 14). The clinically normal cats received the crude extract of *Antidesma acidum* had significantly higher PCV than the clinically normal cats received placebo on day 0 ($p<0.05$). Mean PCV were significantly decrease in the clinically normal cats received the crude extract of *Antidesma acidum* on day 56 when compared to day 0 ($p<0.05$). The mild azotemia cats received placebo had significantly lower PCV than the mild azotemia cats received the crude extract of *Antidesma acidum* (day 14) ($p<0.01$). The moderate azotemia cats received the crude extract of *Antidesma acidum* had significantly lower PCV on day 56 than day 0 of the study ($p<0.05$) (Figure 14).

Figure 14. Mean±S.E.M. of pack cell volume (PCV) in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.



A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum*
Stop = stop the treatment

* *$p<0.05$ when compared between clinically normal cats received placebo and the crude extract of Antidesma acidum.*

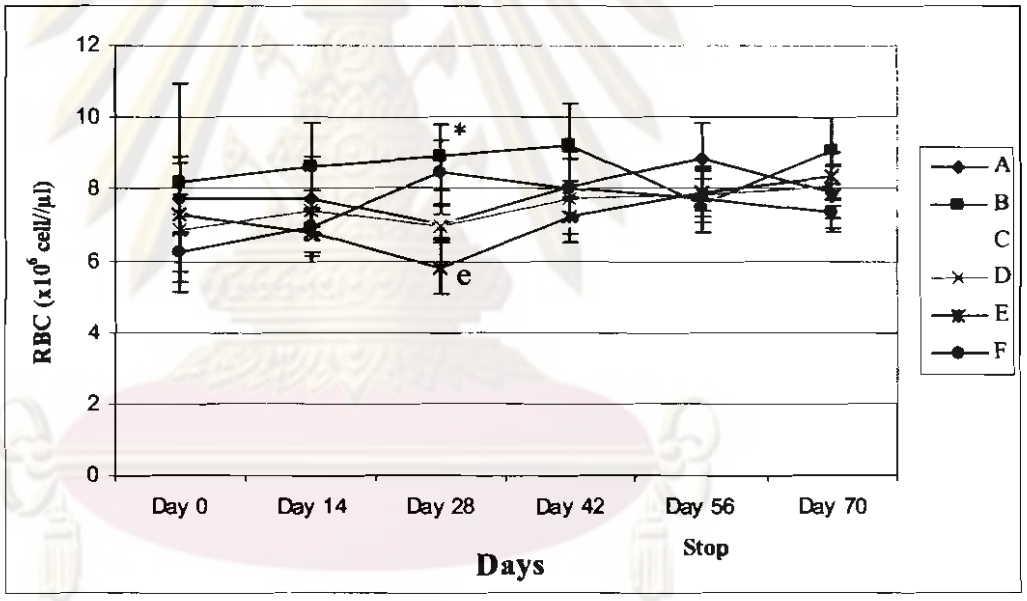
** *$p<0.01$ when compared between mild azotemia cats received placebo and the crude extract of Antidesma acidum.*

b *$p<0.05$ when compared between clinically normal cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 56).*

f *$p<0.05$ when compared between moderate azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 56).*

The red blood cells in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 15). Mean red blood cells were significantly elevated in the clinically normal cats received the crude extract of *Antidesma acidum* when compared with the clinically normal cats received placebo (day 28) ($p<0.05$). The moderate azotemia cats received placebo had significantly decrease red blood cells on day 28 when compared to day 0 ($p<0.05$) (Figure 15).

Figure 15. Mean±S.E.M. of red blood cells (RBC) in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.



A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = moderate azotemia cats received placebo
F = moderate azotemia catsn received the crude extract of *Antidesma acidum*
Stop = stop the treatment

* *$p<0.05$ when compared between clinically normal cats received placebo and the crude extract of Antidesma acidum.*

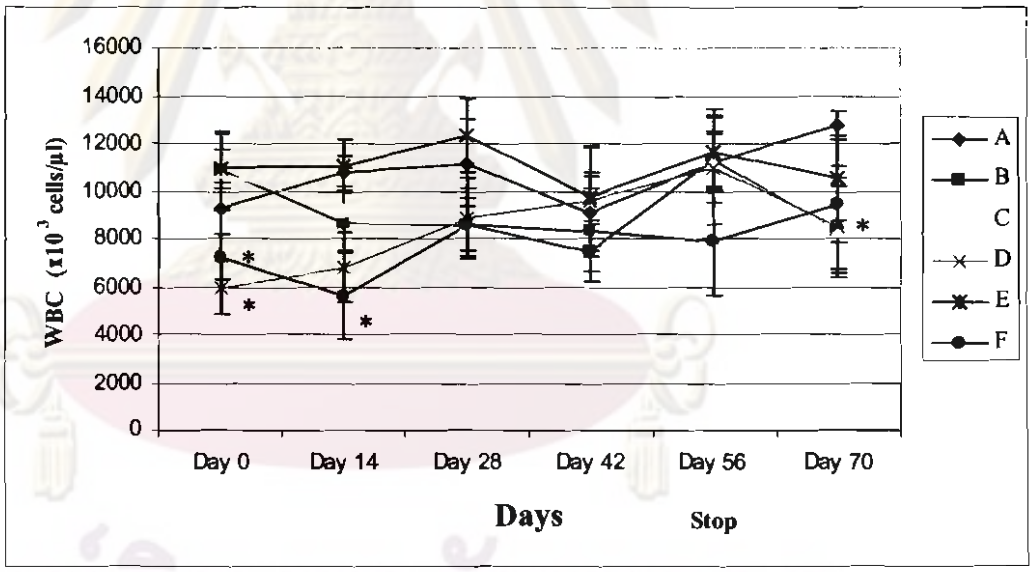
e *$p<0.05$ when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment (day 28).*

The total white blood cells in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 16). The clinically normal cats received the crude extract of *Antidesma acidum* had significantly lower total white blood cells than the clinically normal cats received placebo (day 70) ($p<0.05$).

Mild azotemia cats received the crude extract of *Antidesma acidum* had significantly lower total white blood cells than mild azotemia cats received placebo (day 0) ($p<0.05$).

Moderate azotemia cats received the crude extract of *Antidesma acidum* had significantly lower white blood cells when compared with moderate azotemia cats received placebo (day 0 and 14) ($p<0.05$) (Figure 16).

Figure 16. Mean±S.E.M. of total white blood cells (cells/μL) in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.

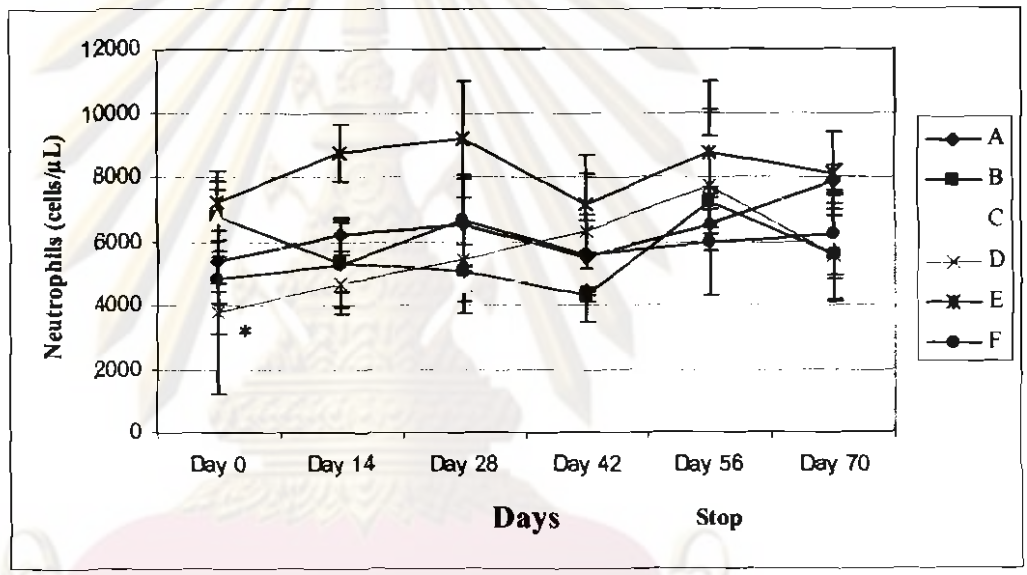


A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum*
Stop = stop the treatment

* *$p<0.05$ when compared between clinically normal, mild azotemia and moderate aotemia cats received placebo and the crude extract of Antidesma acidum.*

The neutrophils in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 17). The mild azotemia cats received the crude extract of *Antidesma acidum* had significantly lower neutrophils than mild azotemia cats received placebo (day 0) ($p<0.05$) (Figure 17).

Figure 17. Mean±S.E.M. of neutrophils (cells/μL) in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.

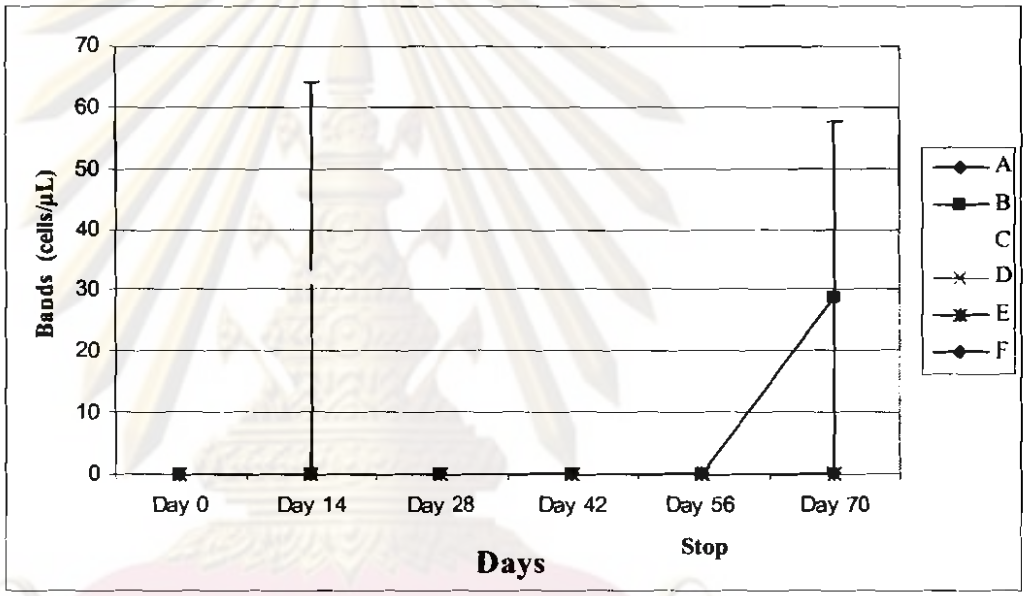


A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum*
Stop = stop the treatment

* *$p<0.05$ when compared between mild azotemia cats received placebo and the crude extract of Antidesma acidum.*

The band cells in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 18).

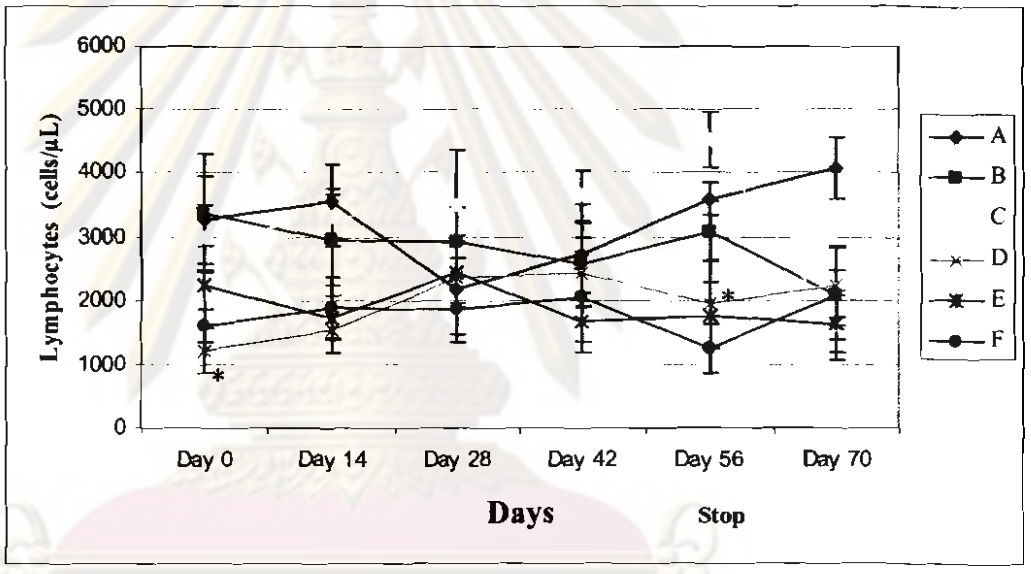
Figure 18. Mean±S.E.M. of band cells (cells/μL) in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.



A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum*
Stop = stop the treatment

The lymphocytes in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 19). The mild azothemia cats received the crude extract of *Antidesma acidum* had significantly lower lymphocytes than mild azotemia cats received placebo (day 0 and 56) ($p<0.05$) (Figure 19).

Figure 19. Mean±S.E.M. of lymphocytes (cells/μL) in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.



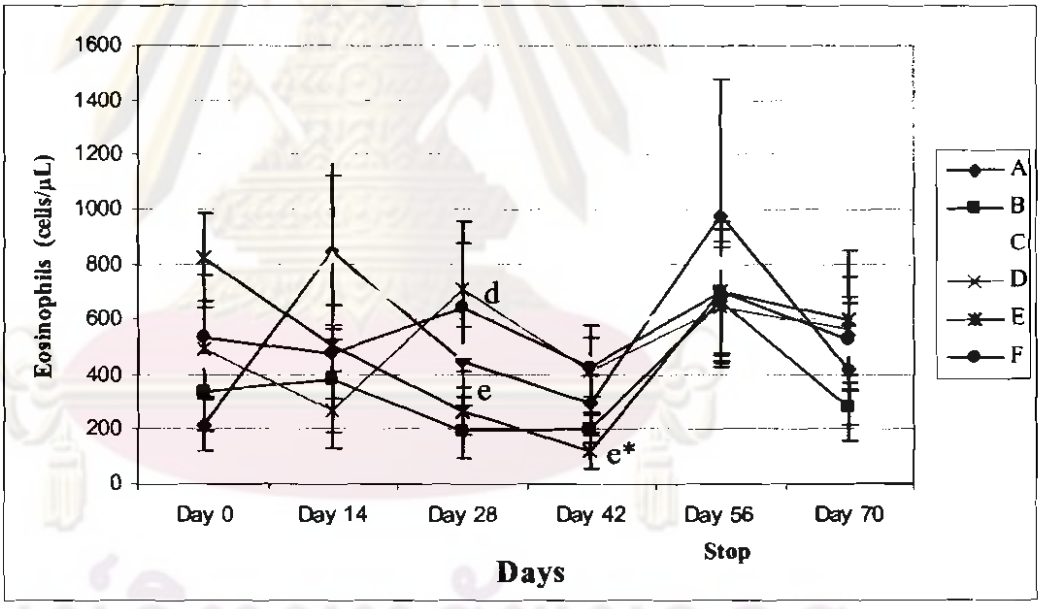
A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = modcrate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum*
Stop = stop the treatment

* *$p<0.05$ when compared between mild azotemia cats received placebo and the crude extract of Antidesma acidum.*

The eosinophils in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 20). Mean eosinophils were significantly increase in the mild azotemia cats received the crude extract of *Antidesma acidum* on day 28 when compared to day 0 ($p<0.05$).

The moderate azotemia cats received placebo had significantly decrease eosinophils on day 28 and 42 when compared to day 0 ($p<0.05$). Mean eosinophils in the moderate azotemia cats received placebo were significantly lower than the moderate azotemia cats received the crude extract of *Antidesma acidum* (day 42) ($p<0.05$) (Figure 20).

Figure 20. Mean±S.E.M. of eosinophils (cells/μL) in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.



A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum*
Stop = stop the treatment

* *$p<0.05$ when compared between moderate azotemia cats received placebo and the crude extract of Antidesma acidum.*

[d] *$p<0.05$ when compared between mild azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 28).*

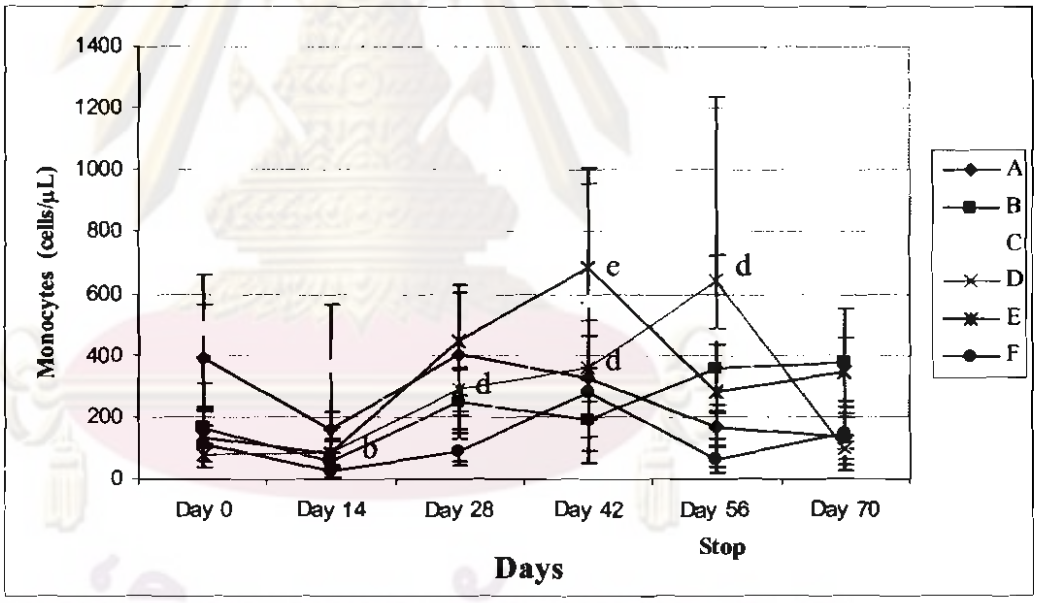
[e] *$p<0.05$ when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment (day 28 and 42).*

The monocytes in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 21). In the clinically normal cats received the crude extract of *Antidesma acidum* had significantly decrease monocytes on day 14 when compared to day 0 ($p<0.05$).

Mean monocytes were significantly increase in the mild azotemia cats received the crude extract of *Antidesma acidum* on day 28, 42 and 56 when compared to day 0 ($p<0.05$).

Moderate azotemia cats received placebo had significantly increase monocytes on day 42 when compared to day 0 ($p<0.05$) (Figure 21).

Figure 21. Mean±S.E.M. of monocytes (cells/μL) in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.



A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum*
Stop = stop the treatment

[b] *$p<0.05$ when compared between clinically normal cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 14).*

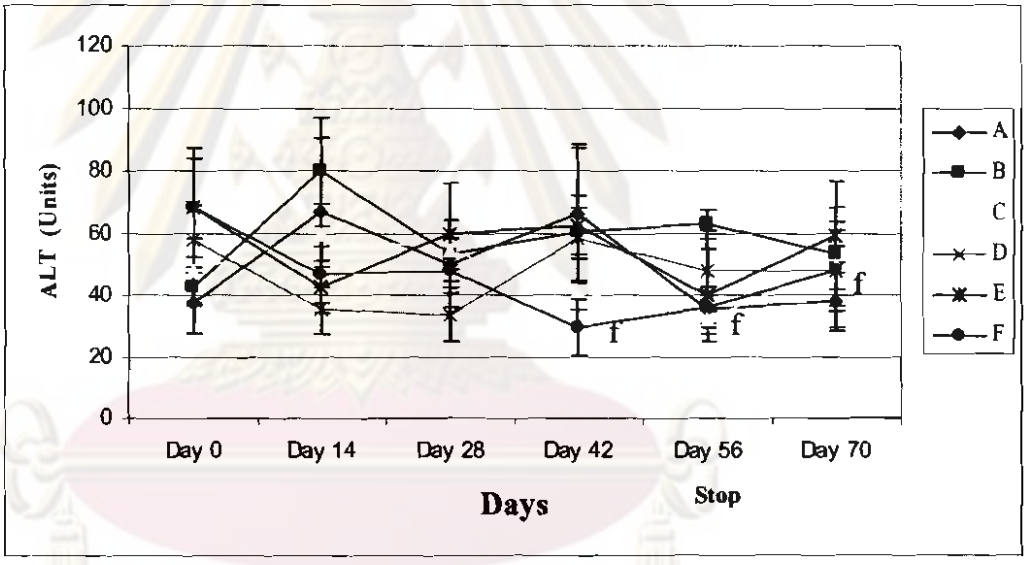
[d] *$p<0.05$ when compared between mild azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 28, 42 and 56).*

[e] *$p<0.01$ when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment (day 42).*

Part IV. Plasma ALT and ALP levels

Plasma ALT in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 22). Moderate azotemia cats received the crude extract of *Antidesma acidum* had significantly decrease plasma ALT levels when compared between before treatment (day 0) and after treatment (day 42, 56 and 70) ($p<0.05$) (Figure 22).

Figure 22. Mean±S.E.M. of ALT levels in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.

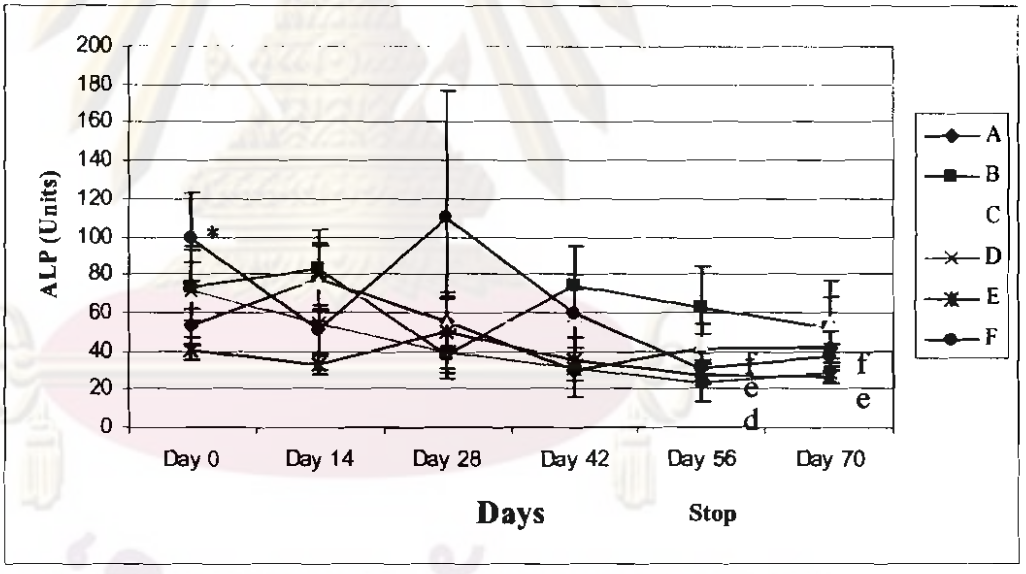


A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum*
Stop = stop the treatment

[f] *$p<0.05$ when compared between moderate azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 42, 56 and 70).*

Plasma ALP in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 23). The mean plasma ALP levels were significantly decrease in the mild azotemia cats received the crude extract of *Antidesma acidum* on day 56 when compared to day 0 ($p<0.05$). Moderate azotemia cats received the crude extract of *Antidesma acidum* had significantly higher plasma ALP than moderate azotemia cats received placebo on day 0 of the study ($p<0.05$). Mean plasma ALP levels were significantly decrease in moderate azotemia cats received placebo on day 56 and 70 when compared to day 0 ($p<0.01$). Moderate azotemia cats received the crude extract of *Antidesma acidum* had significantly decrease in plasma ALP levels on day 56 and 70 ($p<0.05$) when compared to day 0 (Figure 23).

Figure 23. Mean±S.E.M. of ALP levels in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.



A = clinically normal cats received placebo
B = clinically normal eats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum*
Stop = stop the treatment

* *$p<0.05$ when compared between moderate azotemia cats received placebo and the crude extract of Antidesma acidum.*

d *$p<0.05$ when compared between mild azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 56).*

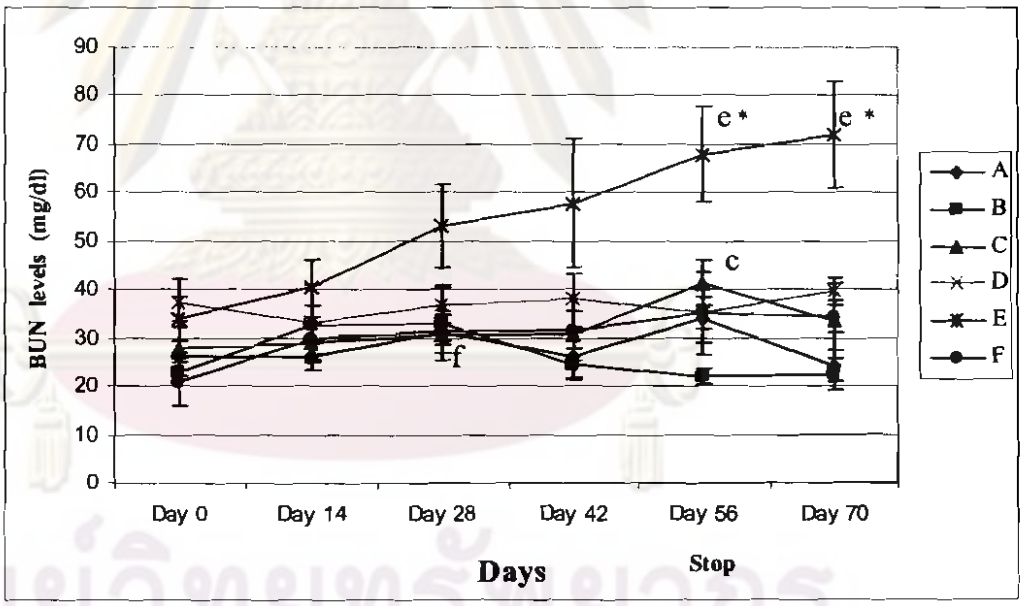
e *$p<0.01$ when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment (day 56 and 70).*

f *$p<0.05$ when compared between moderate azotemia cats received the* crude extract of *Antidesma acidum before treatment (day 0) and after treatment (day 56 and 70).*

Part V. Blood urea nitrogen (BUN), creatinine levels and urinalysis

The BUN levels in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 24). Mean BUN levels in the mild azotemia cats received placebo were significantly elevated on day 56 when compared to day 0 ($p<0.01$). In the moderate azotemia cats received the crude extract of *Antidesma acidum* had significantly increase BUN levels on day 28 when compared to day 0 ($p<0.05$). In the moderate azotemia cats received placebo had significantly increase in BUN levels on day 56 and 70 when compared to day 0 ($p<0.05$). Mean BUN levels were significantly increase in the moderate azotemia cats received placebo when compared to the moderate azotemia cats received the crude extract of *Antidesma acidum* (day 56 and 70) ($p<0.05$) (Figure 24).

Figure 24. Mean±S.E.M. of blood urea nitrogen levels in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.



A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum*
Stop = stop the treatment

* *$p<0.05$ when compared between moderate azotemia cats received placebo and the crude extract of Antidesma acidum.*

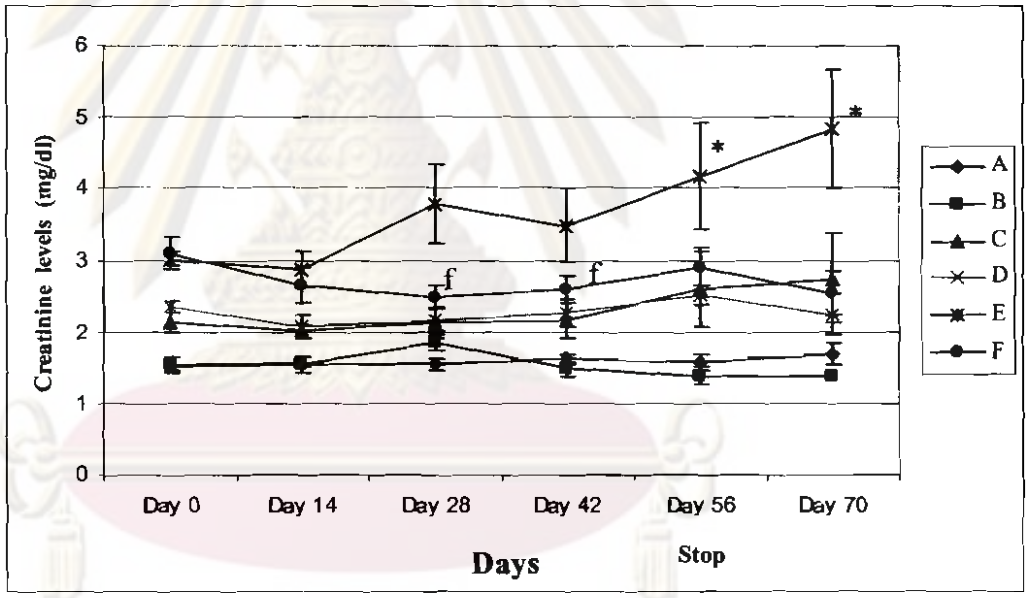
c *$p<0.01$ when compared between mild azotemia cats received placebo before treatment (day 0) and after treatment (day 56).*

e *$p<0.05$ when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment (day 56 and 70).*

f *$p<0.05$ when compared between moderate azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 28).*

The creatinine levels in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 25). In the moderate azotemia cats received the crude extract of *Antidesma acidum* had significantly decrease creatinine levels on day 28 and 42 when compared to day 0 ($p<0.05$). The mean creatinine levels were significantly increase in moderate azotemia cats received placebo when compared to moderated azotemia cats received the crude extract of *Antidesma acidum* (day 56 and 70) ($p<0.05$) (Figure 25).

Figure 25. Mean±S.E.M. of creatinine levels in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.



A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum*
Stop = stop the treatment

* $p<0.05$ *when compared between moderate azotemia cats received placebo and the crude extract of Antidesma acidum (day 56 and 70).*

f $p<0.05$ *when compared between moderate azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 28 and 42).*

Urinalysis

The urinalysis in the clinically normal cats on day 0 were presented (Table 4). Mean±SEM of the urine specific gravity (SG) in the clinically normal cats on day 0 were 1.040±0.002. The mean±SEM of the urine pH were 6.85±0.2.

The mild azotemia cats on day 0 were presented (Table 5). Mean±SEM of the urine specific gravity (SG) in the mild azotemia cats on day 0 were 1.027±0.003. The mean±SEM of the urine pH were 6.85±0.2.

The moderate azotemia cats on day 0 were presented (Table 6). Mean±SEM of the urine specific gravity (SG) in the moderate azotemia cats on day 0 were 1.027±0.003. The mean±SEM of the urine pH were 6.95±0.17. The UPC ratio in cats number 24 and 25 were 0.34 and 0.87, respectively.

The mean urine specific gravity in the clinically normal cats were significantly higher than the mild and moderate azotemia group on day 0 of the study ($p<0.01$).

The urinalysis in the clinically normal cats on day 56 were presented (Table 7). Mean±SEM of the urine specific gravity (SG) in the clinically normal cats on day 56 were 1.033±0.004. The mean±SEM of the urine pH were 6.92± 0.24.

The mild azotemia cats on day 56 were presented (Table 8). Mean±SEM of the urine specific gravity (SG) in the mild azotemia cats on day 56 were 1.018±0.001. The mean±SEM of the urine pH were 6.68±0.22.

The moderate azotemia cats on day 56 were presented (Table 9). Mean±SEM of the urine specific gravity (SG) in the moderate azotemia cats on day 56 were 1.021± 0.002. The mean±SEM of the urine pH were 6.66 ± 0.25.

The mean urine specific gravity in the clinically normal cats were significantly higher than the mild and moderate azotemia group on day 56 of the study ($p<0.05$).

Table 4. Urinalysis* in the clinically normal cats on day 0.

Number	Color	SG	pH	Blood	Bilirubin	Urobilinogen	Ketone	Glucose	Protein	Nitrite	Leucocyte	Note
1	yellow	1.040	7.0	-	-	-	-	-	+2	-	+3	RBC 0-10 hpf
2	yellow	1.028	7.0	-	-	-	-	-	+1	-	+3	
3	yellow	>1.050	7.0	-	-	-	-	-	+2	-	+3	+ cocci bacteria, RBC 0-20 hpf
4	yellow	1.040	7.5	-	-	-	-	-	+2	-	+3	RBC 0-5 hpf, WBC 0-10 hpf
5	yellow	1.030	6.0	-	-	-	-	-	+1	-	+3	-
6	yellow	1.045	6.0	-	-	-	-	-	+2	-	+3	-
7	yellow	>1.050	7.5	-	-	-	-	-	+1	-	+3	RBC 0-10 hpf
8	yellow	1.038	7.5	-	-	-	-	-	+2	-	+3	RBC 0-5 hpf
9	yellow	1.040	7.0	-	-	-	-	-	+1	-	+3	-
10	yellow	1.036	6.0	-	-	-	-	-	+1	-	+3	-

* Voiding
SG = specific gravity
hpf = high power field
RBC = red blood cell
WBC= white blood cell

Table 5. Urinalysis* in the mild azotemia cats on day 0.

Number	Color	SG	pH	Blood	Bilirubin	Urobilinogen	Ketone	Glucose	Protein	Nitrite	Leucocyte	Note
11	yellow	1.020	6.5	-	-	-	-	-	+1	-	+3	-
12	light yellow	1.014	6.5	-	-	-	-	-	+1	-	+3	-
13	yellow	1.043	7.5	-	-	-	-	-	+1	-	+3	RBC 0-30 hpf, struvite crystal
14	yellow	1.030	7.0	-	-	-	-	-	+1	-	+3	-
15	yellow	1.025	6.0	-	-	-	-	-	+1	-	+3	-
16	yellow	1.030	7.0	-	-	-	-	-	+1	-	+3	-
17	yellow	1.045	7.5	-	-	-	-	-	+2	-	+3	RBC 0-10 hpf, hyaline cast
18	yellow	1.025	7.5	-	-	-	-	-	+2	-	+3	RBC 0-5 hpf
19	yellow	1.022	6.5	-	-	-	-	-	+1	-	+3	-
20	Yellow	1.020	7.5	-	-	-	-	-	+1	-	+3	-

* Voiding
SG = specific gravity
hpf = high power field
RBC = red blood cell

Table 6. Urinalysis* in the moderate azotemia cats on day 0.

Number	Color	SG	pH	Blood	Bilirubin	Urobilinogen	Ketone	Glucose	Protein	Nitrite	Leucocyte	Note
21	light yellow	1.012	6.0	-	-	-	-	-	+1	-	+3	-
22	yellow	1.020	6.5	-	-	-	-	-	+1	-	+3	-
23	yellow	1.022	6.0	-	-	-	-	-	+1	-	+3	-
24	yellow	1.018	6.0	-	-	-	-	-	+1	-	+3	urine creatinine 87 mg/dl, urine protein 3 mg%
25	light yellow	1.008	5.0	-	-	-	-	-	-	-	+3	urine creatinine 46 mg/dl, urine protein 4 mg%
26	light yellow	1.010	7.5	-	-	-	-	-	+1	-	+3	-
27	yellow	1.022	7.5	-	-	-	-	-	+1	-	+3	RBC 0-10 hpf, hyaline cast
28	yellow	1.020	7.5	-	-	-	-	-	+2	-	+3	RBC 0-5 hpf
29	yellow	1.022	6.5	-	-	-	-	-	+1	-	+3	-
30	yellow	1.018	6.5	-	-	-	-	-	+1	-	+3	-

* Voiding
SG = specific gravity
hpf = high power field
RBC = red blood cell

Table 7. Urinalysis* in the clinically normal cats on day 56.

Number	Color	SG	pH	Blood	Bilirubin	Urobilinogen	Ketone	Glucose	Protein	Nitrite	Leucocyte	Note
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	yellow	1.050	7.0	-	-	-	-	-	+1	-	+3	RBC 0-5 hpf
5	yellow	1.025	6.0	-	-	-	-	-	+1	-	+3	-
6	yellow	1.030	7.0	-	-	-	-	-	+1	-	+3	RBC 0-5 hpf. small amounts debris
7	-	-	-	-	-	-	-	-	-	-	-	-
8	yellow	1.025	7.5	-	-	-	-	-	+2	-	+3	small amounts debris
9	yellow	1.035	6.5	-	-	-	-	-	+1	-	+3	-
10	Yellow	1.040	7.5	-	-	-	-	-	+1	-	+3	-

* Voiding
SG = specific gravity
hpf = high power field
RBC = red blood cell
WBC= white blood cell

Table 8. Urinalysis* in the mild azotemia cats on day 56.

Number	Color	SG	pH	Blood	Bilirubin	Urobilinogen	Ketone	Glucose	Protein	Nitrite	Leucocyte	Note
11	Yellow	1.018	6.0	-	-	-	-	-	+1	-	+3	-
12	Yellow	1.020	6.5	-	-	-	-	-	+1	-	+3	-
13	Yellow	1.025	6.5	-	-	-	-	-	+1	-	+3	-
14	Yellow	1.022	6.0	-	-	-	-	-	+1	-	+3	-
15	Light yellow	1.012	6.0	-	-	-	-	-	-	-	+3	RBC 0-10 hpf, WBC 0-5 hpf
16	Light yellow	1.010	7.5	-	-	-	-	-	+1	-	+3	-
17	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-
19	Yellow	1.022	6.5	-	-	-	-	-	+1	-	+3	-
20	Yellow	1.016	6.0	-	-	-	-	-	+1	-	+3	RBC 0-10 hpf

* Voiding
SG = specific gravity
hpf = high power field
RBC = red blood cell
WBC= white blood cell

Table 9. Urinalysis* in the moderate azotemia cats on day 56.

Number	Color	SG	pH	Blood	Bilirubin	Urobilinogen	Ketone	Glucose	Protein	Nitrite	Leucocyte	Note
21	-	-	-	-	-	-	-	-	-	-	-	-
22	Light yellow	1.018	6.0	-	-	-	-	-	+1	-	+3	-
23	-	-	-	-	-	-	-	-	-	-	-	-
24	Yellow	1.024	7.0	-	-	-	-	-	+1	-	+3	Small amount debris, waxy cast
25	Yellow	1.020	6.0	-	-	-	-	-	+1	-	+3	-
26	Yellow	1.030	7.0	-	-	-	-	-	+1	-	+3	WBC 0-5 hpf
27	-	-	-	-	-	-	-	-	-	-	-	-
28	Light yellow	1.015	7.5	-	-	-	-	-	+2	-	+3	-
29	Yellow	1.018	6.5	-	-	-	-	-	+1	-	+3	-
30	-	-	-	-	-	-	-	-	-	-	-	-

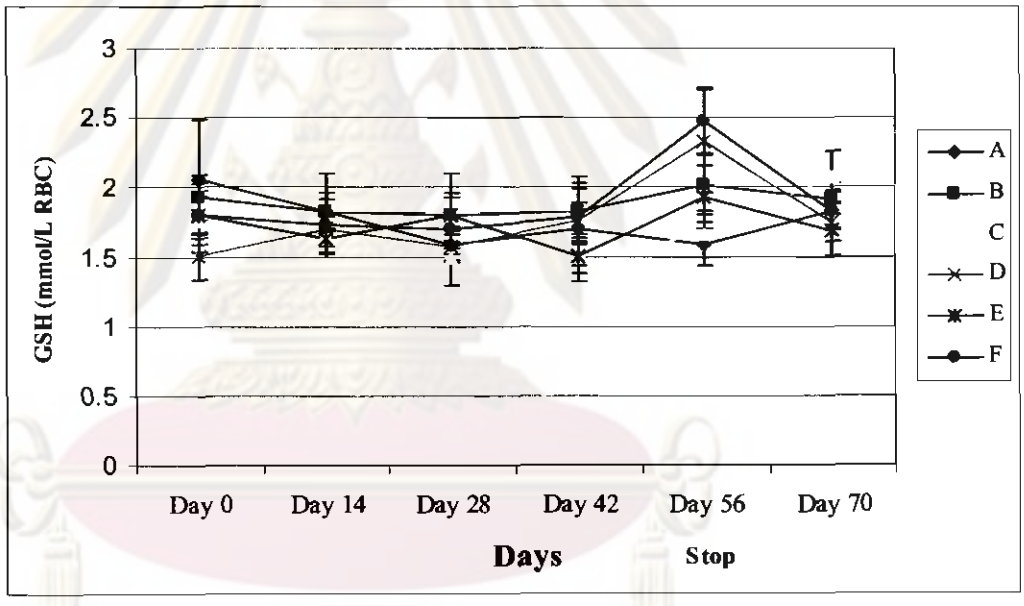
* Voiding
SG = specific gravity
hpf = high power field
WBC= white blood cell

Part VI. Oxidative stress markers

The mean±SEM glutathione levels in the clinically normal cats were 2.0±0.22 mmoles/L RBC.

The glutathione (GSH) levels in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 26).

Figure 26. Mean±S.E.M. of glutathione levels (mmol/L RBC) in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.

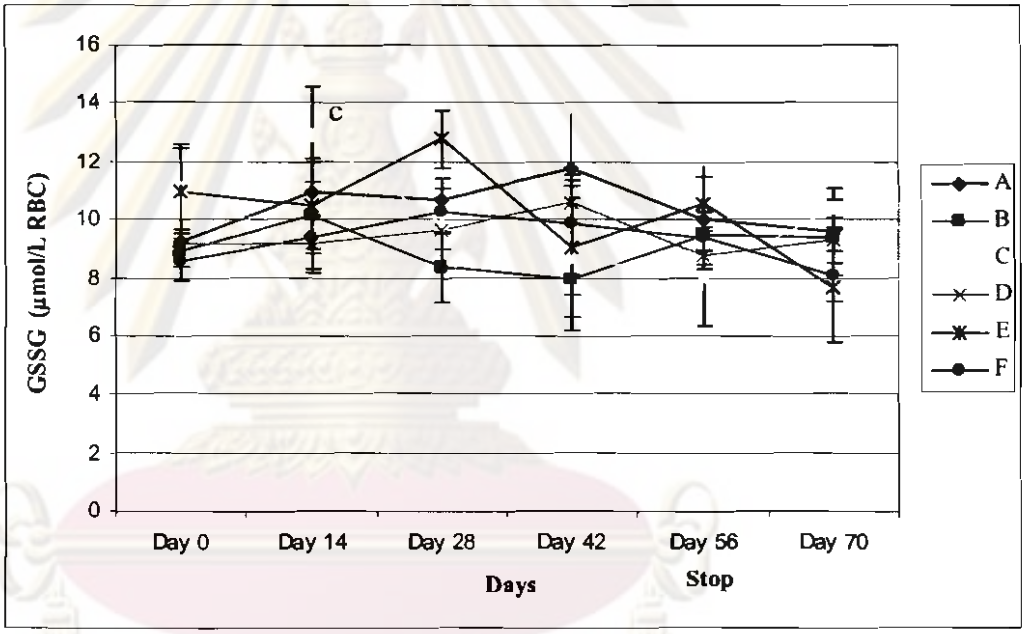


A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum.*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum.*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum.*
Stop = stop the treatment

The oxidized glutathione (GSSG) levels in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 27).

The mild azotemia cats received placebo had significantly increase GSSG on day 14 when compared to day 0 ($p<0.05$).

Figure 27. Mean±S.E.M. of oxidized glutathione levels (µmol/L RBC) in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.



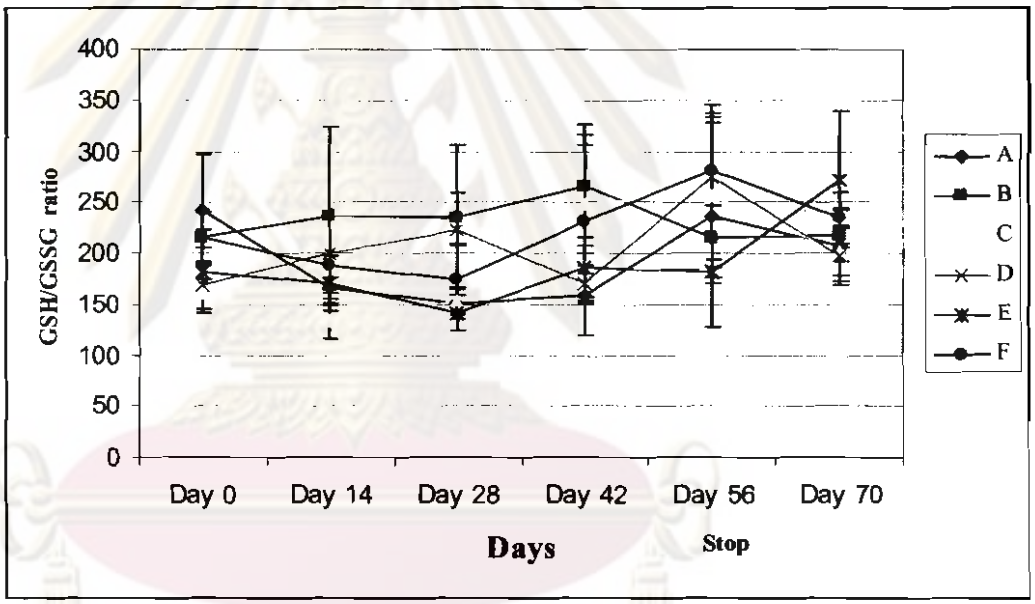
A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum.*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum.*
E = moderate azotemia eats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum.*
Stop = stop the treatment

[c] *$p<0.05$ when compared between mild azotemia cats received placebo before treatment (day 0) and after treatment (day 14).*

The GSH/GSSG ratio in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 28).

The mean GSH/GSSG ratio in the clinically normal, mild azotemia and moderate azotemia cats were 228.8, 164.3 and 199.3, respectively. Mean GSH/GSSG ratio in the mild azotemia cats were significantly lower than the clinically normal cats on day 0 of the study.

Figure 28. Mean±S.E.M. of GSH/GSSG ratio in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.

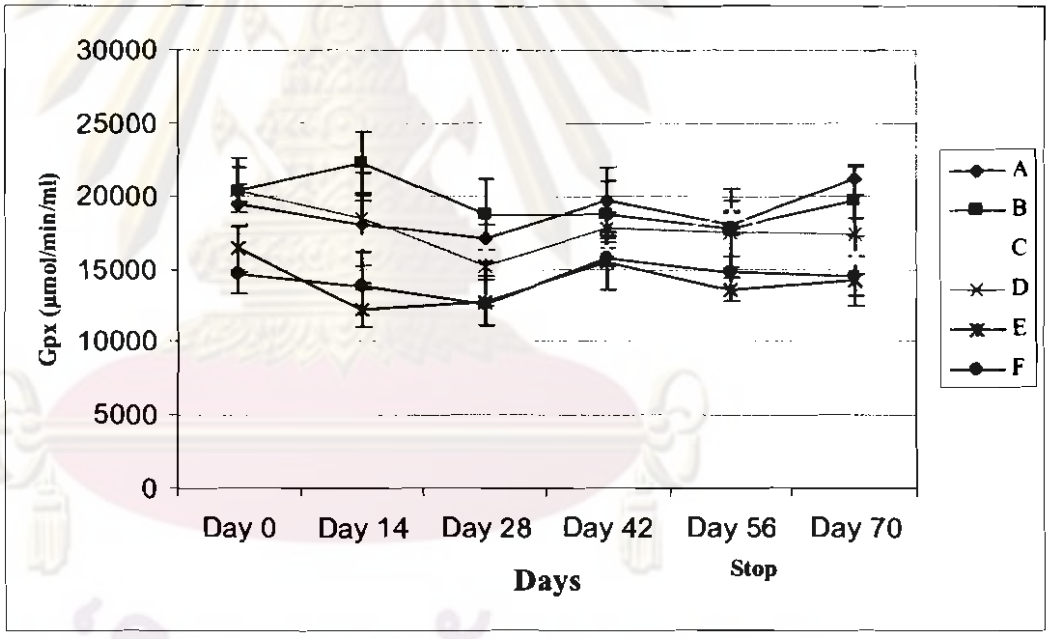


A = clinically normal cats received placebo
B = clinically normal cats received the crude extract of *Antidesma acidum.*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum.*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum.*
Stop = stop the treatment

The glutathione peroxidase (Gpx) (µmol/min/ml) in each group of the cats on day 0, 14, 28, 42, 56 and 70 were presented (Figure 29).

The mean glutathione peroxidase activity in the clinically normal, mild azotemia and moderate azotemia cats were 19,899.6, 19,384.4 and 15,552.6 µmol/min/ml, respectively. Mean glutathione peroxidase in the moderate azotemia cats were significantly lower than the clinically normal cats on day 0 of the study.

Figure 29. Mean±S.E.M. of glutathione peroxidase (Gpx) (µmol/min/ml) in the clinically normal cats received placebo (A), the clinically normal cats received the crude extract of *Antidesma acidum* (B), mild azotemia cats received placebo (C), mild azotemia cats received the crude extract of *Antidesma acidum* (D), moderate azotemia cats received placebo (E) and moderate azotemia cats received the crude extract of *Antidesma acidum* (F) on day 0, 14, 28, 42, 56 and 70.



A = clinically normal cats received placebo
B = clinically normal eats received the crude extract of *Antidesma acidum.*
C = mild azotemia cats received placebo
D = mild azotemia cats received the crude extract of *Antidesma acidum.*
E = moderate azotemia cats received placebo
F = moderate azotemia cats received the crude extract of *Antidesma acidum.*
Stop = stop the treatment

CHAPTER V

DISCUSSION

Part I. Radical scavenging activity and the effective concentration

The crude extract of *Antidesma acidum* were as effective DPPH radical scavengers as quercetin hydrate at the concentration of 100 µg/ml. The percentages scavenging activity of quercetin hydrate and crude extract were 91.90 and 91.36, respectively. Whereas the percentages scavenging activity of BHT was 71.79. The effective concentration (EC_{50}) which can decrease 50 percents DPPH concentration of quercetin hydrate and the crude extract were 3.13 and 5.47 µg/ml, respectively. Both quercetin hydrate and crude extract of *Antidesma acidum* were more effective than BHT at approximately 14 and 8 times. The percentages radical scavenging of both standard antioxidants depended on its concentration. It was interested that even at various concentration of quercetin hydrate and the crude extract, both showed strong radical scavenger properties. The crude extract of *Antidesma acidum* at the concentration of 25 µg/ml. had the highest percentage radical scavenging in vitro. The studies of many plants such as Lavander *(Lavandula angustifolia)*, Garden sage (*Salvia officinalis)*, German chamomile *(Matricaria recutita)* and Walnut tree *(Juglans regia)* demonstrated that the amount of total phenolic compounds in the plant extracts were often correlated with the radical scavenging activity (Miliauskas et al., 2004). One study evaluated the antioxidant activity in eighty-four species of Thai indigenous vegetable by β-carotene bleaching method demonstrated that the leaves of *Antidesma acidum* had a very high potency of the antioxidant activity which contained substance of more than 100 mg of BHA (butylated hydroxyanisole) equally in 100 g of fresh vegetable (Trakoontivakorn & Saksipitak, 2000).

Quercetin (3,3',4',5,7-pentahydroxyflavone) is one of the natural dietary antioxidant flavonol compound found in onion, cranberry, red apple, fruits and vegetables. It is rich in the polyphenolic substance of antioxidant phytochemicals. Flavonoids is the representative active substance of the most widely occurring group of phenolic phytochemicals. Flavonoids are characterized by a phenylbenzon (γ)pyrone-derived structure of two benzene rings and classified according to the oxidation level of their linked a heterocyclic pyran or pyrone ring. These variations define the families of anthocyanidin, flavone (flavonol), flavanone and proanthocyanidin oligomers (Rice-Evan, 2001). Flavonoids are reported to function as the antioxidant agents by scavenging reactive oxygen species (ROS) (Hanasaki et al., 1994) and reactive nitrogen species (RNS) (Vanacker et al., 1995) by the in vitro study. It act as the antioxidants by donating an electron to an oxidant.

When $DPPH^{\bullet}$ reacts with an antioxidant compound, these compound can donate hydrogen, then DPPH gives to the reduced from. The percentages scavenging activity are indicated by the ability to act as antioxidant in vitro. The compounds of crude extract of *Antidesma acidum* have antioxidant

ability nearly as quercetin hydrtate. When we compared the ability to act as the antioxidants of the substances in this study from the lowest to the highest the ability by DPPH scavenging assay, there were BHT, crude extract of *Antidesma acidum* and quercetin hydrate, respectively. The crude extract of *Antidesma acidum* had demonstrated strong antioxidant by this in vitro study. The crude extract of *Antidesma acidum* had been shown to composed of methoxy flavone, phenolic compounds (Smittinan, 1980) and other chemical compound such as anthrocyanin, β-isbesterol, tannins, stigmasterol, triterypenes (Sanansieng, 1998). Further study is needed to study the mechanism of *Antidesma acidum* on the radical scavenging activity.

Part II. Signalment

Ten clinically normal cats and twenty cats with CRF were studied. For the clinically normal cats, there were six males, two females and two female neutered. The CRF cats were consisted of eight males, eleven females neutered and one intact female. The mean age of the CRF cats (10.9 years old) was older than the CRF cats in our previous study (7.2 years old) (Pusoonthornthum et al., 2001). Various studies have reported the mean age of the CRF cats were 7.5 years old (Polzin et al,. 1989) and 12.6 years old (Elliot and Barber, 1998). It showed that 53% of old cats develop CRF were older than 7 years old (DiBartola et al., 1987). In the study of the age distribution of CRF in cats, 37% cats were younger than 10 years, 31% cats were between 10-15 years, and 32% cats were older than 15 years (Lulich et al., 1992). Difference in the mean of age of the CRF cats in many studies may reflect the different cat population in many countries. The CRF cats in this study were mostly domestic short hair except only one cat which was American Short Hair breed. The present study, had no significant difference in bodyweight in each group. All groups had elevated bodyweight when finished the treatment on day 56 of the study. One study of cats with CRF reported that CRF in cats were found with increase frequency in Maine coon, Abyssinian, Siamese, Russian blue, and Burmese cats (Polzin et al., 2000).

Part III. Hematology

The clinically normal cats received placebo had increased levels of PCV on day 56 and day 70 but these values were within the normal range. On day 42, the mild azotemia cats received the crude extract of *Antidesma acidum* had higher pack cell volume than the mild azotemia cats received placebo. The moderate azotemia cats received the crude extract of *Antidesma acidum* had elevated in the red blood cells on day 28. These results in various group of cats supported that the crude extract of *Antidesma acidum* may benefit in the elevation of the red blood cells in the CRF cats. Previous study in normal cats received the crude extract of *Antidesma acidum* leaves for 42 days had demonstrated the effect of erythrocytosis. This erycythrosis

effect was found only in the crude extract of *Antidesma acidum* leaves but did not find in the stem and bark parts (Sonkami et al., 2007).

Before treatment (day 0), the percentages of pack cell volume and red cell in the clinically normal, mild azotemia and moderate azotemia were not different between groups. The mild azotemia group had decrease in PCV but normal in red blood cells on day 14 and increase to normal on day 28. The CRF cats with moderate to advance chronic renal failure have a characteristic of a progressive hypoproliferative anemia. Anemia is caused by multifactorial, and the cause of anemia in human and animals with chronic renal failure is erythropoietin deficiency (Polzin et al., 2000). The anemic cats with CRF have been reported to have erythropoietin levels parallel to those of normal cats (Cook and Lothrop, 1994). Other causes of anemia in cats with chronic renal failure are iron deficiency and chronic gastrointestinal blood loss (Polzin et al., 2000).

Cats are sensitive to erythrocytes oxidative injury. Oxidized hemoglobin is denatured, causes microscopic visible aggregates (Heinz body) to attach inner of erythrocytes membrane leading to the deformability and shorter erythrocyte life span (Allison et al., 2000). Feline hemoglobin is more susceptible to oxidative damage than other mammalian species (Harvey and Kaneko, 1975; Allison et al., 2000). The hemoglobin molecule has been attributed to eight reactive sulfhydryl groups and dislocation of hemoglobin from the tetramers to dimers form. The ultrastructure of the feline spleen are non- sinusoid and inefficient in removing of the Heinz body containing RBC from the circulation. Although RBC life span may be shortened, these may develop without concurrent anemia (Christopher et al., 1990). In the present study, the moderate azotemia cats received the crude extract of *Antidesma acidum* had increase in red blood cells on day 28. On the contrary, the moderate azotemia cats received placebo had decrease red blood cells value. These results reflected that the crude extract of *Antidesma acidum* had no adverse effects on the erythrocyte oxidative injuries in cats. Bioflavonoid are ubiquitously compounds from many plants including the *Antidesma acidum* (Smittinan, 1980). Allison et al., (2000) demonstrated that bioflavonoid had no adverse effects on erythrocyte oxidative injury. In contrast, many plants such as onion, or many drugs such as acetaminophen, phenylbuthazone, propylene glycol were recognized to cause Heinz body formation and the decreased of RBC survival in cats (Christopher et al., 1990).

The clinically normal cats received the crude extract of *Antidesma acidum* had higher total white blood cells than the clinically normal cats received placebo on day 70. Previous study in rats received the crude extract of *Antidesma acidum* for 90 days had increase in total white blood cells (Thamaree et al., 2003). Normal cats received the crude extract of *Antidesma acidum* had increase white blood cells mainly lymphocytosis in one previous study (Sonkami et al., 2007). But in the present study, the mild and moderate azotemia cats that received the crude extract of *Antidesma acidum* did not

have total white blood cells elevation. At day 0 of the study, the clinically normal cats had higher total white blood cells but their value were in the normal range. In the mild azotemia cats received the crude extract of *Antidesma acidum* had decrease total white blood cells and a decreased in neutrophils. In mild azotemia cats had increase in eosinophils on day 28 but not persistent increase in eosinophils. The persistent eosinophillia in cats is an indicator of the systemic hypersensitivity including: parasitic diseases with a systemic phase (flea bite dermatitis, heartworm disease), feline asthma, allergic gastroenteritis and disseminated eosinophillic granoluma complex in cats. The gastrointestinal tract parasitic disease does not cause systemic persistent eosinophillic in cats. The mild azotemia cats received the crude extract of *Antidesma acidum* had persistent increase in monocytes on day 28, 42 and 56, and a decrease in lymphocytes on day 56 and the moderate azotemia cats received placebo had an increase in monocytes on day 42. A sick cats under chronic stress may leads to a decrease in lymphocytes and increase in neutrophil and monocytes. The mild azotemia cats received the crude extract of *Antidesma acidum* in this study may be in the chronic stress stage.

Part IV. Plasma ALT and ALP levels

After forty-two days of treatment, the results demonstrated that the moderate azotemia cats received the crude extract of *Antidesma acidum* had decrease hepatic enzyme including ALT and ALP levels. This results were the same with our previous study in normal cats received the crude extract of *Antidesma acidum* which had decrease in ALT levels on day 57 but increased in ALP levels on day 8 and 50. The changes of ALT and ALP levels in that study were within the normal range (Sonkami et al., 2007).

In the study of three various plants with flavonoids, (quercetin, silybin and morin), its was found that these plants could reverse the hepatotoxic effects of microcystin-induced hepatotoxic in mice (Jayarai et al., 2007). Silymarin, a silybin flavonoid had also been shown to have the hepatoprotective activity against ethanol-induced liver damage. The animals received silymarin did not show significant changes in ALT, AST and gamma glutamyl transferase levels (Wang et al., 1996). Similarly, the study in the patients with elevated serum liver enzyme due to ethanol ingestion, silymarin was found to significantly lower the liver enzyme including AST and ALT after four weeks of treatment (Salmi and Sarna, 1982). Hepatoprotective mechanism of silymarin are varied and including antioxidation, anti-lipid peroxidation (Halim et al., 1997), anti-inflammatory effect by inhibition of enzyme lipoxygenase (Fiebrich and Koch, 1979). To date, the exact mechanism of the crude extract of *Antidesma acidum* on the decrease in ALT and ALP levels in cats are unknown and needed further study.

Part V. Blood urea nitrogen (BUN), creatinine levels and urinalysis

The creatinine and blood urea nitrogen levels are used as the screening tests for the glomerular function in this study. Urea is the product of hepatic urea cycle that relative with amino acid metabolism and nitrogen excretion. Urea is passively reabsorbed in the renal tubules and the production and excretion of urea by the kidney is not in a constant rate. Whereas creatinine which is a product of phosphocreatinine from the muscle, not affected by diets and not metabolyted by the kidney. The rate of creatinine excretion is absolutely constant in the steady stage. The determination of plasma creatinine is considerated as a good estimate of glomerular function in the clinical practices (DiBartola, 2000; Brown, 2004).

In this study, the CRF cats were randomized into 2 subgroups (received placebo and the crude extract of *Antidesma acidum*). To collect blood for blood urea nitrogen determination, cats were recommended to be fasted for 8-12 hours after a high protein meal to avoid the effects of feeding on urea production (DiBartola, 2000). The CRF cats in one previous study fed with the renal diet were associated with a reduction in the BUN levels during the 12-24 months intervals but the creatinine levels in that study were not effect when giving the renal diet (Ross et al., 2006). Results of the present study showed that the moderate azotemia cats received the crude extract of *Antidesma acidum* had decrease creatinine levels on day 28 and 42. The CRF cats in this study were not require to receive the special renal diet throughout the project. The 70% (14/20) of the CRF cats in this study received homemade diet and 30 % (6/20) received the renal diet. The decrease in creatinine levels after receiving the crude extract of *Antidesma acidum* in the moderate CRF cats in this study may reflect the improvement in their glomerular function in the cats with moderate azotemia group.

Glomerulosclerosis and endothelial dysfunction occurs in the patients with CRF. The patients with CRF had elevated lipid peroxide products and decreased levels of antioxidant that may impaired endothelium dependent vasodilation (Annuk et al., 2001). Flavonoid compounds help in the protective effect by promote vascular relaxation. Quercetin promoted vascular relaxation by inhibit the synthesis of prostanoid vasoconstrictors in the vascular endothelium (Furchgott and Vonhoutte, 1989). Grape skin extract and tannic acid promoted relaxation and vasodilation by stimulating the NO-cGMP system (Formica and Regelson, 1995). The anti-inflammatory mechanisms of flavonoids compound are inhibits cyclooxygenase and lipoxygenase activities, reducing the formation of these inflammatory metabolites (Robak and Gryglewski, 1996), inhibit eicosanoid such as prostaglandin biosynthesis (Formica and Regelson, 1995) and reduce the release of arachidonic acid by neutrophils and other immune cells (Hoult et al., 1994). These evidences suggested that cats with moderate chronic renal failure received the crude extract of *Antidesma acidum* had improvement in the glomerular function resembling the flavonoids effects on the vascular relaxation and reducing glomerulonephritis via inhibit

inflammatory mediator. The exact mechanism of how the crude extract of *Antidesma acidum* which contain flavonoids as one of its compositions may decrease the creatinine levels in the cats with moderate azotemia remains to be investigated.

From the urinalysis, urine was collected by naturally voiding. Light yellow to yellow urine colorization were shown in each groups of the study. Normal urine is yellow due to the presence of urochrome pigment. The dilute urine may be colorless. Urine specific gravity is the reflection of the total solid concentration of urine. The mean of the urine specific gravity in clinically normal cats were significantly higher than the mild and moderate azotemia cats. Cats with early renal insuffiency may have minimal ability to concentrate urine (SG < 1.035 in cats) and concentrating ability generally decrease before the development of azotemia (Robertson and Seguin, 2006). The animals in renal failure are unable to dilute or concentrate their urine usually have specific gravity in the range of 1.008-1.012 (Jagger, 2002).

The urine pH in the clinically normal cats are 5.0 to 7.5 (DiBartola, 2000). In the clinically normal cats, the mild azotemia and moderate azotemia cats had urine pH in the normal range. Urine pH varies with the diet and acid-base balance (DiBartola, 2000).

Urine dip-stick test can be used to test glucose, bilirubin, ketone, pH, protein, urobilinogen, nitrite and leucocytes in the urine sample. However, urine leucocytes dip-stick was not a reliable test and the results may give false positive in cats (Jagger, 2002).

The dip-stick urinary protein test also give qualitative results. In the clinically normal cats with (+1) to (+2) urine protein and having active sediment (such as bacteriuria, white blood cells, red blood cells or casts) should be further diagnosed for the underline cause of inflammatory renal disease, lower urinary disease or genital tract disease. One cat in the clinically normal group with bacteriuria and active sediment, the follow up of bacterial urinary tract infection should be considered.

Proteinuria in the absence of urine sediment abnormalities are suggestive of glomerular disease. If the present of proteinuria with inactive sediment is observed, a urine protein:creatine ratio (UPC) should be performed to rule out the glomerular disease. Two cats in the moderate azotemia were presented with proteinuria (+1) and inactive sediment, that cats (UPC=0.34) had borderline proteinuria and the other cat with UPC=0.87 had proteinuria and need further studied.

Part VI. Oxidative stress markers

Glutathione (GSH) is a tripeptide composed of glutamine, glycine and cystein. GSH is particularly function on the degradation of hydrogenperoxide

and hydroperoxides. In a catalyzation process by the enzyme glutathione peroxidase (Gpx), reduced glutathione are converted to the oxidize gluthathione (GSSG). Gluthathione is the compound found intracellularly in various tissue such as erythrocytes, liver, spleen, kidney, leukocytes and ocular lens (Pastore et al., 2003).

The mean±SEM of glutathione levels in the clinically normal cats in this study (2.0±0.22 mmoles/L RBC) were closed to the mean of glutathione levels (1.97±0.07 mmoles/L RBC) in previous study by using the same method as in this study (Harvey and Kaneko, 1975). The various study on plasma glutathione by HPLC found the mean of glutathione levels in the clinically healthy cats were between 1.85 and 2.39 mmoles/L (Allison et al., 2000) and 4.51 μM (Denzoin et al., 2007). The variability of the results may be related to the different methodology, differences in sample process or various factors affecting the plasma glutathione concentration (Denzoin et al., 2007).

In the present study, the GSH levels in the clinically normal cats and the CRF cats were not significantly different. The mild azothemia cats received placebo had increase level of GSSG on day 14 and a slighty decrease level of GSSG on day 28, 42 and 56. These suggested that the cats with mild azotemia may had compensatory response to oxidative stress.

In human with progressive chronic renal failure, it was found that total glutathione levels were significant decrease and the GSSG levels remain constant or increase during the progressive of CRF and had a positive correlation between the creatinine clearance and the plasma glutathione, plasma glutathione peroxidase and plasma selenium concentration (Ceballos-Picot et al., 1996). Human with CRF had impaired antioxidant system which may result from several factors including uremia, lower concentrations of some antioxidant such as vitamin E, C and glutathione (Ross et al., 1997).

The decreasing in the ratio of GSH/GSSG may occur because of GSH become depleted after the oxidative damage. In the present study, the clinically normal cats had significantly higher in GSH/GSSG ratio than the mild azotemia cats on day 0 of the study. These may be results of the GSH depleted in mild azotemia cats.

There were many studies on human with renal disease and the investigation on the role of GSH, GSSG and the Gpx activity. On the otherhand, the study of the GSH, GSSG and Gpx in cats with CRF were very rare. Other parameter of the oxidative stress that had been studied in the cats with CRF indicated that the higher level of serum 8-hydroxy-2'-deoxyguanosine (8-oHdG), the derivative of oxidative DNA damage, the higher levels of serum malondialdehyde (MDA), a lipid peroxidation derivative was found (Yu and Paetau-Robinson, 2006). In dogs, the renal oxidative stress as measured by U-MDA/Cr was enhanced in the renal azotemia dogs and related to degree of renal dysfunction (Buranakarl et al., 2009). In rats, there was no change in both kidney and urinary MDA in gentamycin-induced renal injuries

(Thongchai et al., 2008). Its was shown that gentamycin-enhanced superoxide anion and hydrogen peroxide generation and causing renal vasoconstriction but did not change in lipid peroxidation (Nagajima et al., 1994).

Previous study in dogs with renal disease had demonstrated that the RBC catalase, superoxide dismutase and glutathione peroxidase were decrease. This finding suggested that the dogs with renal disease may associated with the occurring of oxidative stress (Kargin and Fidanci, 2001).

The glutathione peroxidase activities in this study were significantly decrease in the moderated azotemia cats. This result supported that the CRF cats had the oxidative stress. In one study of the characteristic of the enzyme glutathione peroxidase identified two types of glutathione peroxidase in the blood; erythrocyte Gpx (E-Gpx) and plasma Gpx (P-Gpx). The E-Gpx was reduced in the nephrotic syndrome and the patients with renal impairment but showed no significant correlation with either serum creatinine or blood urea nitrogen (Mohamed et al., 2005). The P-Gpx was reduce in patients with renal impairment and P-Gpx had a highly significantly negative correlation with either serum creatinine or blood urea nitrogen (Mohamed et al., 2005).

The P-Gpx activity is proposed to be an important factor to assess the oxidative damage in the kidney disease and the progression of renal disorder in human (Zachara et al., 2004; Mohamed et al., 2005; Zachara et al., 2006). In addition a decrease in plasma Gpx activity in renal disease may due to the synthesis of this enzyme in the kidney impairment causing a decrease in the enzyme activity (Zachara et al., 2004). A progressive decline in P-Gpx activity is related with the decrease in the enzyme synthesis in the proximal tubular cells of the kidney which it is secreted into the extracellular fluid (Avissar et al., 1994). The decreasing in P-Gpx activity had also demonstrated in the polycystic kidney models. There was a decrease in the mRNA expression of antioxidant enzyme extracellular glutathione peroxidase, superoxide dismutase, catalase, and glutathione S-transferase during the progressive of polycystic kidney disease. Lack of appropriate expression of protective enzyme genes and oxidative stress are general pathologic mechanisms of cystic kidney progression (Maser et al., 2002).

However, E-Gpx were measured in this study to assess the oxidative stress in the CRF cats. The E-Gpx was used as the marker of oxidative stress in cats because of their sensitive to erythrocytes oxidative injuries more susceptible than other species (Allison et al., 2000). At day 0 of the study, the moderate azotemia cats had lower levels of the Gpx activity than the clinically normal cats. Reduction in Gpx, antioxidant enzyme contribute to the oxidative stress in the moderate azotemia cats. The mild azotemia cats had no significantly different in Gpx activity suggested that this stage cats may had a compensatory stage of antioxidant mechanism.

The effects of the crude extract of *Antidesma acidum* on GSH in the mild azotemia and moderate azotemia cats received the crude extract of

Antidesma acidum had the trend to increase in GSH levels on day 56. The moderate azotemia cats received the crude extract of *Antidesma acidum* had no change in Gpx activity. These results may be due to the effects of the crude extract of *Antidesma acidum* on an inhibition of glutathione (GSH) depletion. The exact mechanism of the crude extract of *Antidesma acidum* on GSH level in the cats with CRF remains to be investigated.

Antidesma acidum is compose of methoxy flavone, phenolic compounds (Smittinan, 1980) and other chemical compounds such as anthrocyanin, β-isbesterol, tannins, stigmasterol and triterypenes (Sanansieng, 1998). Flavonoid, silymarin had demonstrated the effect of protection against glutathione depletion by acute acetaminophen toxic in rats' liver (Campos et al., 1989). However, quercetin and its derivatives prevented the oxidative damages by either increasing glutathione which is indispensable for the Gpx activity. Other plants such as *Gongronema latifolium*, had been reported as the antioxidant which increase the GSH level, Gpx, SOD and G-6PDH activity in the hepatocytes of rat models with non-insulin dependent diabetes mellitus (Ugochukwu and Babady, 2002). The antioxidant mechanisms of flavonoids (catechin, epicatechin, 7,8-dihydroxy flavone, and rutin) had shown the free radical-scavenging abilities. Their anti-radical property is direct toward $OH^{\cdot}$ and $O_2^{\cdot-}$, which are highly reactive oxygen species implicated in the initiation of lipid peroxidation. Thus their effects of flavonoids compound are by inhibit lipid peroxidation and suppressive against cytotoxicity cause by the reactive oxygen species (Hanasaki et al., 1994; Nagata et al., 1999). Intracellular antioxidative function of flavonoids requires the interaction with Gpx. Among antioxidative defenses, Gpx are known to act directly by reduce and eliminate intracellulay occurring hydrogen peroxide and lipid peroxides. The Gpx plays the very important role in the protection against oxidative stress. The antioxidative function of flavonoids play a role as the synergist effects and promote the interaction with Gpx and selenium (Nagata et al., 1999).

The mild and moderate azotemia cats received the crude extract of *Antidesma acidum* had the trend to increase in the GSH levels. These results were hypothesized to cause by 1). The effects on inhibition of glutathione (GSH) depletion 2). flavonoids, the composition compound of the crude extract of *Antidesma acidum* had the effects on direct reactive oxygen species by the activation of Gpx and function similar to the GSH. These leads to increase in the GSH levels and cause no changes in Gpx activity.

Although the crude extract of *Antidesma acidum*, which contained the flavonoids as one of its compositions had demonstrated the high radical scavenger by the DPPH assay in vitro nearly as quercetin, the mechanism of action of the compounds in the crude extract of *Antidesma acidum* needs further study.

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APPENDICES

Appendix A. Total numbers of cats and places of the study.

Total number of cats	Small Animal Hospital Chulalongkorn University	Private Animal Hospitals
30	12	18

Appendix B. Age, gender, weight and breed in the clinically normal, mild azotemia and moderate azotemia cats.

Number	Code	Age (years)	Gender	Weight (kg)		Breed
				Day 0	Day 56	
Clinically normal cats						
1.	A1	5	M	3.4	3.8	DSH
2.	A2	5	M	5.2	6.0	DSH
3.	A3	6	M	6.2	6.5	DSH
4.	A4	5	M	4.0	4.5	DSH
5.	A5	3	F	2.8	3.0	DSH
6.	B1	3	F	3.0	3.2	DSH
7.	B2	5	FS	3.5	3.8	DSH
8.	B3	3	M	3.5	3.8	DSH
9.	B4	5	FS	3.2	3.6	DSH
10.	B5	3	M	3.0	3.8	DSH
Mild azotemia cats						
11.	C1	6	F	3.0	3.2	DSH
12.	C2	7	M	5.5	5.8	DSH
13.	C3	10	FS	4.5	4.7	DSH
14.	C4	10	FS	3.2	3.0	DSH
15.	C5	14	FS	6.8	6.8	DSH
16.	D1	12	M	4.0	4.5	DSH
17.	D2	12	FS	4.2	4.5	ASH
18.	D3	13	FS	4.0	4.3	DSH
19.	D4	10	M	3.5	4.2	DSH
20.	D5	14	FS	4.0	4.8	DSH
Moderate azotemia cats						
21.	E1	12	M	4.2	4.2	DSH
22.	E2	10	M	4.0	4.3	DSH
23.	E3	10	M	4.5	4.8	DSH
24.	E4	12	FS	3.8	4.2	DSH
25.	E5	10	FS	2.8	3.0	DSH
26.	F1	14	FS	4.2	4.5	DSH
27.	F2	10	FS	4.0	4.2	DSH
28.	F3	10	FS	3.8	4.0	DSH
29.	F4	10	M	3.5	3.5	DSH
30.	F5	12	M	3.8	4.0	DSH

M = Male cats
F = Female cats
FS = Female neutered cats
ASH = American Short Hair
DSH = Domestic Short Hair

Appendix C. Mean±SEM of hematology and blood chemistry results in the clinically normal, mild azotemia and moderate azotemia cats on day 0.

Parameter	Normal# value	Day 0					
		Clinically normal		Mild azotemia		Moderate azotemia	
		Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
PCV (%)	38-55	38.0±0.95	45.4±2.77 *	40.2±1.62	41±0.89	40.00±0.55	42.6±1.08
RBC ($x10^6$cells/µl)	5.22-8.46	7.76±0.67	8.17±0.81	7.29±0.59	6.87±0.74	7.32±0.55	6.23±0.13
WBC ($x10^3$cells/µl)	6,000-17,000	9,254±1,096.17	10,870±1,648.07	10,818±1,575.70	5,990±1,103.48 *	10,950±831.11	7,260±938.03 *
Neutrophils (cells/µl)	3,000-11,500	5,367±686.38	6,762±858.21	6,764±1,079.74	3,788.4±680.48 *	7,260±938.0	4,804.6±720.39
Bands (cells/µl)	0-300	0	0	0	0	0	0
Lymphocytes (cells/µl)	1,500-5,000	3,264±686.38	3,370±921.65	2,981±506.14	1,213.1±361.48 *	2,242.9±622.31	1,603.1±243.51
Eosinophils (cells/µl)	100-1,000	216.98±91.6	337.80±142.67	448±284.54	494±152.79	823.80±159.86	537.4±219.95
Monocytes (cells/µl)	0-2,000	391.10±172.40	170±52.49	485±176.72	79.7±22.05	136.70±97.94	115.4±57.59

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

* *$p<0.05$ when compared between cats received placebo and the crude extract of Antidesma acidum.*

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. 3-20.

Appendix D. Mean±SEM of hematology and blood chemistry results in the clinically normal, mild azotemia and moderate azotemia cats on day 14.

Parameter	Normal# value	Day 14					
		Clinically normal		Mild azotemia		Moderate azotemia	
		Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
PCV (%)	38-55	40.2±1.28	44.4±2.48	31.80±7.02	40.6±0.81	39.00±1.18	39.8±0.66
RBC ($x10^6$cells/µl)	5.22-8.46	7.76±1.17	8.62±1.20	6.46±0.49	7.39±0.58	6.78±0.66	6.98±0.71
WBC ($x10^3$cells/µl)	6-17	10,850±674.19	8,700±1,293.61	9,210±1,762.82	6,800±1,466.08	11.080±1,052.68	5,633±1,783.97*
Neutrophils (cells/µl)	3,000-11,500	6,222.1±537.18	5,308.1±1,293.61	4,862.1±853.51	4,673.6±900.50	8,771.2±900.96	5,300.4±1,370.77
Bands (cells/µl)	0-300	0	0	32.1±32.1	0	0	0
Lymphocytes (cells/µl)	1,500-5,000	3,560.6±583.39	2,951.2±708.77	3,311.9±453.34	1,541.9±359.52	1,720±353.0	1,895.2±481.74
Eosinophils (cells/µl)	100-1,000	846±278.45	381.2±195.03	823.7±340.86	270.7±143.90	505.40±19.83	479.2±168.97
Monocytes (cells/µl)	0-2,000	157.90±63.60	59.7±24.98[b]	368.4±199.19	81.8±39.02	83.4±43.04	24.1±15.68

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cat s received the crude extract of *Antidesma acidum*

* *p<0.05 when compared between moderate azotemial cats received placebo and the crude extract of Antidesma acidum.*

b *p<0.05 when compared between control cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 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. Louls. 3-20.

Appendix E. Mean±SEM of hematology and blood chemistry results in the clinically normal, mild azotemia and moderate azotemia cats on day 28.

Parameter	Normal# value	Day 28					
		Clinically normal		Mild azotemia		Moderate azotemia	
		Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
PCV (%)	38-55	41.2±1.77	38.6±8.89	40.2±0.80	42.4±0.75	37.80±0.49	40.6±1.17
RBC ($x10^6$cells/µl)	5.22-8.46	7.02±0.52	8.88±0.91	7.11±0.48	6.95±0.36	5.81±0.71 [e]	8.47±0.87*
WBC ($x10^3$cells/µl)	6-17	11,190±1,851.71	8,630±1,496.63	8,680±1,413.67	8,860±1,664.34	12,340±1,507.51	8,593.2±1,113.1
Neutrophils (cells/µl)	3,000-11,500	6,522.2±1,424.82	5,069±838.65	4,654.2±522.96	5,465.5±1,009.61	9,185±1,805.94	6,658.2±1,374.30
Bands (cells/µl)	0-300	0	0	0	0	0	0
Lymphocytes (cells/µl)	1,500-5,000	2,181.7±830.74	2,913.8±549.63	3,510.8±842.47	2,387.2±497.73	2,437.6±477.10	1,853.4±376.40
Eosinophils (cells/µl)	100-1,000	447.1±125.92	193.20±99.89	485.6±210.86	706.1±251.40 [c]	269.8±85.83 [e]	642.9±230.64
Monocytes (cells/µl)	0-2,000	404.9±199.70	251±103.96	108.4±52.31	288.7±71.35 [c]	447.6±178.91	87.3±41.83

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

* *p<0.05 when compared between moderate azotemia cats received placebo and the crude extract of Antidesma acidum.*

[c] *p<0.05 when compared between mild azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 28).*

[e] *p<0.05 when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment (day 28).*

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. 3-20.

Appendix F. Mean±SEM of hematology and blood chemistry results in the clinically normal, mild azotemia and moderate azotemia cats on day 42.

Parameter	Normal# value	Day 42					
		Clinically normal		Mild azotemia		Moderate azotemia	
		Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
PCV (%)	38-55	40.6±1.94	45±1.61	39.20±0.37	42.2±0.49 *	43.60±2.25	41.2±1.02
RBC ($x10^6$cells/µl)	5.22-8.46	8.10±0.99	9.26±1.15	7.12±0.62	7.72±0.54	7.25±0.53	8.01±0.86
WBC ($x10^3$cells/µl)	6-17	9,080±1,595.24	7,500±1,294.89	9,700±1,088.92	9,580±2,341.52	9,760±2,066.12	8,360±1,754.72
Neutrophils (cells/µl)	3,000-11,500	5,540±1,119.64	4,326.7±828.0	4,911.13±755.92	6,352.6±1,767.64	7,176.4±1,520.32	5,589.3±1,253.81
Bands (cells/µl)	0-300	0	0	0	0	0	0
Lymphocytes (cells/µl)	1,500-5,000	2,712.1±795.77	2,557.6±664.72	3,656.13±400.45	2,439.2±543.52	1,653.5±451.59	2,060.4±707.56
Eosinophils (cells/µl)	100-1,000	293.4±102.80	205.0±53.25	444±135.06	415±162.87	121.1±61.86 [e]	429.6±107.80 *
Monocytes (cells/µl)	0-2,000	330.6±239.60	193.5±58.1	575.6±380.15	357.2±107.46	686.6±324.76	281±230.19

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

* *$p<0.005$ when compared between cats received placebo and the crude extract of Antidesma acidum.*

[e] *$p<0.05$ when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment (day 42).*

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

Appendix G. Mean±SEM of hematology and blood chemistry results in the clinically normal, mild azotemia and moderate azotemia cats on day 56.

Parameter	Normal# value	Day 56					
		Clinically normal		Mild azotemia		Moderate azotemia	
		Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
PCV (%)	38-55	41.8±1.28[a]	38.2±8.71	40.2±0.80	41.6±0.75	41.80±1.74	40.6±1.08[f]
RBC ($x10^6$cells/µl)	5.22-8.46	8.85±1.0	7.57±0.30	7.79±0.71	7.86±0.44	7.91±0.67	7.73±0.92
WBC ($x10^3$cells/µl)	6-17	11,220±1,268.17	11,330±1,783.44	11,220±1173.31	11,000±2,417.41	11,650±1,505	7,930±2,281.82
Neutrophils (cells/µl)	3,000-11,500	6,548.4±807.70	7,254.8±1,426.98	9,000±1998.37	7,770±1,517.07	8,788.4±1,325.2	5,983±1,687.20
Bands (cells/µl)	0-300	0	0	0	0	0	0
Lymphocytes (cells/µl)	1,500-5,000	3,606±259.75	3,080.2±468.54	4,516.3±443.15	1,966.3±674.20*	1,763.5±500.08	1,260±385.22
Eosinophils (cells/µl)	100-1,000	973.8±500.68	664.7±217	803.80±316.41	642.2±217.22	702.5±226.84	701±255.87
Monocytes (cells/µl)	0-2,000	163.8±52.96	360.7±125.61	468.90±254.94	640±598.77	280.5±153.24	62±43.17

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

* *$p<0.05$ when compared between mild azotemia cats received placebo and the crude extract of Antidesma acidum.*

[a] *$p<0.05$ when compared between clinically normal cats received placebo before treatment (day 0) and after treatment (day 56).*

[f] *$p<0.05$ when compared between moderate azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 56).*

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. 3-20.

Appendix H. Mean±SEM of hematology and blood chemistry results in the clinically normal, mild azotemia and moderate azotemia cats on day 70.

Parameter	Normal# value	Day 70					
		Clinically normal		Mild azotemia		Moderate azotemia	
		Placebo (n=5)	Extract (n= 5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
PCV (%)	38-55	41.6±0.97[a]	43.6±1.83	41.2±0.80	42.8±0.80	43.2±2.54	41.0±0.45
RBC ($x10^6$cells/µl)	5.22-8.46	7.90±0.71	9.09±0.94	7.39±0.58	8.10±0.58	8.37±0.66	7.33±0.39
WBC ($x10^3$cells/µl)	6-17	12,800±593.94	8,330±1,627.24*	8,370±1,831.43	8,470±2,078.86	10,560±1,793.28	9,424±1,615.43
Neutrophils (cells/µl)	3,000-11,500	7,903±482.88	5,571.2±1,576.96	6,162±1,355.38	5,584±1,373.02	8,099.6±1,318.38	6,246.2±1,290.6
Bands (cells/µl)	0-300	0	28.80±28.80	0	0	0	0
Lymphocytes (cells/µl)	1,500-5,000	4,079.3±471.03	2,096±369.52*	1,609.1±546.92	2,230±640.16	1,637.4±451.81	2,101.4±736.12
Eosinophils (cells/µl)	100-1,000	420±263.09	279.6±59.48	489.40±131.52	561.8±189.67	597.4±251.11	529.36±129.55
Monocytes (cells/µl)	0-2,000	33.8±24.45	380.7±170.78	95.6±48.03	93.2±28.33	344.80±111.1[e]	147.02±49.73

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

* *p<0.05 when compared between control cats received placebo and the crude extract of Antidesma acidum.*

a *p<0.005 when compared between control cats received placebo before treatment (day 0) and after treatment (day 70).*

e *p<0.005 when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment (day 70).*

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. 3-20.

Appendix I. Mean±SEM of ALT and ALP levels in the clinically normal, mild azotemia and moderate azotemia cats on day 0.

Parameter	Day 0					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
ALT (Units)	37.08±.85	42.38±5.11	47.68±10.87	57.94±10.85	72.23±21.39	68.22±15.71
ALP (Units)	53.12±8.96	74.28±18.89	69.22±26.01	71.64±15.51	43±6.21	100±22.65*

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

* $p<0.05$ *when compared between moderate azotemia cats received placebo and the crude extract of Antidesma acidum.*

Appendix J. Mean±SEM of ALT and ALP levels in the clinically normal, mild azotemia and moderate azotemia cats on day 14.

Parameter	Day 14					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
ALT (Units)	66.76±23.41	79.88±17.48	59.42±9.81	35.28±7.66	47.45±7.83	46.46±9.30
ALP (Units)	78.6±17.90	83.02±21.13	75.58±19.74	54.44±24.47	37.18±4.18	51.08±13.49

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

Appendix K. Mean±SEM of ALT and ALP levels in the clinically normal, mild azotemia and moderate azotemia cats on day 28.

Parameter	Day 28					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
ALT (Units)	50.14±9.78	53.38±5.01	54.32±9.81	43.54±5.53	69.75±15.24	48.04±11.88[f]
ALP (Units)	55.68±14.61	63.28±12.71	54.16±12.92	39.64±10.45	59.53±18.09	109.62±67.01

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

[f] *$p<0.05$ when compared between moderate azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 28).*

Appendix L. Mean±SEM of ALT and ALP levels in the clinically normal, mild azotemia and moderate azotemia cats on day 42.

Parameter	Day 42					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
ALT (Units)	65.95±19.10	60.64±7.54	47.18±14.85	58.04±13.79	109.4±31.18	29.7±9.17[f]
ALP (Units)	29.85±4.69	75.32±19.74	50.84±14.31	31.4±15.78	38.43±6.5	60.06±20.07[f]

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

[f] *$p<0.05$ when compared between moderate azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 42).*

Appendix M. Mean±SEM of ALT and ALP levels in the clinically normal, mild azotemia and moderate azotemia cats on day 56.

Parameter	Day 56					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
ALT (Units)	35.24±4.48	63.1±4.68	32.13±5.24	47.8±12.87	50.93±12.8	35.82±6.55[f]
ALP (Units)	42.14±6.83	63.10±21.04	49.2±10.66	28.02±8.32[d]	30.65±5.8[e]	31.14±8.9[f]

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

[d] *$p<0.05$ when compared between mild azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 56).*

[e] *$p<0.01$ when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment (day 56).*

[f] *$p<0.05$ when compared between moderate azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 56).*

Appendix N. Mean±SEM of ALT and ALP levels in the clinically normal, mild azotemia and moderate azotemia cats on day 70.

Parameter	Day 70					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
ALT (Units)	38.34±9.01	53.23±2.70	39.85±10.33	35.92±4.34	144.98±66.75	48.14±20.25[f]
ALP (Units)	42.1±8.22	52.1±16.25	73.1±21.0	28.84±5.44	27.45±3.87[e]	37.14±7.0

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

[e] *$p<0.01$ when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment on day 70.*

[f] *$p<0.05$ when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment on day 70.*

Appendix O. Mean±SEM of blood urea nitrogen and creatinine levels in the clinically normal, mild azotemia and moderate azotemia cats on day 0.

Parameter	Day 0					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
BUN (mg/dl)	25.98±1.06	22.80±0.65	27.84±5.73	37.24±5.12	33.88±4.38	21.02±4.95
Creatinine (mg/dl)	1.51±0.04	1.54±0.11	2.13±0.14	2.35±0.09	3.02±0.10	3.10±0.22

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

Appendix P. Mean±SEM of blood urea nitrogen and creatinine levels in the clinically normal, mild azotemia and moderate azotemia cats on day 14.

Parameter	Day 14					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
BUN (mg/dl)	26.22±3.10	32.82±7.96	29.02±3.54	33.18±3.68	40.30±5.87	29.74±3.27
Creatinine (mg/dl)	1.54±0.02	1.56±0.11	2.01±0.10	2.08±0.17	2.88±0.24	2.65±0.24

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

Appendix Q. Mean±SEM of blood urea nitrogen and creatinine levels in the clinically normal, mild azotemia and moderate azotemia cats on day 28.

Parameter	Day 28					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
BUN (mg/dl)	31.26±4.34	33.06±7.81	30.54±0.97	36.90±3.60	53.26±8.47	31.66±3.04 [f]
Creatinine (mg/dl)	1.54±0.08	1.86±0.11	2.13±0.23	2.17±0.14	3.79±0.56	2.50±0.16 [f]

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

[f] *$p<0.05$ when compared between moderate azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 28).*

Appendix R. Mean±SEM of blood urea nitrogen and creatinine levels in the clinically normal, mild azotemia and moderate azotemia cats on day 42.

Parameter	Day 42					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
BUN (mg/dl)	26.25±4.74	26.25±4.74	30.58±5.13	37.94±5.54	57.86±13.34	31.7±4.06
Creatinine (mg/dl)	1.62±0.08	1.48±0.09	2.16±0.24	2.26±0.19	3.49±0.51	2.60±0.20[f]

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

[f] *$p<0.05$ when compared between moderate azotemia cats received the crude extract of Antidesma acidum before treatment (day 0) and after treatment (day 42).*

Appendix S. Mean±SEM of blood urea nitrogen and creatinine levels in the clinically normal, mild azotemia and moderate azotemia cats on day 56.

Parameter	Day 56					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
BUN (mg/dl)	33.76±4.78	22.16±1.76	41.40±4.74[c]	35.16±8.42	67.74±9.84[e*]	35.20±3.37
Creatinine (mg/dl)	1.57±0.11	1.39±0.12	2.60±0.53	2.52±0.14	4.18±0.75	2.90±0.29

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

* $p<0.05$ *when compared between moderated azotemia cats received placebo and crude extract of Antidesma acidum.*

[c] $p<0.01$ *when compared between mild azotemia cats received placebo before treatment (day 0) and after treatment (day 56).*

* $p<0.05$ *when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment (day 56).*

Appendix T. Mean±SEM of blood urea nitrogen and creatinine levels in the clinically normal, mild azotemia and moderate azotemia cats on day 70.

Parameter	Day 70					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
BUN (mg/dl)	24.08±3.37	22.50±3.23	33.56±7.73	39.60±2.83	71.92±10.9 [e*]	34.52±3.25
Creatinine (mg/dl)	1.70±0.14	1.38±0.05	2.74±0.62	2.25±0.30	4.84±0.83	2.54±0.30

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*

* *p<0.05 when compared between moderated azotemia cats received placebo and crude extract of Antidesma acidum.*

* *p<0.05 when compared between moderate azotemia cats received placebo before treatment (day 0) and after treatment (day 70).*

Appendix U. Mean±SEM of blood GSH, GSSG, GSH/GSSG ratio and Gpx levels in the clinically normal, mild azotemia and moderate azotemia cats on day 0.

Parameter	Day 0					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
GSH (mg/dl RBC)	2.06±043	1.93±0.16	1.76±0.22	1.52±0.17	1.81±0.22	1.79±0.13
GSSG(μmol/L RBC)	9.28±1.21	8.94±0.59	11.14±1.48	9.21±0.81	10.99±1.49	8.55±0.63
GSH/GSSG	242.4±54.23	215.2±8.94	160.4±14.46	168.2±21.87	182.2±40.81	216.4±24.21
Gpx (μmol/min/ml)	19,384±1,423	20,415±1,609	20,350±2,294	18,418±3,583	16,486±3,121	14,619±1,424

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*
GSH = Reduced glutathione
GSSG = Oxidized glutathione
GSH/GSSH = Reduced glutathione/ oxidized glutathione ratio
Gpx = Glutathione peroxidase

Appendix V. Mean±SEM of blood GSH, GSSG, GSH/GSSG ratio and Gpx levels in the clinically normal, mild azotemia and moderate azotemia cats on day 14.

Parameter	Day 14					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
GSH (mmol/L RBC)	1.83±0.13	1.84±0.26	1.66±.139	1.71±0.21	1.64±0.10	1.74±0.05
GSSG (µmol/L RBC)	10.98±0.35	10.15±1.97	12.95±1.61	9.22±0.91	10.47±1.50	9.41±0.57
GSH/GSSG	166.6±10.52	236.84±87.8	134.6±17.45	199.7±37.68	171.2±27.24	187.45±11.38
Gpx (µmol/min/ml)	18,096±1,996	22,282±2,090	18,418±3,161	16,872±2,873	12,236±1,308	13,846±966

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*
GSH = Reduced glutathione
GSSG = Oxidized glutathione
GSH/GSSH = Reduced glutathione/ oxidized glutathione ratio
Gpx = Glutathione peroxidase

Appendix W. Mean±SEM of blood GSH, GSSG, GSH/GSSG ratio and Gpx levels in the clinically normal, mild azotemia and moderate azotemia cats on day 28.

Parameter	Day 28					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
GSH (mmol/L/ RBC)	1.59±0.13	1.81±0.15	1.49±0.2	1.58±0.23	1.81±0.3	1.71±0.23
GSSG (µmol/L RBC)	10.67±0.52	8.39±1.24	9.88±0.87	9.7±1.76	12.77±1.01	10.31±0.80
GSH/GSSG	150.72±14.11	234.33±25.83	153.4±12.18	222.9±83.93	141.62±17.71	175.37±33.4
Gpx (µmol/min/ml)	17,062±1,632	18,740±2,468	15,263±2,754	16,164±1,851	12,808±1,736	12,558±1,385

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*
GSH = Reduced glutathione
GSSG = Oxidized glutathione
GSH/GSSH = Reduced glutathione/ oxidized glutathione ratio
Gpx = Glutathione peroxidase

Appendix X. Mean±SEM of blood GSH, GSSG, GSH/GSSG ratio and Gpx levels in the clinically normal, mild azotemia and moderate azotemia cats on day 42.

Parameter	Day 42					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
GSH (mmol/L RBC)	1.70±0.33	1.84±0.24	1.49±0.174	1.76±0.13	1.51±0.78	1.79±0.2
GSSG (µmol/L RBC)	11.77±1.88	7.94±1.27	8.69±2.49	10.63±0.74	9.06±1.67	9.84±1.76
GSH/GSSG	159.98±39.17	266.91±59.9	248.32±67.62	169.94±19.13	186.26±29.08	230.81±74.77
Gpx (µmol/min/ml)	19,638±2,347	18,736±2,299	17,839±1,021	16,164±913	15,520±1,965	15,706±1,272

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*
GSH = Reduced glutathione
GSSG = Oxidized glutathione
GSH/GSSH = Reduced glutathione/ oxidized glutathione ratio
Gpx = Glutathione peroxidase

Appendix Y. Mean±SEM of blood GSH, GSSG, GSH/GSSG ratio and Gpx levels in the clinically normal, mild azotemia and moderate azotemia cats on day 56.

Parameter	Day 56					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
GSH (mmol/L RBC)	1.59±0.16	2.02±0.21	1.66±0.17	2.33±0.37	1.93±0.23	2.48±0.23
GSSG(µmol/L RBC)	10.02±1.85	9.45±0.54	8.06±1.70	8.8±0.5	10.56±0.95	9.38±0.92
GSH/GSSG	237.27±109.1	219.12±30.67	256.59±80.83	275.24±59.34	182.22±11.64	280.8±46.76
Gpx (µmol/min/ml)	18,091±780	17,774±2,695	17,452±1,566	18,740±952	13,592±810	14,812±939

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*
GSH = Reduced glutathione
GSSG = Oxidized glutathione
GSH/GSSH = Reduced glutathione/ oxidized glutathione ratio
Gpx = Glutathione peroxidase

Appendix Z. Mean±SEM of blood GSH, GSSG, GSH/GSSG ratio and Gpx levels in the clinically normal, mild azotemia and moderate azotemia cats on day 70.

Parameter	Day 70					
	Clinically normal		Mild azotemia		Moderate azotemia	
	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)	Placebo (n=5)	Extract (n=5)
GSH (mmol/L RBC)	1.83±0.14	1.93±0.11	2.07±0.19	1.76±0.14	1.69±0.17	1.84±0.14
GSSG (µmol/L RBC)	9.60±1.23	9.37±1.28	10.42±0.71	9.31±0.79	7.72±1.95	8.09±0.85
GSH/GSSG	208.7±36.3	218.08±25.15	201.55±23.76	197.96±29.51	272.59±67.61	234.63±25.29
Gpx (µmol/min/ml)	21,116±1,010	19,642±2,355	17,388±2,713	15,778±2,616	14,232±1,677	14,490±1,138

Note: Clinically normal cats = Healthy cats
Placebo = Cats received placebo
Extract = Cats received the crude extract of *Antidesma acidum*
GSH = Reduced glutathione
GSSG = Oxidized glutathione
GSH/GSSH = Reduced glutathione/ oxidized glutathione ratio
Gpx = Glutathione peroxidase

BIOGRAPHY

Miss Numfa Fungbun was born on November 13, 1978. She graduated from the Faculty of Veterinary Science, Khon Kean University, Thailand. She received the degree of Doctor of the Veterinary Medicine in 2002. She has worked for the veterinary clinicians. In 2006, she joined the teaching staff for the small animal internal medicine at the Faculty of Veterinary Medicine, Khon Kean University.