การทำหน้าที่ของไตและภาวะความเครียดออกซิเดชันภายหลังจากการได้รับ ฮอร์โมนอิริโทรโปอิติน เหล็ก และวิตามินอีในหนูที่ถูกเหนี่ยวนำให้ไตเสียหายโดยใช้เยนตามัยซิน

นางสาว พัชรินทร์ ธงไชย

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RENAL FUNCTION AND OXIDATIVE STRESS FOLLOWING GENTAMICIN INDUCED RENAL INJURY IN RATS TREATED WITH ERYTHROPOIETIN, IRON AND VITAMIN E

Miss Patcharin Thongchai

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By	Miss Patcharin Thongchai
Field of Study	Animal Physiology
Thesis Advisor	Associate Professor Chollada Buranakarl D.V.M., Ph.D.
Thesis Co-advisor	Professor Narongsak Chaiyabutr D.V.M., Ph.D.

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

.....Dean of the Faculty of Veterinary Science

(Professor Annop Kunavongkrit , D.V.M., Ph.D.)

THESIS COMMITTEE

..... Chairman

(Associate Professor Kris Angkanaporn, D.V.M., Ph.D.)

..... Thesis Advisor

(Associate Professor Chollada Buranakarl, D.V.M., Ph.D.)

..... Thesis Co-advisor

(Professor Narongsak Chaiyabutr, D.V.M., Ph.D.)

..... Member

(Associate Professor Somchit Eiam-Ong, Ph.D.)

..... Member

(Sirakarnt Dhitavat, D.V.M., Ph.D.)

พัชรินทร์ ธงไชย : การทำหน้าที่ของไตและภาวะความเครียดออกซิเดชันภายหลังจากการได้รับฮอร์โมนอิริโทรโปอิติน เหล็กและวิตามินอีในหนูที่ถูกเหนี่ยวนำให้ไตเสียหายโดยใช้เยนตามัยซิน (RENAL FUNCTION AND OXIDATIVE STRESS FOLLOWING GENTAMICIN-INDUCED RENAL INJURY IN RATS TREATED WITH ERYTHROPOIETIN, IRON AND VITAMIN E) อ. ที่ปรึกษา : รศ. สพ.ญ. ดร. ชลลดา บูรณกาล, อ.ที่ปรึกษาร่วม : ศ.น.สพ.ดร. ณรงค์ศักดิ์ ชัยบุตร 69 หน้า

การศึกษาการทำหน้าที่ของไตและภาวะความเครียดออกซิเดชันภายหลังจากการได้รับฮอร์โมนอิริโทรโปอิติน เหล็ก และ วิตามินอีในหนูที่ถูกเหนี่ยวนำให้ไตเสียหายโดยใช้เยนตามัยซิน โดยแบ่งหนูออกเป็น 5 กลุ่ม กลุ่มควบคุมฉีดน้ำเกลือในวันที่1 ถึง วันที่ 12 กลุ่มที่ 2 ฉีด เยนตามัยซินขนาด 100 มก./กก. เข้าใต้ผิวหนัง ในวันที่ 5-12 กลุ่มที่ 3 ฉีดเยนตามัยซินขนาด 100 มก./กก.และ ฮอร์โมนอิริโทรโปอิติน ขนาด 100 ยูนิต./กก. เข้าใต้ผิวหนังในวันที่ 5-12 กลุ่มที่ 4 ฉีดเหล็ก ขนาด 500 มก./กก. เข้าช่องท้อง ในวันที่ 4 ฉีดเยนตามัยซินขนาด 100 มก./กก. และฮอร์โมนอิริโทรโปอิติน ขนาด 100 ยูนิต./กก. เข้าใต้ผิวหนัง ในวันที่ 5-12 กลุ่มที่ 5 ป้อน วิตามิน อี ขนาด 250 ยูนิต./กก. ในวันที่ 1-3ฉีดเหล็ก ขนาด 500 มก./กก. เข้าช่องท้อง ในวันที่ 4 ฉีดเยนตามัยซินขนาด 100 มก./กก. และ ฮอร์โมนอิริโทรโปอิติน ขนาด 100 ยูนิต./กก. เข้าใต้ผิวหนัง ในวันที่ 5-12 จากผลการศึกษาพบว่าในหนูกลุ่ม 2,3,4 และ 5 ที่ได้รับเยนตามัยซินพบระดับครีเอตินีนและยูเรียไนโตรเจนในเลือดเพิ่มสูงขึ้น และพบว่าอัตราการกรองผ่านกลอเมอรูลัสและอัตราการไหลของพลาสมาที่ไปเลี้ยงไตลดต่ำลงอย่างมีนัยสำคัญทางสถิติ สัดส่วนการขับทิ้งของอิเล็คโทรไลต์ทั้งโซเดียมและโพแทสเซียมและการขับทิ้งโปรตีนเพิ่มสูงขึ้นในหนูกลุ่ม 2,3,4 และ 5 หนูกลุ่ม 3 ที่ได้รับฮอร์โมนอิริโทรโปอิตินไม่พบการเพิ่มขึ้นของอัตราการกรองผ่านกลอเมอรูลัสและอัตราการไหลของพลาสมาที่ไปเลี้ยงไต อย่างไรก็ตามในหนูกลุ่ม 3 พบว่าการทำงานของกลูตาไทโอนเริ่มลดลงเมื่อเปรียบเทียบกับหนูกลุ่ม 2 หนูกลุ่มที่ได้รับเหล็กร่วมด้วยไม่มีการเปลี่ยนแปลงการทำงานของไตและความเครียดออกซิเดชันเมื่อเปรียบเทียบกับกลุ่มที่ 3 แต่ในหนูกลุ่มที่ 5 ที่ได้รับวิตามินอีร่วมด้วยมีการเพิ่มขึ้นของอัตราการไหลของพลาสมาที่ไปเลี้ยงไต โดยสรุป การฉีดเยนตามัยซินทำให้เกิดความเสียหายของไตส่วนกลอเมอรูลัสและหลอดไตฝอยอย่างรุนแรงและยังพบการเปลี่ยนแปลงภาวะความเครียดออกซิเดชัน ฮอร์โมนอิริโทรโปอิตินและเหล็ก ไม่เปลี่ยนแปลงการทำหน้าที่ของไตแต่การให้วิตามินอีสามารถช่วยเพิ่มเลือดไปเลี้ยงไตได้

ภาควิชาสรีรวิทยา ลายมือชื่อนิสิต.....
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PATCHARIN THONGCHAI : (RENAL FUNCTION AND OXIDATIVE STRESS FOLLOWING GENTAMICIN INDUCED RENAL INJURY IN RATS TREATED WITH ERYTHROPOIETIN,IRON AND VITAMIN E) THESIS ADVISOR : ASSOC. PROF. CHOLLADA BURANAKARL, Ph.D., THESIS COADVISOR : PROF. NARONGSAK CHAIYABUTR, Ph.D., 69 pp.

Effects of erythropoietin, iron, and vitamin E on renal function and oxidative stress induced by gentamicin in rats were investigated. Rats were divided into 5 groups; group 1 (control: NSS injection on day 1-12); group 2 gentamicin injection (100 mg/kg s.c. on day 5-12); group 3 gentamicin plus erythropoietin (EPO) (gentamicin 100 mg/kg s.c., EPO 100 i.u./kg s.c. on day 5-12) group 4 gentamicin plus EPO and iron (iron 500 mg/kg i.p. on day 4, gentamicin 100 mg/kg, EPO 100 i.u./kg s.c. on day 5-12 and) ; group 5 gentamicin plus EPO, iron, and vitamin E (vitamin E 250 i.u./kg orally on day 1-3, iron 500 mg/kg i.p. on day 4, gentamicin 100 mg/kg, EPO 100 i.u./kg s.c. iron 500 mg/kg i.p., on day 5-12). The results showed that plasma creatinine and BUN concentrations of rats in group 2,3,4, and 5 were significantly increased while glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) decreased. The fractional excretion of Na^+ and K^+ and protein excretion were higher in groups 2,3,4 and 5. Group 3 with EPO did not improve GFR and ERPF. However, kidney GSH was decreased compared with group 2. Rats receiving iron showed no changes in renal function and oxidative stress compared with group 3. However, supplement with vitamin E in group 5 caused higher ERPF. In conclusion, gentamicin induced nephrotoxicity by causing severe damage of both glomerulus and renal tubular cells with alteration of oxidative stress. EPO and iron did not alter renal function but vitamin E supplementation could improve blood flow to the kidney.

Department Physiology Student's signature.....

Field of study Animal physiology Advisor's signature.....

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CONTENTS

LIST OF TABLES

Tables **page**

LIST OF FIGURES

LIST OF ABBREVIATIONS

γ-GT	gamma glutaryl transferase
ARF	acute renal failure
ANOVA	analysis of variance
BH	butylated hydroxyltoluene
BUN	blood urea nitrogen
CAT	catalase
C_{H2O}	free water clearance
Cl^-	choride
C_{osm}	osmolar clearance
DP	diastolic pressure
CRF	chronic renal failure
Cu	copper
EDTA	ethylenediaminetetra-acetic acid
ET-1	endothelin-1
EPO	erythropoietin
EPOR	erythropoietin receptor
ERBF	effective renal blood flow
ERPF	effective renal plasma flow
FE	fractional excretion
FF	filtration fraction
GFR	glomerular filtration rate
GSH	glutathione
H_2O	water
Hct	hematocrit
HNE	4-hydroxynonenal
i.m.	intramuscular injection
i.v.	intravenous injection
i.p.	intraperitoneal injection
K^+	potassium
LPO	lipid peroxide
MABP	mean arterial blood pressure

MDA	malondialdehyde
mg	milligram
mg%	milligram percent
min	minute
mM	millimolar
mRNA	messenger RNA
Na^+	sodium
NAG	N-acetyl-β-D glucosaminidase
NPSH	non-protein sulhydryl
NSS	normal saline solution
PAH	para-aminohippuric acid
PCNA	proliferating cell nuclear antigen
PP	pulse pressure
ROS	reactive oxygen specie
SDS	sodium dodecyl sulfate
SOD	superoxide dismutase
SP	systolic pressure
s.c.	subcutaneous injection
U	unit
UV	ultraviolet
µg	microgram
µl	microliter
µmol	micromol
Zn	zinc

CHAPTER I

INTRODUCTION AND AIMS

Gentamicin is an aminoglycoside antibiotic that has a good activity against variety of bacteria especially gram negative bacilli. It is obtained from cultures of *Micromonaspora purpurae*. Gentamicin has seriously adverse effects notably nephrotoxicity shown by acute tubular necrosis (Mazzon et al., 2001). The mechanism of this drug is probably related to interference with phospholipids metabolism in the lysosome of proximal renal tubular cells resulting in leakage of proteolytic enzymes into the cytoplasm (Plumb, 2005). Nephrotoxicity is usually manifested by increased concentrations of blood urea nitrogen (BUN), creatinine, non-protein nitrogen in the serum and increased urinary enzyme both *N*-Acetyl-*β*-D-glucosaminidase (NAG) and gamma glutamyl transferase (γ-GT). The decreases in urine specific gravity and creatinine clearance with proteinuria were concomitant with the present of casts in the urine. Since gentamycin is nephrotoxic aminoglycoside, using this drug requires caution and patient monitoring.

Administration of erythropoietin (EPO) improves anemic condition, renal function and acts as a growth factor by accelerating tubular cell regeneration (Bagnis et al., 2001). EPO has been used in many models of renal failure especially chronic renal failure (CRF). In 1990, Nagano and co-workers studied the effects of recombinant human erythropoietin on new anemic model rat induced by gentamicin. They found that EPO can improve both anemia and renal function. In 2000, Sommerberg and co-workers found that EPO decreased plasma levels of lipid peroxidation and malondialdehyde (MDA) in CRF patients. However, accelerated red

blood cell production using EPO requires iron supplementation. It has been demonstrated that intravenous iron promotes oxidative stress by catalyzing the fenton reaction (Lim and Vaziri, 2004) leading to glomerulosclerosis, tubular atrophy, interstitial fibrosis and renal failure (Zhou et al., 2000). Thus, giving iron may aggravate the renal function. Supplementation of antioxidant such as vitamin E was suggested to improve renal damage as a consequence of iron. However, the study on the combination effects of EPO , iron and vitamin E on renal function and oxidative stress in gentamycin induced nephrotoxicity has not yet been demonstrated. Therefore, the aims of this study were to investigate; first, to study the effects of EPO administration on renal function and oxidative stress in rat with gentamicin-induced renal injury, second, to study the effect of iron supplementation with EPO on erythropoiesis, renal function and oxidative stress, and third, to study the combination of EPO, iron and vitamin E on these parameters.

CHAPTER II

LITERATURE REVIEWS

Pharmacological of Gentamicin

Gentamicin antibiotics had been the mainstays of treatment of serious infection due to aerobic gram-negative bacilli. However, since their use was limited by serious toxicity. Gentamicin that are derived from Steptomyces. The action of Gentamicin is believed to inhibit bacterial protein synthesis by mechanism determined for streptomycin and then gentamicin binds to separated 30s ribosome subunit, interfering with assembly of the functional ribosomal apparatus; or causing the 30s subunit of the complete ribosome to misread the genetic code. Polysomes become depleted because gentamicin interrupt the process of polysome desegregations and assembly (Mycek et al., 1997)

Gentamicin is bactericidal and effective only against aerobic organism since anaerobes lack the oxygen-requiring transport system. The highly polar, polycationic structure of the gentamicin prevents adequate adsorption after oral administration. Therefore, gentamicin must be parenterally to achieve adequate serum levels.

The levels of gentamicin achieved in most tissue is low and penetration into most body fluids is variable. Concentrations in cerebrospinal fluid are inadequate even when the meninges are inflamed. High concentration accumulate in the renal cortex and in the endolymph and perilymph of the inner ear which may account for their nephrotoxic and ototoxic potential (Bunnett, 1996). It is important to monitor peak plasma levels of gentamicin to avoid concentrations that cause related-toxicities. Patient factors, such as old age, previous exposure to aminoglycosides, gender and

liver disease, tend to predispose patients to susceptible to nephrotoxicity and ototoxicity. Nephrotoxicity of gentamicin causes retention of drug by the proximal tubular cell disrupts calcium-mediated transport processes and result in kidney damage ranging from mild renal impairment to severe acute tubular necrosis which can be irreversible (Mcglynn and Ryan., 1990).

Gentamicin on renal function

Nephrotoxicity induced by gentamicin manifests clinically as acute renal failure with rise in serum BUN and creatinine, reduction in GFR and renal plasma flow. (Leclercq et al., 1999, Baylis , 1980)

After filtered at the glomerulus, a small proportional filtered gentamicin (2-5%) binds and actively transports into proximal tubular cell. The drug can be taken up into the cell from both luminal and basolateral surfaces and exposed to mitochondria and microsome. After penetration into cytoplasm, the drug accumulates in the lysosomes, endosomes and golgi complexes (Ali, 1995, Paule et al., 1999). Subsequently, gentamicin remain with a long half life in the renal proximal tubular cell, leading to renal damage such as structural change and functional impairment of the plasma membrane mitochondria and lysosome (Mingeot-Leclercq and Tulkens, 1999). A direct effect of gentamicin on the glomerulus has been seen at high dose (Baylis et al., 1979). The impairment of glomerular filtration rate (GFR) is probably due to abnormalities of renal circulation or reduction of glomerular capillary ultrafiltration (Nakajima et al., 1994). Baylis and co-worker (1977) studied pathological mechanisms responsible for impairment of GFR in gentamicin mediated nephropathy. They found many abnormalities of renal circulation such as renal vasoconstriction and reduction of glomerular capillary ultrafiltration coefficient (Kf) resulting in decreased

GFR. In addition, treatment of experimental animals with gentamicin results in tubular cell injury and necrosis. These functional alterations suggest an impairment of tubular reasorption capacity resulting in increase in urinary excretion of sodium and potassium (Cojocel et al., 1984). In laboratory animal, after administration therapeutic dose (10-20 mg/kg b.w.) only for a few days can induce lysosome of proximal tubular cell alteration inconsistent with the accumulation of polar lipid myeloid bodies (Begg and Barclay, 1995). These changes are preceded and accompanied by signs of tubular dysfunction or alteration such as release of lysosomal enzyme from brush border, decrease reabsorbtion of filtered protein, wasting of K^+, Mg^+, Ca^+ and glucose, and excretion of phospholipid and cast (Gilbert, 1995). Gentamicin can also produce disturbance of plasma electrolyte homeostasis resulting in hypomagnesemia, hypocalcemia and hypokalemia (Ali et al., 1992, Foster et al., 1992). These electrolytes disturbances may be responsible for renal impairment.

High dose of gentamicin (40 mg/kg bw) in animals rapidly induced extended cortical necrosis and overted renal dysfunction (Kosek et al., 1974). The alterations of both structure and function are observed in proximal tubular cell in initial stage. In addition, another effect that can also be found is inhibition of protein synthesis and modulation of gene expression resulting in mitochondrial alteration.

Gentamicin on oxidative stress

The mechanisms of gentamicin induced neprotoxicity remain unknown but the current information revealed a role of reactive oxygen metabolites have been implicated (Silan et al., 2007). Sequential reduction of oxygen pathway leads to the generation of superoxide anion, hydrogen peroxide, hydroxyl radical, and water.

These are important mediators of tissue injury and dysfunction. Gentamicin has been shown to enhance the generation of superoxide anion and hydrogen peroxide by renal cortical mitochondria (Walker et al., 1999). The interaction between superoxide anion and hydrogen peroxide in the presence of metal catalyst can lead to generation of hydroxyl radical (Walker and Shah, 1988). Gentamicin has been shown to induce the release of iron from renal cortical mitochondria and enhance generation of hydroxyl radical (Walker and Shah, 1987). Reactive oxygen species cause cellular injury and necrosis by several mechanisms including peroxidation of membrane lipid, protein denature and DNA damage (Ali, 2003). Cuzzocrea and co-workers (2002) studied the role of superoxide in gentamicin-mediated nephropathy in rats and found that gentamicin could induce renal failure by significant decrease in creatinine clearance and increase plasma creatinine levels, fractional excretion of sodium, lithium, urine glutamyl tranferase, daily urine volume, kidney myeloperoxidase and lipid peroxidase activity. Administration of gentamicin in rat caused reduction in renal blood flow and inulin clearance as well as marked tubular damage. However, administration of superoxide dismutase (SOD), a free radical scavengers, improved both glomerular filtration rate and renal blood flow (Nakajima et al., 1994).

An enhanced production of ROS has been demonstrated to be involved in the glomerular and tubular alterations characteristic of acute renal failure induced by gentamicin (Saldago et al., 2002). At the glomerular site, ROS induces glomerulonephritis with microthrombotic and microangiopathic lesions. The morphologic changes occurred such as oedema and dequamation of endothelium, denuding of basement membrane, thrombi, mesangiolysis, foot process fusion and epithelial vacuolarization (Gwinner et al., 2000). Functional changes consist of

increased permeability with proteinuria and changes in intracellular haemodynamics (Shah, 2001).

At the tubular site, ROS may cause swelling and detachment of cell from basement membrane resulting in cell lysis. Functional changes are increasing permeability, alteration in transmembrane potential and proliferative response. These lesions were present in study of reperfusion injury caused by gentamicin and cisplatinum.

Pharmacological of Erythropoietin (EPO)

Erythropoietin (EPO) is a 34–kD glycoprotein which stimulates erythroid cell proliferation, differentiation and maturation of red blood cells in the bone marrow (Ng et al., 2003). Human and murine EPO genes have been cloned and their amino acid sequence is highly evolutionarily conserved. EPO is synthesized primarily by peritubular interstitial cells in the cortex or the outer medulla of the kidney (Fisher, 2003). Beside, it can also produce in the liver particularly during fetal and neonatal life and can be greatly enhanced in response to low oxygen tension in arterial blood of both kidney and liver (Cheung and Miller, 2001). Ratcliffe and colleagues (1992) were the first report that EPO is produced in organs other than the kidney and the liver. Morever its receptor were expressed in the multiple tissues, including the vasculature, brain, uterus, heart and skeletal muscle (Sasaki., 2003, Sharples and Yaqoop., 2006). The constant level of the hormone concentration approximately 2 picomol/liter of EPO evokes the production of erythrocytes enough to maintain a sufficient oxygenation of organ and tissues. During anemia and other conditions of reduced oxygen supply to the kidney the rate of EPO secretion increases , which enhances the production of new erythrocytes. EPO functions by binding to the EPO receptor

result in homodimerization of the receptor, followed by activation of several signal transduction pathway (Ng et al., 2003). There was now abundant experimental evidence demonstrated that EPO interacts with its receptor in many tissues to induce a range of cytoprotective cellular response. The cytoprotective cellular response were include mitogenesis, angiogenesis, inhibition of apoptosis and promotion of vascular repair (Johnson et al., 2006). The other effects of EPO include a hematocrit-independent, vasoconstriction-dependent hypertension, increased endothelin production, upregulation of tissue renin, change in vascular tissue prostaglandins production (Fisher, 2003).

Erythropoietin on renal function

Acute renal failure (ARF) is associated with the development of anemia (Rainford et al., 1992). Tan and co worker (1996) studied the EPO production in rats with post-ischemic acute renal failure.They found that acute renal failure rats had mild anemia in associated with relative EPO deficiency. In the post ischemic kidney, a substantial capacity for EPO response to blood oxygen availability was significantly reduced. Serum EPO concentration was markedly depressed relative to the degree of anemia in 10 patients with acute tubular necrosis with decreased renal function. The low serum EPO is due to defective synthesis rather than increased catabolism (Nielson and Thaysen, 1990). The level of EPO in patients with acute renal failure of various etiology rapidly decreased to a level inappropriately low for the hemoglobin value persisted throughout the oligulic phase and for up to 2 week after the restoration of normal renal function (Lipkin et al., 1990). Whether EPO is the crucial hormone related to tubular injury has been proposed.

In the recent study, experimental data have demonstrated several pleiotropic actions of EPO administrate red acutely such as limitation of apoptosis, promotion of neovascularization and anti-inflammatory effects (Covic and Tatomir., 2007). Bahlman and colleaques (2004) suggested that EPO can ameliorate chronic as well as acute renal failure. A number of studies *in vivo* investigations have shown that EPO can reduce the renal dysfunction and injury caused by oxidative stress, hypoxia and hemorrhagic shock generally by reducing caspase activation and apoptotic cell death (Abdelrahman et al., 2004 , Bagnis et al., 2001). Renoprotective effects and anti-apoptotic effects of EPO have also been reported in animal model of acute renal failure (Yang et al., 2003 , Westenfelder et al., 2002). Sharples and co-worker (2004) reported that EPO protects the rat kidney in a model of severe ischemia-reperfusion injury with inhibition of caspase-3,-8, and -9 activation and reduced apoptotic cell death. The administration of a single dose of EPO in rats model before the onset of renal ischemic reperfusion injury showed a significant reduction in tubular injury by a marked ameriolation of renal impairment and cytoprotective effect by anti-apoptotic action (Spandou et al., 2001) Bahlmann and colleagues (2005) recently investigated whether low dose therapy with darbepoetin-α protects against renal dysfunction and injury in rats with chronic renal failure induced surgically with no significant change in systemic blood pressure or packed cell volume. Bagnis and co-workers (2001) studied the effect of EPO after cisplatin-induced acute renal failure in rats. The results demonstrated that a combination of cisplatin and EPO increased renal blood flow and glomerular filtration rate. After 9 days of treatment, tubular regeneration occurred with significant enhancement of tubular cell proliferation by increasing number of positive cell, which detected from proliferating cell nuclear antigen (PCNA). These results suggested that EPO may act as a growth factor on

tubular cell leading to accelerated recovery of renal function (Vazari et al., 1994). The renoprotective effects of EPO were not only ameliorated renal hypoxia by correcting anemia but also enhanced renal microvascular cell survival and angiogenesis (Kang et al., 2005).

Erythropoietin on oxidative stress

Oxidative stress is a prominent cause of ischemic renal disease. In addition ischemia induced tubulo-interstitial fibrosis, progression of renal failure, dysfunction of renal vasculature mediated by glomerular-capillary sclerosis and loss of peritubular capillaries (Pillebout et al., 2001).

Sommerburg and co-workers (2000) investigated the role of renal anemia on oxidative stress by measuring two lipid peroxidation (LPO)products,malondialdehyde (MDA) and 4-hydroxynonenal (HNE) in the plasma of hemodialysis patients who received EPO therapy. They found that EPO can correct anemia and rejuvenate red blood cells. It significantly decreased plasma level of LPO products, MDA, and increase in whole blood antioxidant capacity in patients with CRF.

Pharmacological of Iron

Iron dextran is a complex of ferric oxyhydroxide with low molecular weight. The commercially available injectable form appeared as a dark brown, slightly vicious liquid that is completely miscible with water or normal saline and has a pH of 5.2-6.5. Iron is necessary for myoglobin and hemoglobin in the transportation and utilization of oxygen. It does not stimulate erythropoiesis nor correct hemoglobin abnormalities. Iron dextran is used in both treatment and prophylaxis of iron deficiency anemia either as a result of enhanced utilization or insufficient absorption

of dietary iron (Plumb, 2005). Total body iron stores in healthy individuals amount to 800 to 1200 mg but vary according to body size (Eschbach, 2005). Normal blood contains approximately 130 to150 mg of hemoglobin per milliliter and each gram of hemoglobin contains 3.4 mg of iron. Although most of iron in the body is in the form of hemoglobin, iron is also store in tissues as hemosiderin and ferritin. In blood, it is bound to transferrin, a carrier protein. Absolute iron deficiency arises when the total body iron stores are insufficient to meet haematopoietic demands (Clark et al., 1992). Iron deficiency develops when the amount of iron in the body is less than that required for formation of haemoglobin and maintain adequate erythropoiesis. Functional deficiency of iron may occur due to enhanced erythropoiesis after therapy with recombinant human erythropoietin that often necessitates therapy with intravenous iron. However, iron therapy is potentially toxic due to participation of elemental iron in oxidation-reduction reaction (Agarwal et al., 2004)..

Acute iron toxicosis causes cellular damage as a result of the presence of unbound iron in the circulation. Free iron penetrates the cell of the liver, heart, and brain. At cellular levels, free ion causes increased lipid peroxidation with resulting membrane damage to mitochondria, microsomes, and other cellular organelles. Because no mechanism for the excretion of iron exists, the toxicity depends on the amount of iron already present in the body. Consequently, some animals can develop clinical signs of iron toxicosis even when given dose that cause no problem in other animals (Humlee, 2004).

Iron and oxidative stress

Iron is a very reactive transitional metal that can change valences states, from ferrous to ferric and then back to ferrous form, very rapidly. Iron can produce free

radicals. These free radicals can initiate autooxidation of polyunsaturated fatty acids (Connell et al., 1985). It is wildly appreciated that parenteral iron can initiate oxidative reaction. Iron –mediated oxidative stress however depends on its carrier (e.g. increased injury with adenosine diphosphate and decreased injury with ferritin).

Parenteral iron (Fe) is frequently used in conjunction with erythropoietin for the treatment of anemia of CRF. However, concerns have been raised about excess parenteral iron administration, which may overload the catalytically active iron and promote oxidative stress. These may accelerate cardiovascular disease, risk of infection and allergic reaction (Afzalib and Goldsmith, 2004). In addition, iron overload can increase production of reactive oxygen species (ROS) and diminish nitric oxide availability. These events can contribute to pathogenesis of renal lesions (Zainal et al., 1999). The generation of highly reactive cytotoxic hydroxyl radical ($^{\cdot}OH$) is facilitated by ferrous ion (Fe^{2+}) which catalyzed fenton reaction resulting in a significant increase in plasma concentration of lipid peroxidase product and malondialdehyde which has been demonstrated in CRF rat (Lim and Vaziri, 2004). Iron was also shown to produce oxidative stress by increasing in plasma concentration and urinary excretion rate of MDA. These was accompanied by ezymuria, protienuria and tubular damage (Agarwal et al., 2004). The reaction were shown as follow ;

$$Fe^{3+} + O^{-}{}_{2}^{\cdot} \rightarrow Fe^{2+} + O_2 \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^{-} \quad (2)$$

$$O^{-}{}_{2}^{\cdot} + H_2O_2 \rightarrow O_2 + {}^{\cdot}OH + OH^{-} \quad (3)$$

Iron is easily oxidized and reduced. The iron catalyzed Haber-Weiss reaction (3) leads to the generation of the highly reactive hydroxyl radical, which is a potent

oxidant. The Haber-Weiss reaction is a composite of the Fenton reaction (2) and an iron catalysed generation of divalent oxygen from its superoxide anion(1). Sequential reductions of oxygen along the univalent pathway lead to the generation of superoxide anion, hydroxyl radical, water, and hydrogen peroxide.

Moreover, iron overload has been linked to the progression of renal disease. Iron overload can lead to glomerulosclerosis, tubular atrophy, interstitial fibrosis and iron deposition in the glomeruli, proximal and distal tubule (Zhou et al., 2000). Administration of parenteral iron has direct glomerular as well as tubular accesses via peritubular capillaries, resulting in progression of glomerular and tubulointerstitial disease (Zager et al., 2004)

Pharmacological of Vitamin E (alpha-tocopherol)

Vitamin E is a lipid soluble vitamin that can be found in either liquid or solid form. The liquid form comes in clear, yellow to brownish red, vicious oils that is insoluble in water but soluble in alcohol and miscible with vetgetable oils (Plumb, 2005). Vitamin E includes eight different related homologous, that is α, β, γ, and δ forms, depending on the number and site of methyl substituents on the chroman ring. Vitamin E is defined as tocopherols and tocotrienols. Alpha-tocopherols is the most abundant active form *in vivo* (Niki and Noguchi, 2004). Also, α- tocopherols is the most potent naturally occurring scavenger of reactive oxygen species (Tucker and Townsend, 2005). Absorption of vitamin E is highly depentdent upon the fatty acid digestion and metabolism. Vitamin E is absorbed as micelle and chylomicron and cross the unstirred water layer follows by releasing its contents into the enterocytes. Vitamin E is a lipid soluble vitamin and therefore over 90% of total body vitamin E

is found in the adipose tissue and is excreted mainly via bile, urine, feces and skin (Traber, 1999).

Vitamin E is also known as alpha tocopherol which is an integral part of cellular membrane. The main role is to defend the cell against oxidation within a cell and organelles especially mitochondria. Vitamin E is the first line of defense against lipid peroxidation and also plays a very important function in red blood cell flexibility, and hemolysis (Plumb, 2005).

Vitamin E is considered to be the major chain breaking antioxidant in membrane and helps to reduce production of prostaglandin such as thromboxane. Bradford et al (2003) has reported two roles of α- tocopheral in membrane 1) as a lipid soluble antioxidant that acts to prevent the free radical damage of unsaturated fatty acid and 2) as a membrane stabilizing agent through its van der waals interaction with membrane phospholipid to prevent the damage from phospholipase. The mechanism of vitamin E to stabilize membrane is possibly by increasing the "oderliness of membrane lipid packaging''. This effect allows a tighter packing of the membrane resulting in greater stability to the cell (Tiddus et al, 1998).

Vitamin E and oxidative stress

In normal physiological condition, the mitochondrial transport system consumes more than 85% of all oxygen used by cell and up to 5% of oxygen consumed by mitochondria is converted to superoxide, hydrogen peroxide and other reactive oxygen species (Chance et al., 1979). There were numerous reports which showed that vitamin E acts as a radical-scavenging antioxidant. Chow et al (1999) studied the link between vitamin E and the production of hydrogen peroxide in mitochondria. They showed that vitamin E can directly regulate hydrogen peroxide

production in mitochondria and suggested that the over production of mitochondrial. ROS is the first event leading to the tissue damage observed in the vitamin E deficiency syndromes. Progression to renal failure is significantly worsen by oxidative stress in kidney disease. The membrane antoxidant vitamin E, α–tocopheral, is examined as a potential therapeutic intervention, that may help to slow the rate of decline of kidney function and collect plasma antioxidant status (Fryer, 1998). In advance CRF, the endogenous antioxidant enzyme (SOD, catalase, glutathione peroxidase) are decreased (Kavutcu et al., 1996). Supplements with antioxidants, vitamin E and C, can be considered as a treatment in CRF . However, when use in large quantities, vitamin C can potentially exacerbate oxidative stress by catalyzing conversion of Fe^{3+} to Fe^{2+}, which favors formation of hydroxy radical (Vazari, 2004). Thus, vitamin E is an option. Intravenous administration of iron sucrose in dialysis patients caused increased total peroxide, free iron, and marker of lipid peroxidation (Agarwal et al., 2004). The improvement was found after supplement with vitamin E (Roob et al., 2000). Moreover, vitamin E supplementation has been associated with amelioration of oxidative stress and anemia in renal disease (Vaziri, 2004). Dillioglugil and co-worker (2005) have shown that cisplatin combination with vitamin E 200, 400 mg/kg decreased MDA and nitric oxide level and increased GSH and SOD level when compared with the cisplatin injection alone. Furthermore, vitamin E deficiency led to progressive oxidative stress damage on renal structure and function. Oxidative renal injury in vitamin E deficient rats is reflected by a marked elevation in the serum creatinine/creatol molar ratio mediated by hydroxyl radicals (Osasa et al, 1997).

Vitamin E and gentamicin

Vitamin E has been used to ameliorate gentamicin nephrotoxicity and oxidative stress. Abdel-Naim and co-worker (1999) showed that vitamin E pretreatment significantly lowered both the elevated serum urea and creatinine levels, and the urinary activity of NAG and γ-glutamyl transferase. In addition, vitamin E ameliorated the rise in renal content of MDA and enhanced the renal content of reduced non-protein sulphydryls (NPSH) as well as SOD activity. Kavutcu and co-worker (1996) demonstrated that the kidney tissue from guinea pigs treated with gentamicin plus vitamin E showed increases in Mn-superoxide dismutase, glutathione peroxidase, catalase and xanthine oxidase compared with group treated with gentamicin alone. Another study revealed that vitamin E can prevent the gentamicin-induced reduction in renal tissue glutathione content (Kadkhodaee et al., 2005).

CHAPTER III

MATERIALS AND METHODS

Experimental Animals

The experiment was performed in accordance with the institutional guidelines and conformed to the Faculty of Veterinary Science, Chulalonkorn University. Male spraque-Dawley rats, weighting between 250-300 g obtained from NLAC. (Salaya District, Nakhonprathom province Mahidol University) were used in this study. The animals were housed under standard conditions of light and dark cycle (L:D=12:12) with free access to regular rat chow and water. The animals were adapted to the laboratory condition before use for seven days and randomly assigned into 5 groups each including 14 animals.

Experimental protocol

Group 1. Control group (n= 14) rats were injected daily with normal saline solution subcutaneously to replace gentamicin and EPO. Intraperitoneal normal saline (NSS) was injected to replace iron dextran. Propylene glycol was fed to replace vitamin E, once daily for 3 consecutive (day 2-4) prior to gentamicin administration on day 5. Group 2-5 were injected with gentamicin daily starting from day 5 for 8 consecutive day.

Group 2. Gentamicin groups (n= 14) rats were injected subcutaneously daily with gentamicin sulfate at a dose of 100 mg/kg for 8 days (day 5-12). NSS was injected to replace EPO and iron. Propylene glycol was fed to replace vitamin E .

Group 3. Gentamicin + EPO (n= 14) In addition to gentamicin**,** rats were daily injected with EPO 100 IU/kg subcutaneously starting from day 5 until day 12. NSS was injected to replace gentamicin and iron. Propylene glycol was fed to replace vitamin E .

Group 4. Gentamicin + EPO + Iron (n= 14) In addition to gentamicin and EPO, rats were injected with a single dose of intraperitoneal iron dextran once at a dose of 500 mg/kg on day five. Propylene glycol was fed to replace vitamin E.

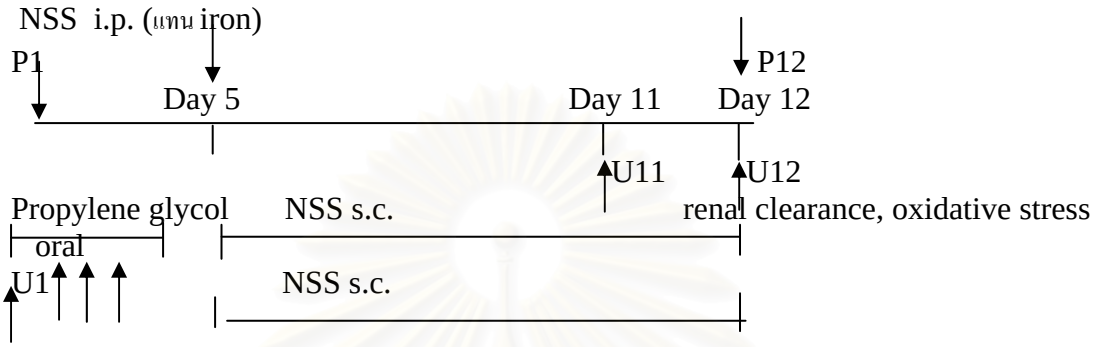
Group 5. Gentamicin + EPO + Iron +Vitamin E (n=14)) In addition to gentamicin, EPO and iron, vitamin E 250 mg/kg was fed once daily for 3 consecutive day prior to gentamicin administration (day 2-4).

Body weight and food intake were recorded daily throughout the experimental period. On the first day of experiment, each animal was kept in metabolic cage with free access of water for measurement of urine volume, urinary protein concentration, electrolyte concentration, osmolarity and malondialdehyde (MDA). Plasma was collected by cutting tip of tail vein for measurement of blood urea nitrogen (BUN), creatinine.

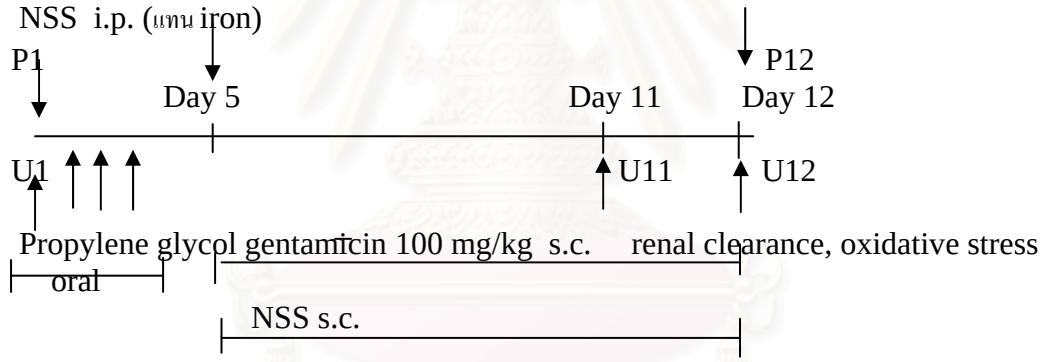
At day 11 urine was collected again from rats for 24 hrs from metabolic cage with free access to water for measurement of urine volume, urinary protein concentration, electrolyte (Na^+, K^+, Cl^-), osmolarity and MDA. Renal clearance study was performed in 5 rats in each groups on day 12 and collected urine for measurement of urine NAG. Seven separated rats were undergone to study oxidative stress at the same protocol without measurement of renal function. Rats were anesthesized by Tiletamine-Zolazepam (zoletil®;50 mg/kg) and cardiac puncture was performed to collect 5 ml of blood for measurement of creatinine, BUN, osmolality and electrolytes (Na^+, K^+, Cl^-). The kidneys were removed immediately. The left kidney was used for

measurement of lipid peroxidation. The cortex of kidney was homogenated and stored at -70˚ C for the determination of MDA, SOD, GSH and catalase activities.

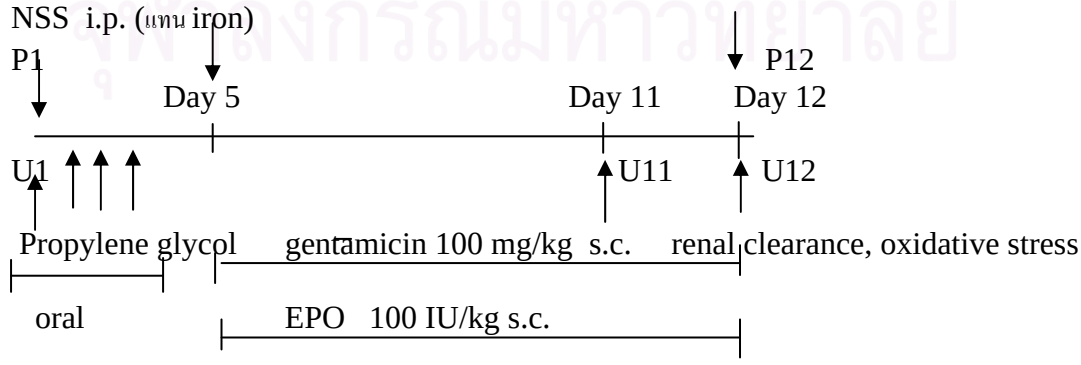
Group I. Control group



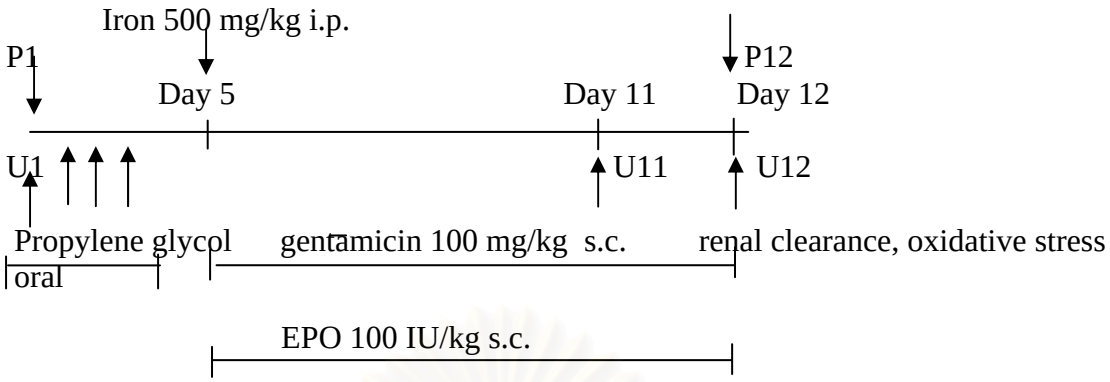
Group II. Gentamicin group



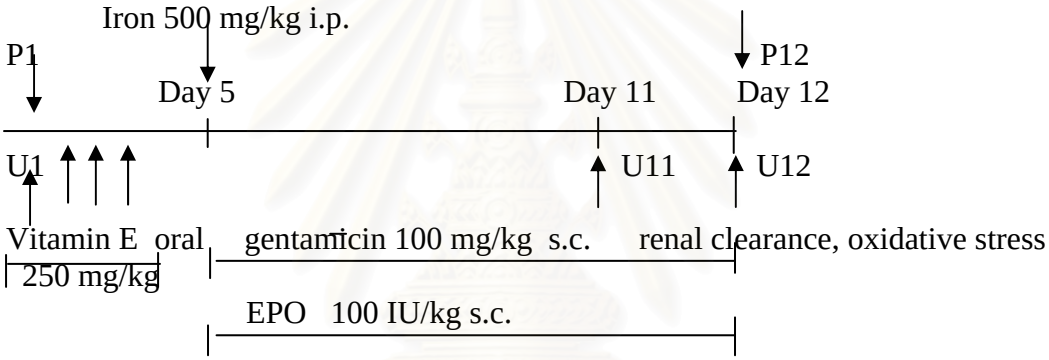
Group III. Gentamicin + EPO group



Group IV. Gentamicin + EPO + Iron group



Group V. Gentamicin + EPO + Iron + Vitamin E group



U1 and U11: Urine sample were collected on day 1 and day 11 for measurements of urinary protein, creatinine, volume, electrolyte (Na^+, K^+, Cl^-), osmolarity, MDA

U12 : Urine sample were collected on day 12 for measurements of urinary NAG

P1 : Blood was collected in day 1 for measurements of Hct,BUN, creatinine

P12 : Blood was collected in day 12 for measurements of Hct,BUN, creatinine, osmolarity, electrolyte (Na^+, K^+ ,Cl^-)

Kidney : Left kidney was collected in day 12 for measurements of MDA, GSH, SOD, CAT

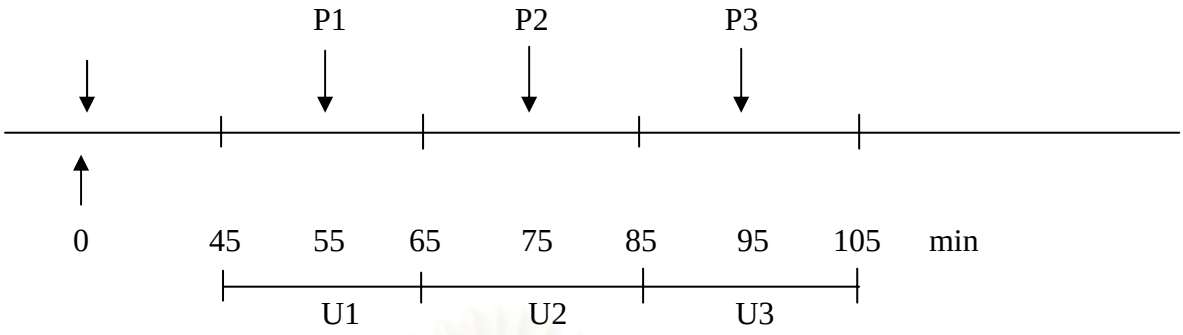
Operative procedure of renal clearance study

For renal clearance study, each rat was anesthesized with Zoretil® (Tiletamine-Zolazepam) at dose of 50 mg/kg bodyweight via an intraperitoneal injection. Tracheotomy was carried out and a short piece of PE 240 catheter was inserted into the trachea for aspirating secretion and use as an artificial airway. The right femoral artery and vein were cannulated with PE50 catheters. The right femoral artery was used for blood collection and also be used to monitor arterial blood pressure by connecting to a pressure transducer with a glass polygraph recorder. A polyethylene catheter was cannulated into the right femoral vein for infusion of inulin and para-aminohippurate (PAH) solution. The abdominal midline incision was perform and urinary bladder was located while the PE250 catheter will be inserted for urine collection. Urine were collected into a pre-weighed eppendrof.

Renal clearance study

The Clearance study was started by infusion a mixture of 1% inulin, 0.2% PAH and 6% mannitol in normal saline at the rate of 1 ml/h/ 100 g bodyweight continuously for 45 minutes to stabilized plasma inulin and PAH concentration. After equilibration period, three time of urine collection (U1, U2, U3) along with arterial blood sampling at midpoint of urine collection (P1, P2, P3) were performed. Bovine serum albumin (6%) was administered after blood collection at the same volume to replace blood losses. Each urine collection period will be 20 minutes. Urine volume was measured from the weight change of pre-weighed eppendrof. Blood sample was use for determination of packed cell volume. Plasma and urine were kept at -20˚ c for further analysis.

Infuse (inulin+PAH+manitol)



P1+P2+P3 : inulin, PAH, Hct

U1+U2+U3 : inulin, PAH

Analytical procedures determination of blood urine and kidney samples

The inulin concentration was determined by the Antrone method as described by Young and Raisz (1952). The PAH concentration was determined by the method of Brun (1951). Sodium and potassium was measured by flame photometer (Flame photometer 410C, Ciba Corning Inc., USA). Chloride was measured by chlorimeter (Chloride analyzer 925, ciba Corning Inc., USA) Osmolarity was measured by osmometer (Osmometer 3D3, Advance Instruments Inc., USA). Urine protein concentration was measured by precipitating with sulfosalicylic while kidney protein concentration was measured according to Lowry et al (1951). The creatinine concentration was analyzed by Jaffe reaction. Plasma urea nitrogen concentration was analyzed by the colorimetric method. Urine and kidney MDA was measured by method as described by Ohkawa et al (1979). Kidney catalase activity was determined following the method of Aebi et al (1983). Kidney SOD activity was measured following the method of Maccord and Fridovich (1969). Kidney glutathione

was measured by a modification method of Beutler et al (1963). Urinary NAG was measured by enzymatic method.

Determination of kidney and urine MDA

Determinations of kidney and urine MDA were performed according to the method described by Ohkawa et al (1979). The renal cortex was removed and immediately placed in iced phosphate buffered saline. Fat and surrounding capsule and fascia was excised, and remaining tissue was homogenized with homogenizer. The homogenized tissue was centrifuged at 600 g for 10 minutes. 100 µl of supernatant was added to a reaction mixture consisting of 50 µl (BHT), 100 µl 8.1% sodium dodecyl sulfate (SDS) , 750 µl 20 % acetic acid, 750 µl 0.5% thiobarbituric acid, and 250 µl of water. This solution was placed in water bath kept at 95º C for 60 minutes. After removal from the water bath 2 ml of n-butanol-pyridine was added and samples were agitated and subsequently centrifuged at 4,000 g for 10 minutes. The upper organic layer was pipetted off and centrifuged at 8,000 g for 10 minutes and then the absorbance of this fraction was read at 532 nm in spectrophotometer. Malondialdehyde tetraethylactal were employed as the standard. MDA values were expressed as nanomol per milligram protein which was determined by using Lowry method.

To determine urine MDA concentration, 250 µl of urine was employed with MDA assay described above.

Determining of kidney GSH

Renal GSH content was measured by a modification of Beutler et al. (1979). Renal cortex tissue (0.2 g) was suspended in 1.8 ml of 100 mM KCl plus 0.003 M

EDTA and homogenized as described above for determination of MDA. The homogenates were centrifuged at 600 g for 10 minutes. The 1 ml of supernatant was added to 1.5 ml metaphospholic acid, and particulate debris was removed by the centrifugation at 3,000 g for 10 minutes. Reduced GSH was measured by adding 500 µl of supernatant to 2.0 ml of 0.2 M phosphate buffer and 0.25 ml 0.04% 5,5´dithio-bis 2-nitrobenzoic acid. The absorbance was read at 410 nm. GSH (sigma) was used as the external standard. GSH content was expressed as nanomoles of GSH per milligram homogenate protein.

Determination of kidney catalase activity

Catalase activity was determined using the method of Aebi et al. (1983). Slice of renal cortex tissue (0.2 g) was transferred to tubes containing 1% Triton x 100 following homogenization. The homogenates were centrifuged at 3,000 rpm for 20 minutes The 100 µl of supernatant was added to 1.9 ml of phosphate buffer and read at 240 nm. One ml of 30 mM H_2O_2 was added to start the reaction. The change in absorbance was read at 240 nm every 30 seconds for 1-2 minutes using UV-Vis spectrophotometer. The catalase was used as external standard catalase as unit of catalase per milligram protein.

Determination of kidney SOD activity

Renal SOD content was measured by a modification of Maccord and Fridovich (1969). Renal cortex tissue (0.1 g) was suspended in 1.5 ml of PBS (100 ml 50 mM phosphate buffer pH. 7.8 containing 0.1 mM EDTA) and homogenized as described for the determination above for MDA. One unit of SOD was defined as that amount of enzyme which inhibits the rate of cytochrome c reduction, under the

specified, by 50 %. To be able to extrapolated accurately to this value of 50% inhibit, one should use several dilutions of one enzyme solution. For relative activity measurements, the data were related to standard preparation utilizing a plot $1/\Delta E$ min^{-1} versus standard. Solution B (xanthine oxidase in 0.1 mM EDTA) is kept on ice. solution A (0.76 mg xanthine and 24.8 mg cytochrome c) was mixed with 100 ml 50 mM phosphate buffer) and kept at 25ºC. The processes in reading were as follows (1) pipet 2.9 ml of solution A into cuvette; (2) add 50 µl of sample or SOD standard; (3) start the reaction with 50 µl of solution B ; (4) after mixing record the absorbance at 550 nm; (5) plot $1/\Delta E$ min^{-1} derived from the linear part of the reaction versus concentration of SOD standard.

Calculation

Mean arterial blood pressure $= DP+\frac{1}{3}(PP)$

Glomerular filtration rate (GFR) $= \frac{U_{in} \times V}{P_{in}}$

Effective renal plasma flow (ERPF) $= \frac{U_{PAH} \times V}{P_{PAH}}$

Effective renal blood flow(ERBF) $= \frac{(ERPF)^{X}(100)}{(100\text{-}PCV)}$

Filtration fraction (FF) $= \frac{GFR \times 100}{ERPF}$

Urinary excretion of substance $= U \times V$

Fractional excretion of electrolyte (FEx) $= \frac{\left(U^{X} \frac{V}{P}^{X} 100 \right)}{GFR}$

$C_{osm} = \frac{U_{osm} \times V}{P_{osm}}$

$C_{H2O} = V - C_{osm}$

Parameters

1. Glomerular filtration rate (GFR)
2. Effective renal plasma flow (ERPF)
3. Effective renal blood flow (ERBF)
4. Mean arterial blood pressure (MABP)
5. Urine flow rate
6. Osmolar clearance
7. Fractional excretion of sodium, potassium and cholide
8. Urinary protein excretion
9. Urinary sodium, potassium, chloride excretion
10. Urinary malondialdehyde excretion
11. Urinary NAG/creatinine ratio
12. Hematocrit
13. Plasma creatinine
14. Plasma urea nitrogen
15. Plasma osmolarity
16. Plasma sodium, potassium and cholide
17. Kidney malondialdehyde
18. Kidney reduced glutathione
19. Kidney superoxide dismutase
20. Kidney catalase

Statistical analysis

Data were expressed as mean ± S.E. Paired t-test design used to compare data in the same group before and after treatment. The data among groups were compared with one-way ANOVA and post hoc analysis with Student Newman-Keuls method were used to compaire the data in all pairwises. Non- parametric method was used to compare same data between groups in which normality failed by one way ANOVA on ranks and Dunn for multiple pairwises. Differences between mean were considered significant at $p < 0.05$. The Sigma-Stat program was used for statistical analysis.

CHAPTER IV

RESULTS

Body weight

The daily body weight of rats in each group throughout the experimental periods are shown in figure 1. The percent decreases in body weight were found on day 12 in rats which received gentamicin plus EPO (group 3), gentamicin plus EPO and iron (group 4), gentamicin plus EPO iron and vitamin E (group 5) when compared with rats in group 1 (normal control group) and group 2 (gentamicin alone).

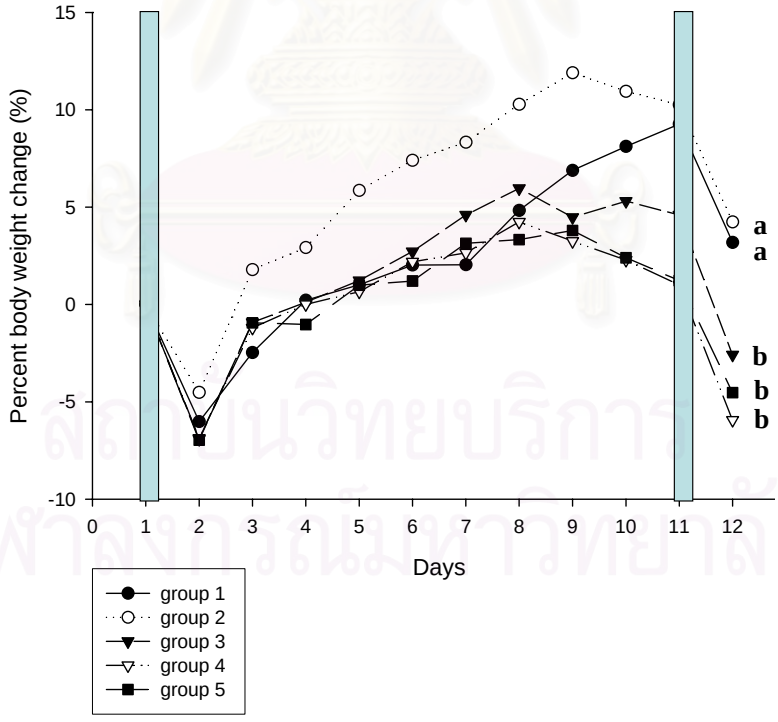


Figure 1. Daily percent change of body weight gain in five groups of rats.
The data are shown as percent change in body weight
[a,b] Means with different superscripts differ significantly ($p<0.05$)

Food intake

Food intake in each group are presented in figure 3. After injected gentamicin, food intakes in goup 2, 3, 4, and 5 were declined when compared with group 1.

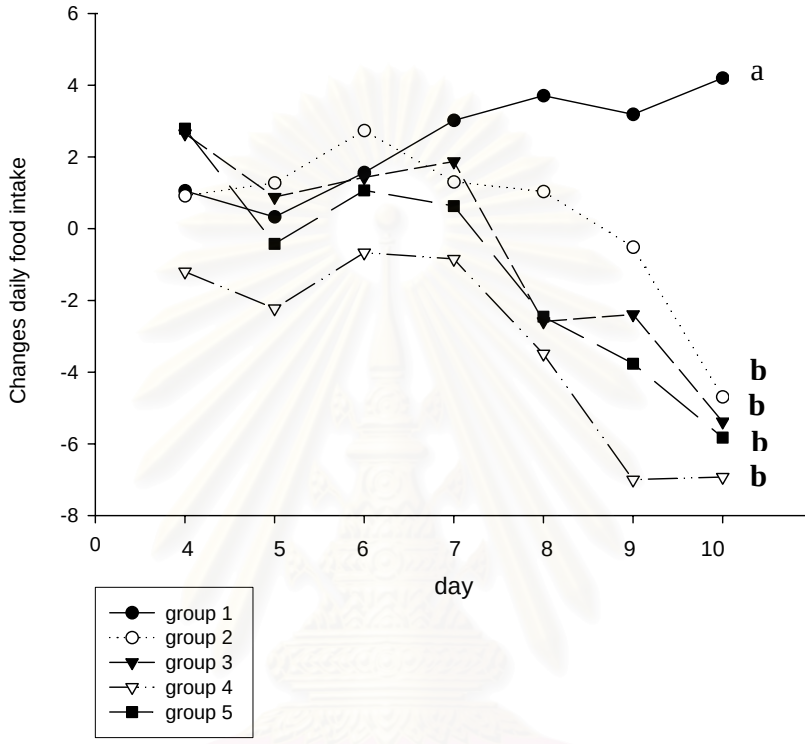


Figure 2. Daily change in mean values for food intake in five groups of rats.

[a,b] Means with different superscripts differ significantly ($p<0.05$)

Plasma creatinine and plasma urea nitrogen (BUN) concentrations

Results of plasma creatinine are shown in table1 and figure 3. By comparing between day 1 before treatment and day 11, group 3, 4, and 5 had significantly higher of the plasma creatinine concentration ($p< 0.01$). By comparing among groups, in group 3 and 4, which received EPO and EPO plus iron, the plasma creatinine concentration were significantly higher than that of control group ($p<0.05$). In rats which received vitamin E, the plasma creatinine concentration tended to be lower than group 3 and 4.

Table 1. Creatinine and BUN before and after treatment in each groups.

	Creatinine (mg%)		BUN (mg%)	
	Day 1	Day 11	Day 1	Day 11
group 1 (n=7)	0.814±0.194	0.557±0.057 ^{A}	21.20±2.62	19.11±1.73^{A}
group 2 (n=8)	0.663±0.084	1.287±0.232 ^{AB}	21.12±0.45	33.66±8.01$^{\dagger\dagger AB}$
group 3 (n=9)	0.611±0.077	2.100±0.252$^{\dagger\dagger B}$	20.46±1.276	53.45±7.27**^{B}
group 4 (n=9)	0.711±0.102	1.818±0.271**^{B}	22.400±0.884	50.36±7.69*^{B}
group 5 (n=9)	0.456±0.024	1.267±0.100$^{\dagger\dagger AB}$	21.11±1.25	39.36±3.04^{***AB}

The data are shown as mean ± S.E.

*= p<0.05 ; **= p<0.01; ***=p<0.001 significantly compared with before treatment using paired t-test

††=p<0.01 significant compared with before treatment using Wilcoxon Signed Rank test

^{A,B} Means with different superscripts differ significantly between groups (p<0.05) by using one way ANOVA on rank

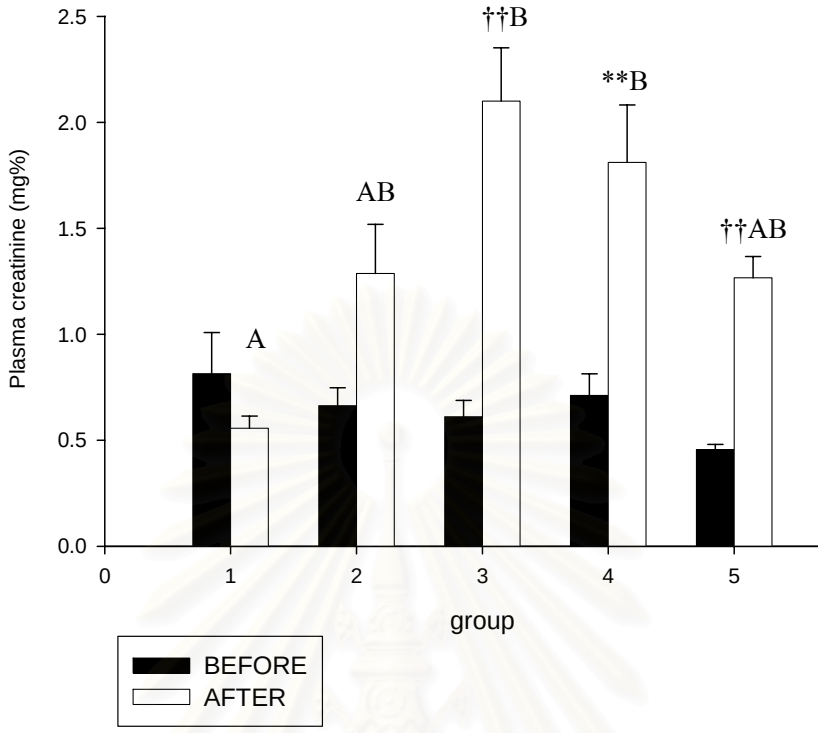


Figure 3. Mean values for the plasma creatinine concentration in five groups of rats before and after treatment.

The data are shown as mean ± S.E.

**= $p<0.01$ significantly compared with before treatment using paired t-test

††=$p<0.01$ significant compared with before treatment using Wilcoxon Signed rank test

A,B Means with different superscripts differ significantly between groups ($p<0.05$) by using one way ANOVA on rank

The plasma urea nitrogen concentrations are presented in table 1 and figure 4. The results of BUN concentrations in group 2, 3, 4 and 5 were significantly higher on day 11 compared to day 1. When comparing between groups, rats receiving EPO with and without iron had significantly higher BUN compared with normal control group ($p<0.01$ and $p< 0.05$, respectively).

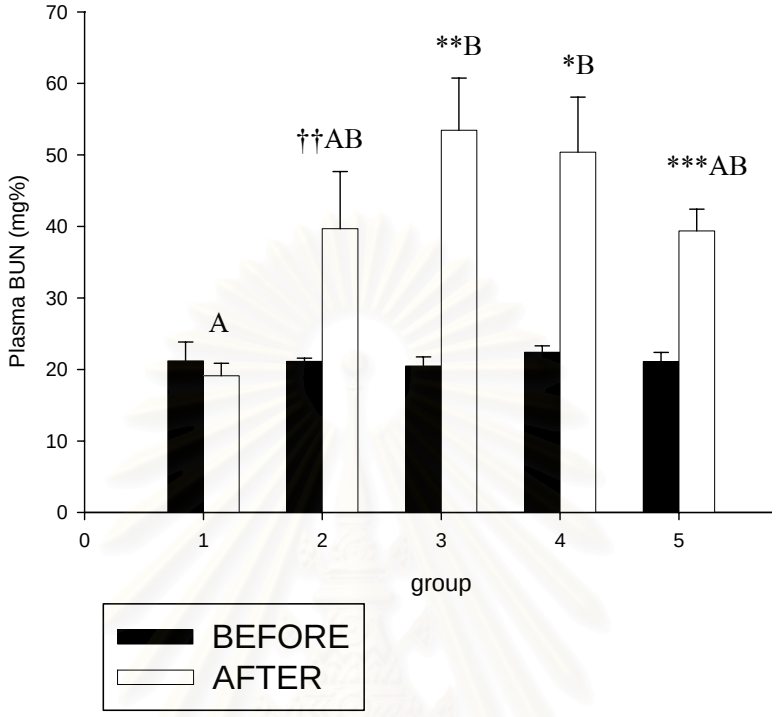


Figure 4. Mean values for BUN in five groups of rats before and after treatment

The data are shown as mean ± S.E.

*= p<0.05 ; **= p<0.01; ***=p<0.001 significantly compared with before treatment using paired t-test

††=p<0.01significant compared with before treatment using Wilcoxon Signed Rank test

^{A,B} Means with different superscripts differ significantly between groups (p<0.05) by using one way ANOVA on rank

Effects on renal hemodynamics

The values of the glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF), filtration fraction (FF), mean arterial blood pressure (MABP) and renal vascular resistance (RVR) in each group of rats are shown in table 2 and figure 5.

GFR was significantly lower in group 2, 3, 4 and 5 that received gentamicin, with and without other treatments. Group 3, 4 and 5 showed a similar reduction of GFR compared with group 2 which received gentamicin alone.

ERPF was markedly reduced in group 2,3,4 and 5. Although the differences were found only in group 3 and group 4. The percent reduction of ERPF was higher than GFR resulting in higher FF values in group 2,3 and group 4. The highest FF was found in group 3 which received EPO. Group 2, 3, and 4 had higher renal vascular resistance when compared with group 1. The ERPF in group 5 which received vitamin E in combination with gentamicin, EPO, and iron was higher than that of group 2. Thus, FF was decreased in group 5. The RVR in group 5 was lower than .group 2, 3, and 4. The values of mean arterial blood pressure were not different among groups (figure 6).

The pack cell volume (PCV) measuring on day 1 in group 1, 2, 3, 4 and 5 were 45.0$\pm$2.3%, 49.2$\pm$2.3%, 44.9$\pm$1.1%, 44.4$\pm$1.2%, 42.9$\pm$0.7% respectively. When measuring PCV on day 11, PCV in group 1, 2, 3, 4 and 5 were 41.8$\pm$1.6%,39.1$\pm$0.7%, 47.7$\pm$2.1%, 44.1$\pm$1.9%, 44.3$\pm$0.9% respectively. Only group 2 had lower PCV significantly compared with day 1 ($p<0.05$). By comparing among groups on day 11, PCV in group 2 was significantly lower than group 3.

Table 2. Glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF), filtration fraction(FF), mean arterial blood pressure (MABP) and renal vascular resistance (RVR)in all groups.

	GFR (µl/g/min)	ERPF (µl/g/min)	ERBF (µl/g/min)	FF (%)	MABP (mmHg)	RVR ($mmHg/\mu l.g^{-1}.min^{-1}$)
group 1(n=7)	3.221 ± 0.444^{a}	17.97 ± 2.54^{A}	28.75 ± 5.71	17.13 ± 1.21	86.86 ± 5.35	3.149 ± 0.591^{A}
group 2(n=8)	1.564 ± 0.263^{b}	9.18 ± 2.17^{A}	16.76 ± 3.99	24.47 ± 5.00	86.36 ± 3.14	11.086 ± 4.431^{AB}
group 3(n=9)	1.016 ± 0.195^{b}	6.02 ± 2.01^{B}	12.16 ± 4.08	25.51 ± 3.85	92.54 ± 2.36	22.387 ± 8.638^{B}
group 4(n=9)	1.261 ± 0.297^{b}	6.93 ± 1.99^{B}	14.02 ± 3.99	23.88 ± 4.88	94.87 ± 6.79	13.439 ± 3.124^{AB}
group 5(n=9)	1.497 ± 0.178^{b}	12.26 ± 2.17^{A}	24.97 ± 4.24	14.88 ± 2.01	91.90 ± 4.05	5.617 ± 1.484^{AB}

The data are shown as mean ± S.E.

^{a,b} Means with different superscripts differ significantly ($p<0.05$)

^{A,B} Means with different superscripts differ significantly between groups ($p<0.05$) by using one way ANOVA on rank

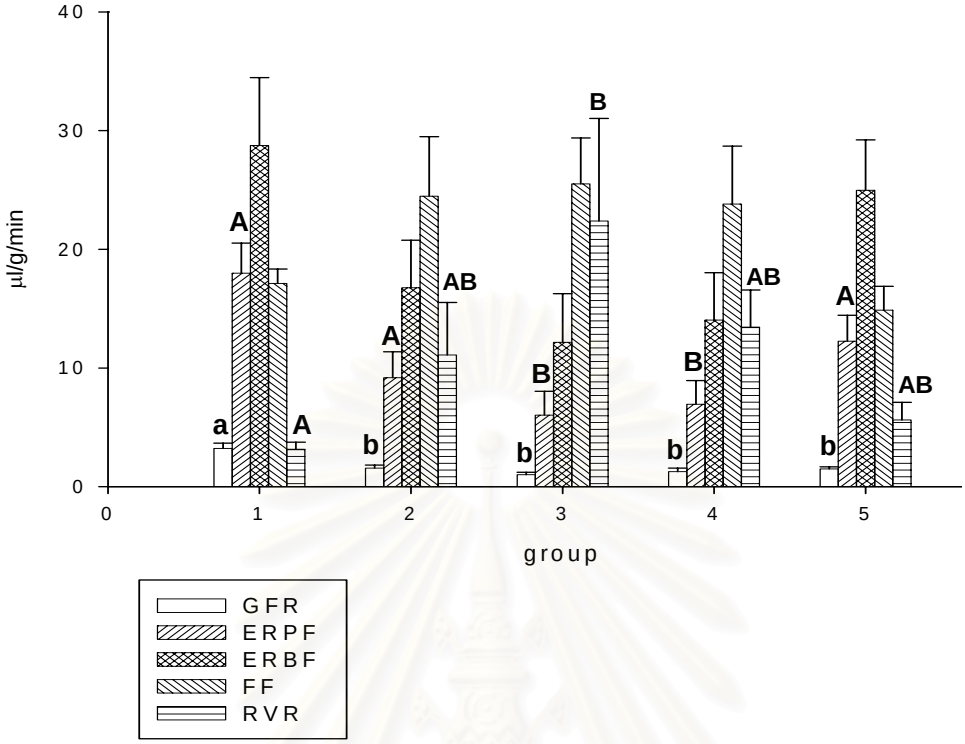


Figure 5. Mean values of glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF), filtration fraction(FF), renal vascular resistance (RVR) in five groups.

The data are shown as mean $\pm$ S.E.

[a,b] Means with different superscripts differ significantly between groups ($p<0.05$)

[A,B] Means with different superscripts differ significantly between groups ($p<0.05$) by using one way ANOVA on rank

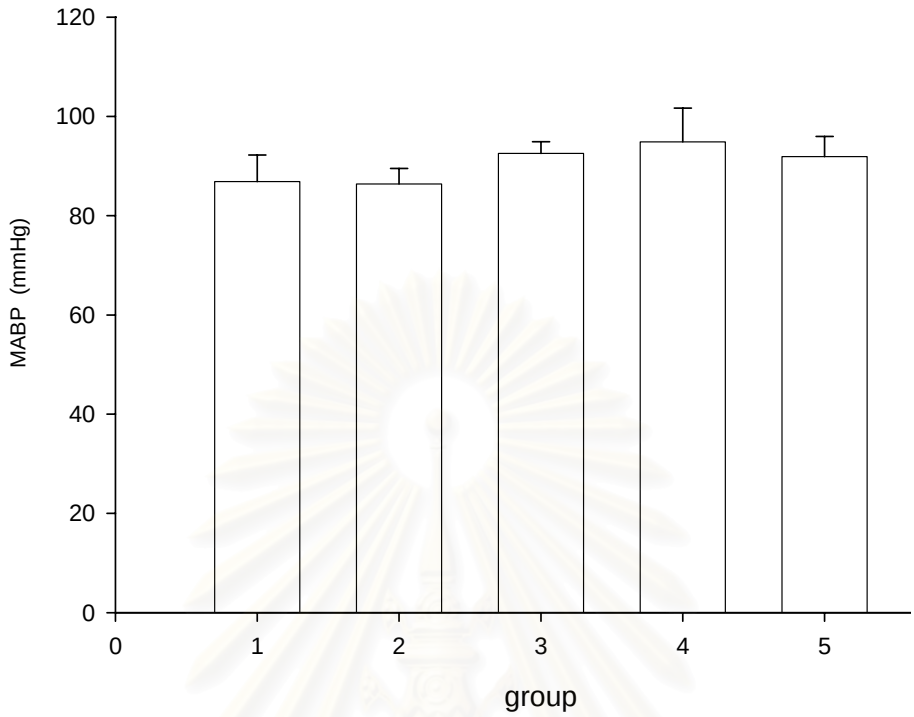


Figure 6. Mean values of mean arterial blood pressure in five groups

The data are shown as mean ± S.E.

Plasma electrolyte concentrations in all groups at day 12 of treatment

The plasma electrolyte concentrations (Na^+, K^+, Cl^-) and plasma osmolarity at day 12 of treatment are presented in table 3. No significant differences were found in plasma (Na^+, K^+, Cl^-) and osmolarity in all groups.

Table 3 . The plasma electrolyte concentrations in all groups at day 12 of treatment

Plasma	Na(mEq/L)	K(mEq/L)	Cl(mEq/L)	Osm(mOsm/L)
group1	130.0 ±6.5 (n=16)	5.31±0.22 (n=16)	105.6±1.3 (n=16)	294.5±10.1 (n=16)
group 2	143.1 ±10.7 (n=14)	5.61±0.52 (n=14)	103.9±1.5 (n=14)	315.0 ±5.7 (n=14)
group 3	133.2 ±16.3 (n=11)	5.34±0.41 (n=9)	102.8±2.0 (n=11)	311.8±5.3 (n=11)
group 4	142.3±8.9 (n=15)	5.30±0.31 (n=15)	102.0±1.5 (n=15)	312.9±4.7 (n=15)
group 5	134.6± 10.9 (n=14)	5.07±0.44 (n=14)	102.0±1.4 (n=14)	313.5±5.4 (n=14)

The data are shown as mean ± S.E.

Urine flow rate, fractional electrolyte excretion(Na^+, K^+, Cl^-), osmolar clearance (C_{osm}) and free water clearance (C_{H2O})

Urine flow rate, fractional excretion of electrolytes (Na^+, K^+, Cl^-), osmolar clearance (C_{osm}) and free water clearance (C_{H2O}) are presented in table 4 figure 7.

Rats in group 2, 3 ,4, and 5 which received gentamicin with and without any other drugs had significantly higher of the urine flow rate as compared with group 1.

At day 12, rats in group 3, 4, and 5 caused significant increased in both fractional excretion of Na^+ and K^+ as compared with normal control group (table 4 figure 8). There were no significant differences of fractional excretion of Cl^- and C_{osm}

among groups. The values of free water clearance were lower in group 2,3,4 and 5 ($p<0.05$) in comparison with that of group 1.

Table 4. Urine flow rate, fractional excretion (Na^+, K^+, Cl^-), osmolar clearance (C_{osm}) and free water clearance (C_{H2O})

Parameter	group1 (n=14)	group 2 (n=14)	group 3 (n=8)	group 4 (n=13)	group 5 (n=14)
Urine flow rate (ml/day)	14.91±2.19 [a]	26.42±2.65 [b]	29.86±3.06 [b]	22.11±2.64 [b]	27.07±2.30 [b]
FE Na^+ (%)	3.37±0.49 [A]	6.56±1.20 [AB]	12.18±1.53 [B]	9.46±1.38 [B]	11.07± 1.46 [B]
FE K^+ (%)	2.64±0.31 [A]	6.48±1.12 [AB]	13.14±2.19 [B]	9.69± 1.30 [B]	10.61±1.29 [B]
FE Cl^- (%)	0.36±0.06	0.37±0.07	0.54±0.08	0.35±0.05	0.60±0.25 [B]
C_{osm} (ml/day)	40.96±4.59	42.74±2.90	40.63±2.9	34.10±2.03	37.22±2.97
C_{H2O} (ml/day)	-26.04±3.74 [a]	-16.13±3.59 [b]	-10.76±2.84 [b]	-11.99±3.24 [b]	-10.14±3.25 [b]

The data are shown as mean ± S.E

[a,b] Means with different superscripts differ significantly ($p<0.05$)

[A,B] Means with different superscripts differ significantly between groups ($p<0.05$) by using one way ANOVA on rank

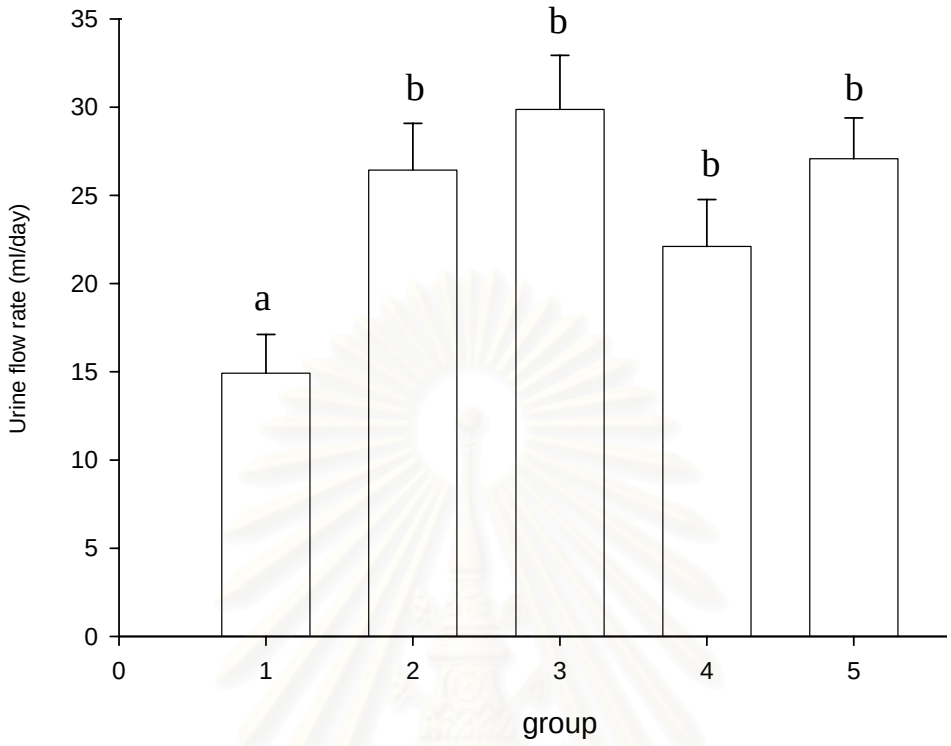


Figure 7. Mean values of urine flow rate measured at day 11 in metabolic cage. The data are shown as mean ± S.E.

[a,b] Means with different superscripts differ significantly ($p<0.05$)

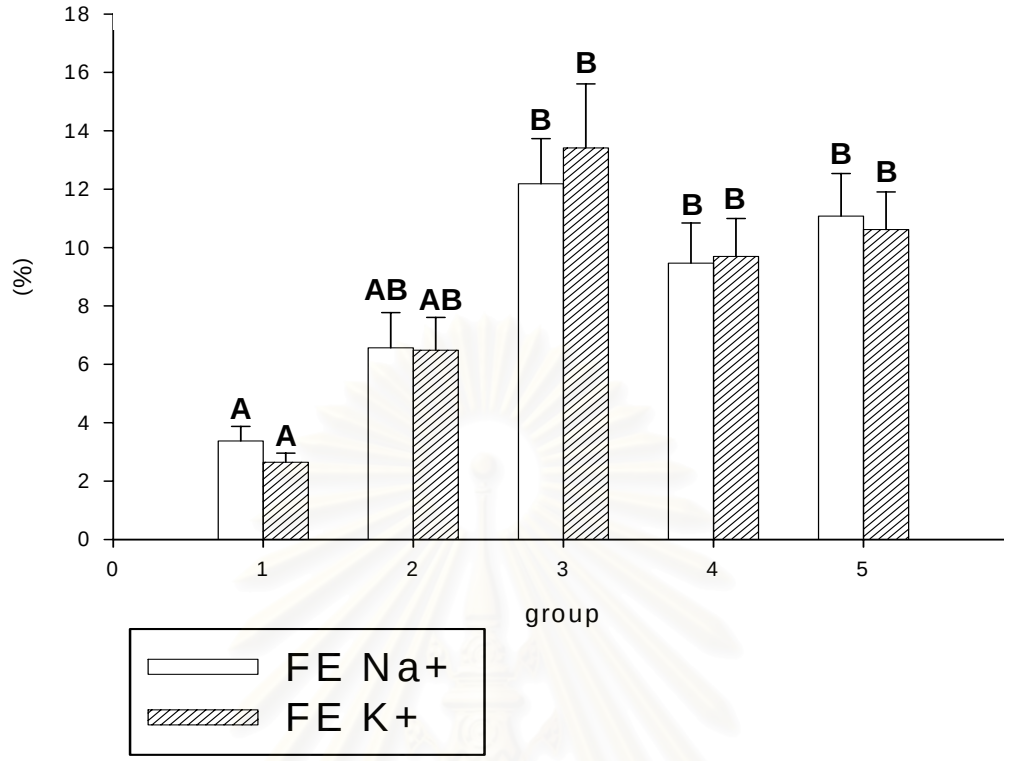


Figure 8. Mean values of Fractional excretion of Na^+, K^+ in five groups

The data are shown as mean ± S.E.

^{A,B} Means with different superscripts differ significantly between groups ($p<0.05$) by using one way ANOVA on rank

Urinary protein excretion and **urinary protein creatinine (UPC) rati**o

Urinary protein excretions and UPC ratio before and after treatment in each groups of rats are presented in table 5 and figure 9 and 10. After treatment on day 11, both urinary protein excretion and UPC ratio were higher significantly in group 2, 3, 4 and 5 compared with before treatment ($p<0.001$). When comparing among groups, group 2 ,3 ,4 and group 5 had higher protein excretion and UPC ratio compared with control groups.

Table 5. Urinary protein excretion and urinary protein creatinine ratio before and after treatment in each groups

	Urinary protein excretion (mg/day)		**UPC ratio**	
	DAY 1	**DAY 11**	**DAY 1**	**DAY 11**
group1 (n=14)	1.992±0.442	1.145± 0.159^{A}	0.181±0.032	0.138±0.016 ^{A}
group2 (n=16)	1.597±0.224	6.488±0.499 ^{***B}	0.141±0.018	0.568±0.041^{***B}
group3 (n=16)	1.460±0.192	7.977±0.137 ^{***B}	0.150±0.016	0.766±0.037^{***B}
group4 (n=13)	2.020±0.491	7.176±0.773$^{\dagger\dagger\dagger B}$	0.179±0.020	0.721±0.078$^{*** B}$
group5 (n=14)	1.390±0.131	8.067±0.898 ^{***B}	0.155±0.011	0.805±0.041^{***B}

The data are shown as mean ± S.E.

***=p<0.001 significantly compared with before treatment using paired t-test

†††=p<0.001 significant compared with before treatment using Wilcoxson Sign Rank test

^{A,B} Means with different superscripts differ significantly between groups (p<0.05) by using one way ANOVA on rank

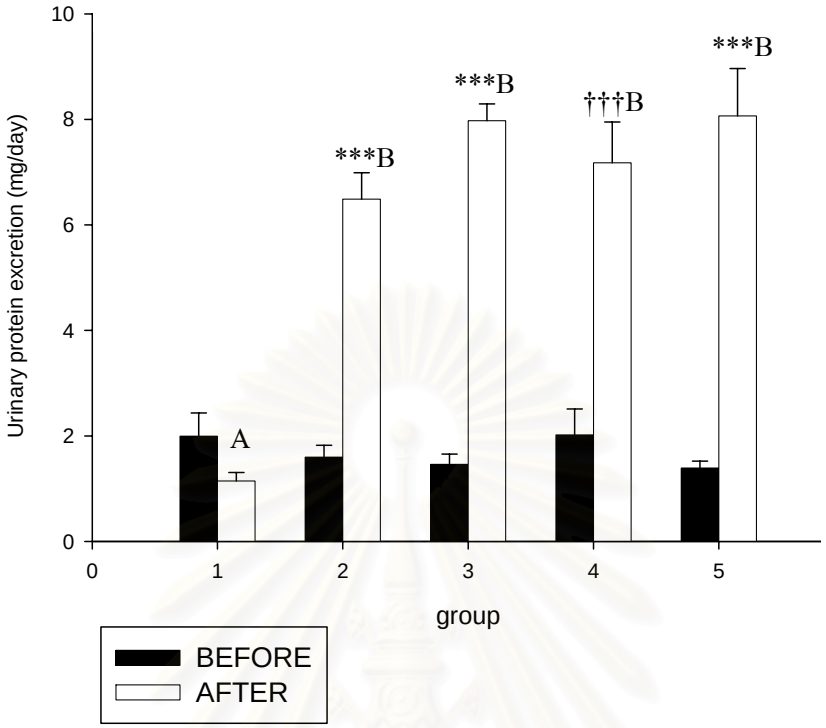


Figure 9. Mean values of urinary protein excretion in five groups

The data are shown as mean ± S.E.

***=p<0.001 significantly compared with before treatment using paired t-test

†††=p<0.001 significant compared with before treatment using wilcoxson

^{A,B} Means with different superscripts differ significantly between groups (p<0.05) by using one way ANOVA on rank

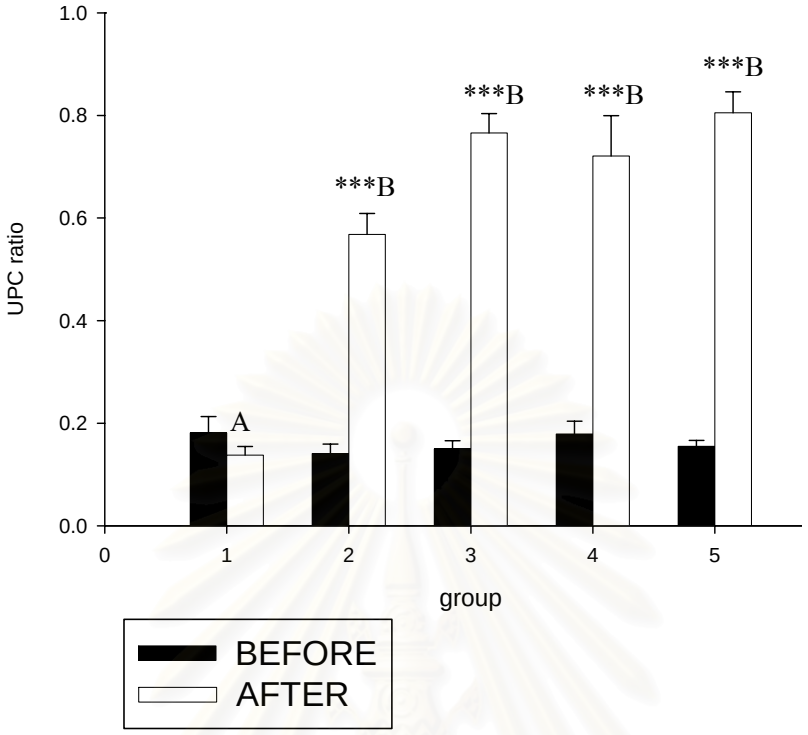


Figure 10. Mean values of urinary protein creatinine (UPC) ratio in five groups

***=p<0.001 significantly compared with before treatment using paired t-test

†††=p<0.001 significant compared with before treatment using Wilcoxson Signed Rank test

^{A,B} Means with different superscripts differ significantly between groups (p<0.05) by using one way ANOVA on rank

Effect of gentamicin, EPO, iron and vitamin E on urinary electrolyte excretions

Electrolyte excretions at day 1 and day 11 of treatment in each group of rats are presented in table 6. On day 11, urinary excretion of Na^+ and K^+ were higher significant in group 3, 4, and 5 compared with day 1. When comparing among groups on day 11, urinary excretion of K^+ significantly higher in group 2,3,4 and 5 compared with group 1. Urinary excretion of Na^+ was higher significantly in group 2 and group 3. However, urinary excretion of Cl^- in group 4 was significantly lower than group 1 and also lower than before treatment (table 6).

Table 6. Urinary excretion of the electrolytes **(**(Na^+, K^+, Cl^-) on day 1 and day 11 of all groups**.**

	Day 1			**Day 11**		
	UNa*V (mEq/day)	**UK*V (mEq/day)**	**UCl*V (mEq/day)**	**UNa*V (mEq/day)**	**UK*V (mEq/day)**	**UCl*V (mEq/day)**
Group 1(n=14)	8.344±1.490^{A}	0.330±0.06	0.750±0.117	6.511±0.920^{a}	0.213±0.026 ^{a}	0.563±0.093 ^{A}
Group 2(n=14)	8.561±0.904 ^{A}	0.333±0.033	0.730±0.080	9.351±0.852^{b}	0.355±0.032 ^{b}	0.418±0.046**^{A}
Group 3(n=8)	7.441±0.742 ^{A}	0.241±0.002	0.558±0.067	9.858±0.551*^{b}	0.379±0.020 ***^{b}	0.345±0.060*^{AB}
Group 4(n=13)	6.353±0.271 ^{A}	0.238±0.008	0.579±0.049	8.218±0.535 **^{ab}	0.315±0.019 ***^{b}	0.212±0.023*** ^{BC}
Group 5(n=14)	6.743±0.594 ^{A}	0.246±0.018	0.533±0.055	8.815±0.746$^{\dagger ab}$	0.325±0.027** ^{b}	0.289±0.037*** ^{AC}

The data are shown as mean ± S.E.

*= p<0.05 ; **=p<0.01 ; ***=p<0.001 significantly compared with before treatment using paired t-test

†= p<0.05 significantly compared with before treatment using paired t-test

^{a,b} Means with different superscripts differ significantly (p<0.05)

^{A,B} Means with different superscripts differ significantly between groups (p<0.05) by using one way ANOVA on rank

Effect of gentamicin, EPO, iron and vitamin E on urinary malondialdehyde (MDA) excretion.

The urinary MDA excretions on day 1 in group 1, 2, 3, 4, and 5 were 448.4 ± 86.9, 580.8 ± 80, 375.7 ± 42.6, 643.8 ± 94.3 and 475.2 ± 61.4 respectively. By measuring urinary MDA excretion were similar to day 1 (280.3 ± 41.6, 733 ± 129.8, 315.6 ± 42.4, 553.0 ± 69.6 and 525 ± 86.1 respectively). Also, no significant differences were found among groups both on day 1 and day 11.

Effect of gentamicin, EPO, iron and vitamin E on kidney malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD)

There was no significant change of kidney malondialdehyde in all groups (table 7). The highest value of kidney GSH was found in group 2 compared with other groups ($p<0.05$) (table 7 and figure 11). The GSH in group 3, 4 and 5 that received EPO showed lower GSH compared with group 2. Catalase was significantly higher in group 2, 3, 4 and 5 compared with group 1 (figure 12). The SOD was significantly higher in group 3,4 compared with group 1and 2. However, the SOD was lower in group 5 compared with group 3 and 4.

Table 7. malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) in kidney on day 12 of treatment.

	MDA (nmol/mg protein)	**GSH** (nmol/mg protein)	**CAT** (unit/mg protein)	**SOD** (unit/mg protein)
group 1	1.272±0.131 (n=6)	34.3677.469 ^{A} (n=7)	38.691±5.829 ^{a} (n=6)	121.028±5.813 ^{a} (n=6)
group 2	1.090±0.0969 (n=8)	79.507±2.639 ^{B} (n=6)	56.172±2.491 ^{b} (n=7)	145.339±8.449 ^{a} (n=8)
group 3	0.886±0.0698 (n=8)	36.977±2.941 ^{AB} (n=8)	54.332±2.584 ^{b} (n=8)	162.120±8.285 ^{b} (n=8)
group 4	1.219±0.162 (n=6)	38.370±2.963 ^{AB} (n=6)	53.042±2.308 ^{b} (n=6)	164.603±12.666 ^{b} (n=6)
group 5	1.218±0.178 (n=6)	29.021±2.723 ^{A} (n=6)	53.667±2.015 ^{b} (n=6)	144.20±15.510 ^{a} (n=6)

The data are shown as mean ± S.E.

^{a,b} Means in the same column with different superscripts differ significantly ($p < 0.05$)

^{A,B} Means with different superscripts differ significantly between groups ($p<0.05$) by using one way ANOVA on rank

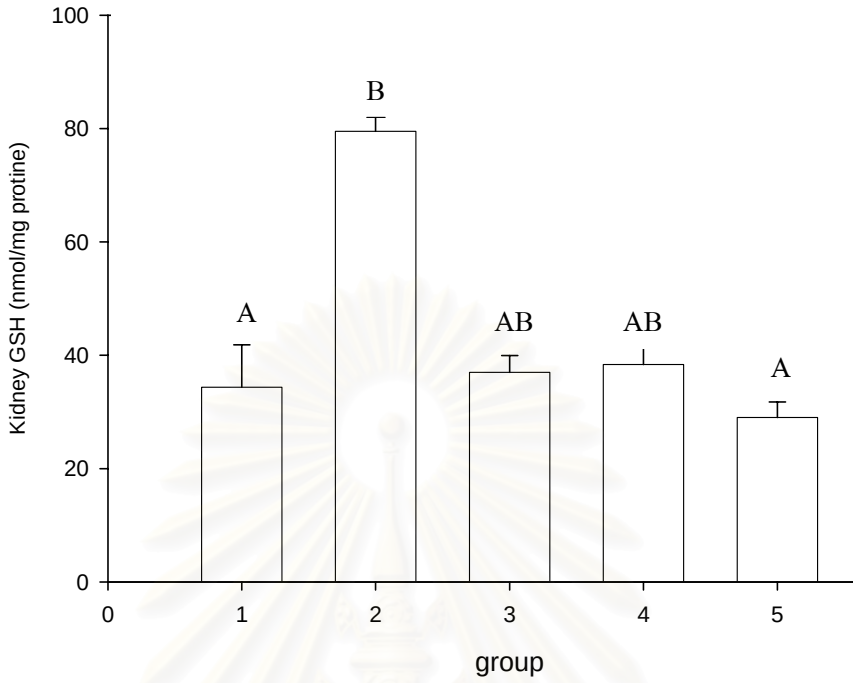


Figure 11. Mean values of kidney GSH activity in five groups

The data are shown as mean ± S.E.

^{A,B} Means with different superscripts differ significantly between groups ($p<0.05$) by using one way ANOVA on rank

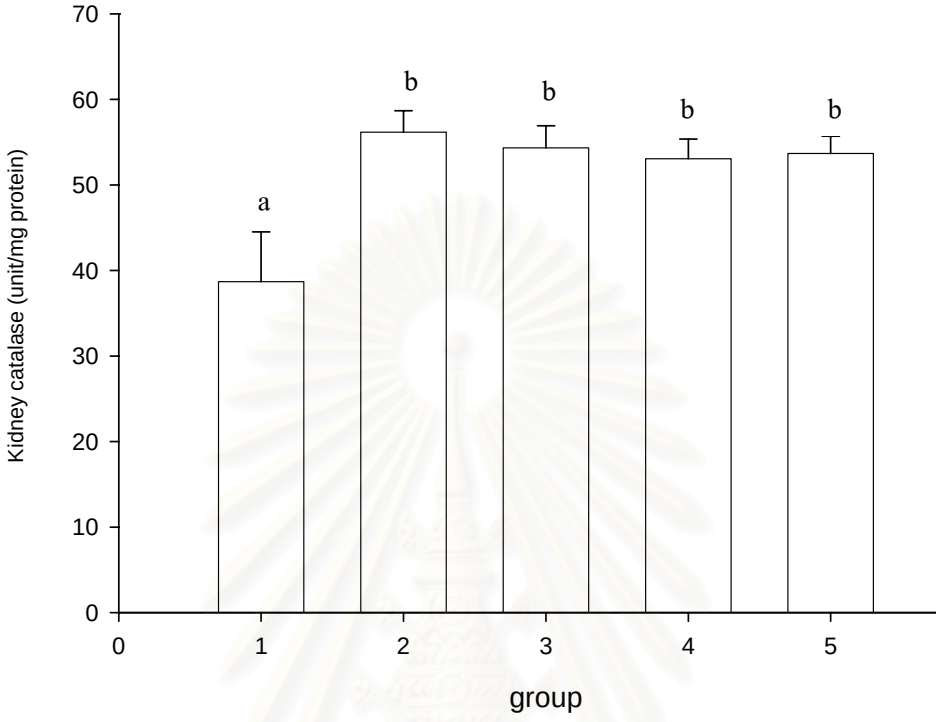


Figure 12. Mean values of kidney catalase activity in five groups

The data are shown as mean ± S.E.

[a,b] Means with different superscripts differ significantly ($p<0.05$)

Effect of gentamicin, EPO, iron and vitamin E on urinary NAG/creatinine

Urinary NAG/creatinine in urine in day 12 after treatment in each group of rats are presented in table 8 and figure 13. The urinary NAG/creatinine ratio was significantly increased in groups 2, 3, 4 and 5 compared with group 1.

Table 8. Urinary N acetyl-β-D glucosaminidase (NAG)/creatinine on day 12 of treatment

	U NAG/creatinine (milliunit/mg creatinine)
group 1 (n=6)	1.08 ± 2.20 [a]
group 2 (n=7)	25.87 ± 4.58 [b]
group 3 (n=8)	29.90 ± 2.34 [b]
group 4 (n=10)	21.73 ± 2.50 [b]
group 5 (n=10)	29.89 ± 3.52 [b]

The data are shown as mean ± S.E.

[a,b] Means with different superscripts differ significantly ($p<0.05$)

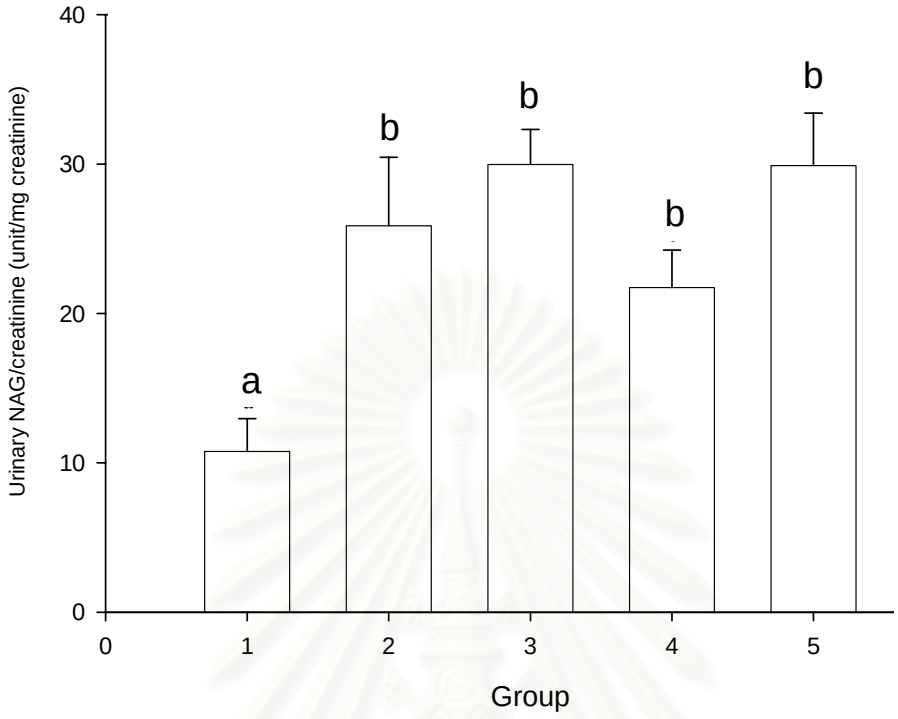


Figure 13. Mean values of urinary NAG in five groups

The data are shown as mean ± S.E..

[a,b] Means with different superscripts differ significantly ($p<0.05$)

CHAPTER V

DISCUSSION

Gentamicin is valuable therapeutic agent for gram negative bacteria infections. Gentamicin induced nephrotoxicity is characterized by direct acute tubular necrosis, primarily localized to the proximal tubule (Silan et al., 2007 , Cuzzocrea et al., 2002). It caused nephrotoxicity which characterized by decreasing in the glomerular filtration rate (GFR), effective renal plasma flow (ERPF) with increasing in blood urea nitrogen (BUN) and creatinine concentration. Morover, the tubular damage was demonstrated by decreased urine concentration capacity, proteinuria, enzymuria, and ultrastructural alteration of glomerular and tubular cell (Cojocel et al., 1984). In addition, gentamicin has been shown to enhance the generation of reactive oxygen species (ROS) which invoked as a pathway for renal injury (Karl et al., 2000).

In the present, the alteration of hemodynamic associated with disproportional less decreased in GFR than in renal plasma flow was found in group 2, 3, and 4.. Therefore, the filtration fraction tended to be increased resulting in glomerular hyperfiltration. The renal efferent arteriole vasoconstriction is suggested. The possible mechanisms of vasoconstriction by gentamicin are stimulating the releasing of vasoconstrictors from activated renin angiotensin system, increasing renal endothelin content or inhibiting the production of vasodilatory prostaglandin PGE2 (Hishida et al., 1994, Assael et al., 1985). Similary, Thurau et al (1976) suggested that gentamicin cause a tubuloglomerular feedback resulting in the alterations of glomerular function. These were mediated by renin angiotensin system which related to pathogenesis of acute renal failure. In addition, Baylis and co-worker (1977)

studied pathological mechanisms responsible for impairment of glomerular filtration rate in gentamicin mediated nephropathy. They found many abnormalities of renal circulation such as renal vasoconstriction and reduction of glomerular capillary ultrafiltration coefficient (Kf) resulting in reduced GFR. In this study, the reduction of GFR did not cause hypertension as shown by many models of renal failure.

Gentamicin damaged tubular cells causing acute tubular necrosis. Increased urinary excretions of Na^+, K^+, NAG were also found in gentamicin treated rats. It might be a result of impaired reabsorbtion of tubular cells. Gentamicin was potent on decreasing tubular reabsorbtion of lysozyme and induced damage to cellular membrane causing leakage of the lysozomal marker enzyme NAG in the urine (Cojocel et al., 1984). Cojocel et al (1983) demonstrated that the polycationic aminoglycosides may affect several processes involved in renal handling of lysozyme including glomerular permeability, tubular reabsorption and intracellular proteolytic degradation. Increased protein excretion in gentamicin treated rats which found in the present study was due to the damaging of basement membrane and the tubular necrosis. Proteinuria of glomerular and/or tubular origin is an index of gentamicin nephrotoxicity (Cojocel et al., 1984).

Oxygen free radicals are considered to be important mediators of gentamicin-mediated nephrotoxicity. Sequential reduction of oxygen along the univalent pathway leads to generation of superoxide anion, hydrogen peroxide, hydroxyl radical and water. Several studied have shown that partially reduced oxygen metabolites are important mediators of gentamicin nephrotoxicity (Walker et al., 1999). Gentamicin has been shown to enhance the generation of superoxide anion and hydrogen peroxide and the superoxide anion can cause renal vasoconstriction (Nakajima et al., 1994). The same study investigated the potential role of superoxide in gentamicin induced

renal toxicity by using M40403, a super oxide dismutase mimetic. They suggested that administration of M40403 attenuated the effect of superoxide induced by gentamicin (Cuzzocrea et al., 2002).Thus, it is possible that superoxide is one factor which may involve in renal failure induced by gentamicin. Morover, it is also possible that renal failure from oxygen depletion may increase oxidative stress. The present demonstrated that glutathione and antioxidant enzymes activity were increased after gentamicin injection. However, the effects of oxidative stress may not be severe because no changes of kidney MDA and urinary MDA excretion were found. Similary, the experiment of Fauconneau and coworker (1995) showed no modification of thiobarbituric acid-reagent substance (TBAR) in rats received gentamicin. Also, In 2002, Saldago found gentamicin induced increase in O^{-}_{2} production and SOD activity but did not induce detectable changes in membrane fluidity and lipid peroxidation. In contrast, Ramsammry (1985) reported that gentamicin injection at the dose of 100 mg/kg/day for 1-4 day induced lipid peroxidation in rat renal cortex. Yamada (1995) was also found increasing of renal MDA after administration of gentamicin at a dose of 120 mg/kg subcutaneously for 7 consecutive days. He was suggested that lipid peroxidation played a possible role in GM-induced renal damage. The differences in the result of many investigators may involve the differences in experimental designs of dose and duration of gentamicin administration.

When rats received EPO and gentamicin, the PCV was increased. However, EPO did not improve but further reduce renal plasma flow and GFR. Hyperfiltration was still maintained in this group. Increased BUN and creatinine after EPO treatment without elevated blood pressure were found. The mechanism of increasing in renal vascular resistance (RVR) was resulted from endothelin-mediated vasoconstriction by

EPO receptor on the surface of vascular endothelial cell of the renal circulation (Slowinski et al., 2002). Moreover, RVR may be high due to blood viscosity after PCV was elevated. Previous report showed that EPO can also worsen glomerular injury and promoted hypertension in rats (Garcia et al.,1988). This study did not demonstrated hypertensions induced by EPO which may be due to high urinary and fractional excretions of Na^+ and K^+.

The possible mechanism of EPO is promotes natriuresis and diuresis in the renal tubule by activated tubular endothelium system. (Malex et al., 1999). Endothelin-1(ET-1) inhibit the activity of the epithelial sodium channel via ET_β receptor, leading to a decrease in sodium and water reabsorbtion in the renal tubule (Ohuchi et al., 2000 , Hocher et al., 2001). EPO can accelerate the development of proteinuria and glomerular sclerosis (Garcia et al., 1988). From the results, EPO did not have a cytoprotective effect on experimental gentamicin-induced nephrotoxicity.

Rats receiving gentamicin plus EPO showed increased SOD and decreased GSH while MDA was unaltered. The previous study showed that EPO decreased the thiobarbituric acid and also increased glutathione peroxidase activity in hypoxic-ischemic brain injury in neonatal rats (Kumral et al., 2005). EPO may affect the oxidative stress by correcting of anemia but may not help improve renal function.

In group 4, renal hemodynamics was similar to group 2 and group 3. The BUN was still high with high protein excretion. The urinary excretion of electrolyte (Na^+ and K^+) were high similar to group 3, 4, and 5. oxidative stress maker, GSH was lower than group 2 while CAT and SOD were elevated than control group. The result suggested that oxidative stress was existed in group 4 but it did not involve in renal hemodynamic or tubular impairment. Moreover, iron did not aggravate renal dysfunction. However, Zager et al (2004) demonstrated that iron sucrose had the most

renal tubular toxicity, while iron dextran caused the highest oxidative stress. Acute iron overload can increased thiobarbituric acid (TBARS) decreased SOD and catalase activity, without any effects in glutathione activity. Moreover, vitamin E was effective in controlling iron dextran induced radical generation in the kidney (Galleano et al., 1994). Argawal et al (2004) suggested that intravenous iron produced oxidative stress and had direct cytotoxic effect on renal tubular function by increasing urinary excretion of protein and MDA. However, no alteration of MDA was found in the present study.

The present study demonstrated that vitamin E did not improve GFR but increased ERPF resulting in the decreased in FF. This means that vasodilation may occur because of decrease in RVR. The BUN and creatinine concentrations was declined compared with group 2, 3, and 4.The mechanism of vasodilation may be mediated via prostanoids, which is a vasodilator. Accordingly, Wu et al (2005) studied the effect of vitamin E on vasodilator. They suggested that vitamin E induced increase in PGI_2 and PGE_2 production which may contributed to its beneficial effect in preserving endothelial function.

Abdel-Naim and co-worker (1999) showed that vitamin E pretreatment before gentamicin could lower BUN and creatinine. The results were similar to this study but the mechanism was remained unclear

The present work demonstrates that vitamin E did not improve tubular function. The urinary protein and electrolyte excretion was still high. There was no change in kidney MDA level. Kidney GSH was lowest in this group while kidney catalase was similar to group 2, 3 and 4.No remarkable changes of oxidative status was found.

Previous studied have shown that dietary Vitamin E reduced generation of superoxide, and protected against oxidative damage by reducing level of superoxide (IBrahim and Chow, 2005). Ramsammy and coworker (1987) have studied the effect of vitamin E on lipid peroxidation induced by gentamicin. Concurrent treatment of rats with vitamin E plus gentamicin for six days cauesd no alteration of renal MDA, SOD, CAT and glutathione. However, in rats with pretreatment with vitamin E for six days prior gentamicin treatment, the declined in renal cortical MDA, GSH, SOD and CAT were found. However, treatments of vitamin E did not prevent the gentamicin-induced rise of serum creatinine or reduced severity of proximal tubular cell lesion and necrosis.

In conclusion, the present study demonstrates that gentamicin has direct effect on hemodynamic, tubular function and oxidative stress by ameliorated GFR and ERPF, increased urine flow rate, urinary excretion of sodium and potassium and lysosomal enzyme (NAG). There were significant increases in antioxidant enzyme activities. EPO administration did not improve renal function. When supplement iron together with gentamicin and EPO, there was no change in kidney function and oxidative stress compared with rats receiving EPO alone. In last group, in which vitamin E was added. Increase in ERPF was demonstrated . In addition, vitamin E did not correct tubular damage. Thus, vitamin E may increase renal plasma flow and slow renal dysfunction in rats receiving gentamicin, EPO, and iron altogether.

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REFERENCES

Abdel-Naim, A.B., Abdel-Wahab, M.H. and Attia, F.F. 1999. Protective effects of vitamin E and probucol against gentamicin-induced nephrotoxicity in rats. Pharmacol. Res. 40: 183-187.

Abdelrahman, M., Sharples, E.J., McDonald, M.C., Collin, M., Patel, N.S., Yaqoob, M.M., and Thiemermann, C., 2004. Erythropoietin attenuates the tissue injury associated with hemorrhagic shock and myocardial ischemia. Shock. 22: 63-9

Afzali, B. and Goldsmith, D.J. 2004. Intravenous iron therapy in renal failure : friend and foe?. J. Nephrol. 17(4) : 487-95.

Agarwal, R., Vasavada, N., Sachs, N.G., and Chase, S. 2004. Oxidative stress and renal injury with intravenous iron in patients with chronic kidney disease. Kidney Int. 65: 2279-2289.

Ali, B.H., Abdel-Naim,A.B., Gayoum, A.A., and Bashir, A.A. 1992. Gentamicin nephrotoxicity in rats : Biochemical correlates. Pharmacol. Toxicol. 70: 419-423.

Ali, B.H. 1995. Gentamicin nephrotoxicity in humans and animals: Some recent research. Gen. Pharmac. 26: 1477-1487.

Ali, B.H. 2003. Agents ameliorating or augmenting experimental gentamicin nephrotoxicity : some recent research. Food Chem. Toxicol. 41: 1447-1452.

Amstrong, D. 1998. Free radical and antioxidant protocol. Newjersy : 299-310.

Assael, B. M., C. Chiabrando, L. Gagliardi, A. Noseda, F. Bamonte, and M. Salmona. 1985. Prostaglandins and aminoglycoside nephrotoxicity. Toxicol. Appl. Pharmacol. 78: 386-394.

Bagnis, C., Beaufils, H., Jacquiaud, C., Adabra, Y., Jouanneau, C., Le Nahour, G., Jaudon, M.C., Bourbouze, R., Jacobs, C., and Deray, G. 2001. Erythropoietin enhances recovery after cisplatin-induced acute renal failure in rat. Nephrol. Dial. Transplant. 16(5): 932-938.

Bahlmann, F.H., Song, R. and Boehm, S.M. 2004. Low-dose therapy with the long acting erythropoietin analogue darbEPOietin alpha persistenly activates endothelial Akt and attenuates progressive organ failure. Ciculation. 110: 1006-12.

Baylis, C. 1980 . The mechanism of decline in glowmerular filtration rate in gentamicin-induced acute renal failure in rat. J. Antimicrob Chemother. 6 : 381-388.

Baylis, C., Rennke, H.R. and Brenner, B.M. 1977. Mechanisms of defect in glomerular ultrafiltration associated with gentamicin administration. Kidney Int. 12: 344-353.

Begg, E.J., and Barclay, M.L. 1995. Aminoglycosides . Br.J. Clin. Pharmacol. 39 : 597-603.

Beutler, E., Duron, O., and Kelly B.M. 1963. Improved method for determination of blood glutathione. J. Lab. Clin. Med. 61(5): 882-888.

Bonventre, J.V. 2002. Kidney ischemic preconditioning. Curr. opinion Nephrol. hypertens. 11 : 43-48

Bradford, A., Atkinson, J., Fuller, N., and Rand, R.P. 2003. The effect of vitamin E on the structure of membrane lipid assemblies. J.Lipid Res. 44 : 1940-1945.

Brun, C.A.1951. Acidosis rapid method for the determination of paraaminohippuric acid in kidney function tests. J. Lab. Clin. Med. 37: 955-958.

Bunnett, C.C. 1996. The aminoglycosides. Prim. Care. Update. Ob/Gyns. 3(6) : 186-191.

Chade, A.R., Krier, J.D., Porcel, M.R., Breen, J.F., Mckusicck, M.A., Lerman, A., and Lerman, L.O., 2004. Comparison of acute and chronic antioxidant interventions in experimental renovascular disease. Am. J. Physiol. Renal. Physiol. 286 : F1079-F 1086.

Chance, B., Sies, H., Boveris, A., Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 59: 527-605.

Cheung, J.Y. and Miller, B.A. 2001. Molecular mechanisms of Erythropoietin signaling. Nephron 87: 215-222.

Chow, C.K., Ibrahim, W., Wei, Z., and Chan, A.C.1999. Vtamin E regurates mitochondrial hydrogen peroxide generation. Free. Radic. Boil. Med. 27 : 580-587.

Clark, W.G., Brater, D.C., and Johnson, A.R. 1992. 13 th ed. Antianemic drugs. Med Pharmacol. 605-609.

Covic, A. and Tatomir, P.G. 2007. Erythropoietin in acute renal failure (and myocardial infarction). Clin Res Cardiol Supp. S75-S80.

Cojocel, C., Docius, n., Maita, K., Sleight, A.D., Hook, J.B.1983. Effects of aminoglycosides on glomerular permeability, tubular reabsorption, and intracellular catabolism of the cationic low-molecular-weight protein lysozymes. Toxicol Appl. Pharmacol. 68: 96-109.

Cojocel, C., Docius, n., Maita, K., Smith, J.H., and Hook, J.B.1984. Renal ultrastructural and biochemical injuries induced by aminoglycosides. Environ Health Perspect. 57: 293-299.

Cuzzocrea, S., Mazzon, E., Dugo, L., Serraino, I., Paola, R.D., Britti, D., Sarro, A.D., Pierpaoli, S., Caputi, A.P., Masini, E. and Salvemini, D. 2002. A role for superoxide in gentamicin-mediated nephropathy in rats. Eur. J. Pharmacol. 450: 67-76.

Dillioglugil, M.O., Kir, H.M., Gulkac, M.D., Kanli, A.O., Ozdogan, H.K. Acar, O., and Dillioglugil, O. 2005. Protective effects of increasing vitamin E and a-dose 0n cisplatin-induced oxidative damage to kidney tissue in rats. Urol Int. 75: 340-344.

Eschbach, J.W., 2005. Iron requirements in erythropoietin therapy. Best. Pract. Res. Clin. Haematolol. 18 (2) : 347-61.

Fauconneau, B., Tallineau, C., Hugeot, F., and Piriou, A. 1995. Gentamicin-induced kidney damage and lipidperoxidation in rats. Toxicol Lett. 76: 127-134.

Fisher, J.W. 2003. Erythropoietin: Physiological and Pharmacological update. Exp. Biol Med. 1-14.

Foster, J.E., Harpur, E.S. and Garland, H.O. 1992. An investigation of the acute effect of gentamicin on the renal handling of electrolytes in the rats. J. Pharmacol. Exp. Ther. 261: 38-43

Frayer, M.J. 2000. Vitamin E as a protective antioxidant in progressive renal failure. Nephrol. 5 : 1-7.

Galleano, M., Farre, S.M., Turrens, J.F., and Puntarulo, S. 1994. Resistance of rats kidney mitochondrial membranes to oxidation induced by acute iron over load. Toxicol. 88(1-3) : 141-9.

Garcia, D.L., Anderson, S., Rennke, H.G., and Brenner, B.M. 1988. Anemia lessens and its prevention with recombinant human erythropoietin worsen glomerular injury and hypertension in rats with reduced renal mass. Proc. Nat. Acad. Sci. USA. 85 : 6142-46.

Gilbert, D.N. 1995. Aminoglycoside. Principle and practice of infectious disease, 4th ed. Churchill Livingstone, New York, 279-306.

Grune, T., Sommerburg, O. and Siems, W.G. 2000. Oidative stress in anemia. Clin. Nephrol. 53: s18-s22.

Gwinner, W., and Grone, H.J. 2000. Role of reactive oxygen species in glomerulonephritis. Nephrol. Dial. Transplant. 15 : 1127-1132.

Hishida, A., T. Nakajima, M. Yamada, A. Kato, and N. Honda. 1994. Role of hemodynamics and tubular factors in gentamicin-mediated nephropathy. Renal Fail. 16:109-116

Henrich, W.L. 2005. Nephrotoxicity of several newer agents. Kidney Int. 94: s107-s109

Hocher, B., Dembowski, C., and Slowinski, T. 2001. Impaired sodium excretion, decreased glomerular filtration rate and elevated blood pressure in endothelin receptor type B deficient rats. J. Mol. Med. 78 : 633-641.

Humlee, K.H. 2004. Iron. Clinical veterinary toxicology. St. Louis, Missouri. 202-204.

Ibrahim, W., and Chow, C.K. 2005. Dietary vitamin E reduces labile iron on rat tissues. J. Biochem. Mol. Toxicol. 19(5) : 298-303.

Johnson, D.W., Forman, C., and Versy, D. 2006. Novel renoprotective actions of erythropoietin: New user for an old hormone. Nephrol. 11: 306-312.

Kadkhodaee, M., Khastar, H., Faghihi, M., Ghaznavi, R. and Zahmatkesh, M. 2005. Effects of co-supplementation of vitamin E and C on gentamicin-induced nephrotoxicity in rat. Exp. Physiol. 90(4): 571-576.

Kang, D.H., Park, E.Y., Yu, E.S., Lee, Y.S. and Yoon, K.I. 2005. Renoprotective effect of erythropoietin (EPO) : possibly via an amelioration of renal hypoxia with stimulation of angiogenesis in the kidney. Kidney Int. 67: 1683.

Kavutcu, M., Canbolat, O., Ozturk, S., Olcay, E., Ulutepe, S., Ekinci, C., Gokhun, I.H., and Durak, I. 1996. Reduced enzymatic antioxidant defense mechanism in kidney tissues from gentamicin-treated guinea pigs: effects of vitamin E and C. Nephron. 72: 269-274.

Keher, JP. 1993. Free radicals as mediators of tissue injury and disease. Crit. Rev. Toxicol. 23: 21-48.

Kosek, J.C., Mazze, R.I., and Cousins, M.J. 1974. Nephrotoxicity of gentamicin. Lab. Invest. 30 : 48-57.

Kumral, A., Gonenc, S., Acikgoz, O., Sonmez, A., Genc, K., Yilmaz, O., Gokman, N., Duman, N., and Ozkan, H. 2005. Erythropoietin increases glutathione peroxidase enzyme activity and decreases lipid peroxidation levels in hypoxic-ischemic brain injury in neonatal rats. Biol. Neonate. 87 : 15-18.

Lim, C.S. and Vaziri, N.D. 2004. Iron and oxidative stress in renal insufficiency. Am. J. Nephrol. 24: 569-575.

Lipkin, G.W., Kendell, R.G., Russon, L.J., Turney, J.H., Norfolk, D.R., and Brownjohn A.M. 1990. Nephrol. Dial. Transplant. 5(11) : 920-2.

Mazzon, E., Britti D., Sarro, A.D., Caputi, A.P., Cuzzocrea, S. 2001. Effects of N-acetylcysteine on gentamicin-mediated nephropathy in rats. Eur. J. Pharmacol. 424: 75-83.

Maccord and Fridovich . 1969. Superoxide dismutase. J. Biol. Chem. 244(22): 6047-6055.

Marex, A.M., Zhang, J., Jiang, J., Alpe,r S.L., and Izumo, S. 1999. Endothelin-1 gene expression by shear stress; pharmacological evaluation of the role of tyrosine kinase, intracellular calcium,cytoskeleton, and mechanosensitive channel. J. Mol. Cell. Cardiol. 31: 387-399.

Mcglynn, H. and Ryan, M.c. 1990 . Gentamicin nephrotoxicity in rat renal proximal tubular cells. Toxicol. Lertters. 53 : 197-200.

Mingeot-Leclercq, M.P. and Tulken, P.M. 1999. Aminoglycoside : Nephrotoxicity. Antimicrobe. Agents. Chemother. 43(5) : 1003-12.

Mycek, M.J., Harvey, R.A., and Champe, P.C. Pharmacology. 2 nd ed. Lippincott .U.S.A. 315-317.

Nagano, N., Koumegawa, J., Arai, H., Wada, M. and Kusaka, M., 1990. Effect of recombinant human erythropoietin on new anemic model rats induced by gentamicin. J. Pharm. Pharmacol. 42(11): 758-762.

Nakajima, A., Hishida, A., and Kato, A., 1994. Mechanism for protective effects of free radical scavengers on gentamicin-mediated nephropathy in rats. Am. J. Physiol. 266: F425-F431.

Nielsen, O.J. and Thaysen, J.H. Erythropoietin deficiency in acute tubular necrosis. J. Intern. Med. 227 : 373-80.

Ng, T., Marx, G., Littlewood, T. and Macdougall, I. 2003. Recombinant erythropoietin in clinical practice. Postgrad. Med. J. 79: 367-376.

O'Connell, M.J., Ward R.J., and Baum H. 1985. The role of iron in ferritin- and hemosiderin-mediated lipid peroxidation in liposomes. Biochem. 229: 135-139.

Ohkawa, T., Ohisi, N., and Yaki, K. 1979. Assay for lipid peroxidation in animals tissue thiobarbituric acid reaction. Anal. Biochem. 95: 531-538.

Ohuchi, T., Yanagisawa, M., and Gariepy, C.E. 2000. Renal tubular effect of endothelin-B receptor signaling: its role in cardiovascular homeostasis and extracellular volume regulation. Curr. Opin. Nephrol. Hypertens. 9 : 435-439.

Osasa, H., Watanabe, T., and Nakamura, K. 1997. Changes in serum levels of creatol and methylguanidine in renal injury induced by lipidperoxide produced by vitamin E deficiency and GSH depletion in rats. Nephron. 75 : 224-9.

Pillbout, E., Burtin, M., Yuan, H.T., Briand P., Woolf, A.S., Friedlander, G., Terzi, F., 2001. Am. J. Pathol. 159 (2) : 547-60.

Plum, D.C. 2005.Veterinary Drug Handbook. 5 th edition. Iowa.Blackwell publishing. 424-425 ; 798-799.

Rainford, D.J., and Stevens, P.E., 1992. The investigative approach of the patients of acute renal failure. Oxford University press. 969-982.

Ramsammry, L., Link, K.Y., Josepovitz, C., Levine, R., and Kaloyanides, G.J., 1985. Effect of gentamicin on lipidperoxidation in rat renal cortex. Biochem. Pharmacol. 34(21): 3895-900.

Roob, L.M., Khoschsorur, G., Tiran, A., Horina, J.H., Holzer, H., and Winklhofer-Roob, B. M. 2000. Vitamin E attenuates oxidative stress induced by intravenous iron in patients on hemodialysis. J. Am. Soc. Nephrol. 11: 539-549.

Sasaki, R. 2003. Pleiotic function of erythropoietin. Internal medicine. 42 : 142-149.

Sies, H. 1997. Oxidative stress : antioxidants. Exp. Physiol. 82 : 291-295.

Silan, C., Uzun, O., Comunoglu, N. U., Gokcen, C., Bedirhan, S., and Gengiz, M. 2007. Gentamicin-induced nephrotoxicity in rats ameliorated and healing effect of resveratrol. Biol. Pharm. Bull. 30(1) 79-82.

Shah S.V. 2001. Role of iron in progressive renal disease. Am. J. Kidney. Dis. 37(1) S2 : 30-33.

Spandou, E., T souchnikas, I., Karkavelas, G., Dounousi, E., Simeonidou, C., Guiba-Tziampirl O., and Tsakiris, D., 2006. Erythropoietin attenuates renal injury in experimental acute renal failure Ischemic/reperfusion model. Nephrol. Dial. Transplant. 21(2). 330-6.

Sharples, E.J., Patel, N., Brown, P., Mota-Philipe, H., Sheaff, M.C., Kieszkiewiez, J., Allen, D., Hardwood, S., and Raftery M. 2004. Erythropoietin protects the kidney against the injury and dysfunction caused by ischemia-reperfusion. J. Am. Soc. Nephrol. 15 : 2115-2124.

Sharples, E.J. and Yaqoob, M.M. 2006. Erythropoietin and acute renal failure. Semin. Nephrol. 26(3) : 325-31.

Slowinski, T., Schulz, N., and Ruschitzka, F.T. 2002. Pattern of prepro-endothelin-1 expression revealed by receptor gene activity in kidney of erythropoietin-overexpressing mice. Clin Sci. 103 : 3561-65.

Soldago, C.M., Eleno, N., Tavares, P., Barbero, A.R., Criado, J.G., Bolanos, J.P., and Novoa, J.M.L. 2002. Involvement of reactive oxygen species on gentamicin-induced mesangial cell activation. Kidney Int. 62: 1682-1692

Sommerburg, O., Grune, T. and Hamp, H., 2000. Does treatment of renal anemia with recombinant erythropoietin influence oxidative stress in hemodialysis patients ? Clin. Nephrol. 53: S23-29.

Tan, C.C., Eckardt K.U., Firth, J.D., and Ratcliffe, P.J. 1992. Feed back moduration of renal and hepatic erythropoietin mRNA in response to graded anemia and hypoxia. Am. J. Physiol. 263: F474-F481.

Tiidus, P.M. 1998. Radical species in inflammation and overtraining. Can. J. Physiol. Pharmacol. 76 : 533-8.

Traber, M.G. 1999. Vitamin E. In: Modern nutrition in health and disease. 9 th ed. Baltimore: William & Wilkins. 347-362.

Tucker, J.M. and Towmsend, D.M. 2005. Alpha-tocopherol: roles in prevention and therapy of human disease. Biomed. Pharmacother. 59: 380-387.

Thurau, K., and Boylan, J.W. 1976. Acute renal success. The unexpected logic oliguria in acute renal failure. Am. J. Med. 61 : 308-315.

Walker, P.D. and Shah, S.V. 1987. Gentamicin enhanced production of hydrogen peroxide by renal cortical mitrochondria. Am. J. Physiol. 253: C495-499.

Walker, P.D. and Shah, S.V. 1988. Evidence suggesting a role for hydroxyl radical in gentamicin-induced acute renal failure in rats. Am. Soc. Clin. Invest. 81: 334-341.

Wang, X., and Quinn, P.J. 1999. Vitamin E and its function in membranes. Prog. Lipid Res.. 38 : 309-336.

Westenfelder, C. 2002Unexpected renal actions of erythropoietin. Exp Nephrol. 10 : 294-298.

Wu D., Liu, L., Meydani M., and Meydani S.N. 2005. Vitamin E increases production of vasodilator prostanoids in human aortic endothelial cells through opposing effects on cyclooxygenase-2 and phospholipase A_2. J. Nutr. 138(8) : 1847-53.

Vaziri, N.D., Zhou, X.J. and Liao, S.Y. 1994. Erythropoietin enhances recovery from cisplatin-induced acute renal failure. Am. J. Physiol. 266: F360-366.

Vaziri, N.D. 2004. Oxidative stress in uremia : Nature, Mechanism, and Potential consequence. Semin. Nephrol. 24 : 469-473.

Yamada, T. 1995. Studies on the mechanisms of renal damages induced by nephrotoxic compounds. Nihon Hoigaku Zasshi. 49(6) : 447-57.

Yang, C.W., Li, C., Jung, J.Y., Shin, S.J.,Choi, B.S., Lim, S.W., Sun, B.K., Kim Y.S., Kim, J., Chang, Y.S., and Bank B.K. 2003. Preconditioning with erythropoietin protects against subsequent ischemia-reperfusion injury in rats kidney. FASEB. 1754-1755

Young, M.K.J. and Raisz, L.G. 1952. An anthrone procedure for determination of inulin in biological fluids. Proc. Soc. Exp. Biol. Med. 80(4) : 771-774.

Zager, R.A., Johnson, A. C.M., and Hanson, S.Y. 2004. Parenteral iron nephrotoxicity: potential mechanisms and consequences. Kidney Int. 66 : 144-156.

Zainal, T.A., Wcindruch, R., Szwcda, L.I. and Oberley, T.D. 1999. Localization of 4-hydroxy-2 nonenal-modified proteins in kidney following iron overload. Free Radic. Biol. Med.26: 1181-1193.

Zhou, X.J., Laszik, Z., Wang, X.Q., Silva, F.G. and Vaziri, N.D. 2000. Association of renal injury with increased oxygen free radical activity and altered nitric oxide metabolism in chronic experimental hemonsiderosis. Lab. Invest. 80: 1905-1914.

BIOGRAPHY

NAME	Miss Patcharin Thongchai
DATE OF BIRTH	22 December 1979
PLACE OF BIRTH	Nakornsawan, Thailand
INSTITUTIONS ATTENDED	Chulalongorn University, 1998-2003 Veterinary Science
RESEARCH GRANT	Chulalongorn University Graduate Scholarship to Commemorate the 72 nd Anniversary of His Majestry King Bhumibol Adulyadej Office of Commission for Higher