ผลของ L-carnitine ต่อการทำหน้าที่ของไตในหนูที่ถูกเหนี่ยวนำให้เกิดความเสียหายของไต และ oxidative stress โดยการใช้ Doxorubicin

นางสาว ดลฤดี บุญสนิท

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสรีรวิทยาการสัตว์ ภาควิชาสรีรวิทยา
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2547

ISBN 974-53-1906-6

EFFECTS OF L-CARNITINE ON RENAL FUNCTION AND OXIDATIVE STRESS IN RATS WITH DOXORUBICIN-INDUCED RENAL INJURY

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A thesis Submitted in Partial Fulfillment of the Requirements

for the degree of Master of Science in Animal Physiology

Department of Physiology

Faculty of Veterinary Science

Chulalongkorn University

Academic Year 2004

ISBN 974-53-1906-6

Thesis Title	Effects of L-carnitine on renal function and oxidative stress in rats with doxorubicin-induced renal injury.
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ดลฤดี บุญสนิท : ผลของ L-carnitine ต่อการทำหน้าที่ของไตในหนูที่ถูกเหนี่ยวนำให้เกิดความเสียหายของไต และ oxidative stress โดย Doxorubicin. (EFFECTS OF L-CARNITINE ON RENAL FUNCTION AND OXIDATIVE STRESS IN RATS WITH DOXORUBICIN INDUCED RENAL INJURY) อ. ที่ปรึกษา: รศ.สพ.ญ.ดร. ชลลดา บูรณกาล, อ. ที่ปรึกษาร่วม : รศ.สพ.ญ.ดร. สุมลยา กาญจนะพังคะ : 63 หน้า ISBN 974-53-1906-6

การศึกษาผลของ L-carnitine ต่อการทำหน้าที่ของไตในหนูที่ถูกเหนี่ยวนำให้เกิดความเสียหายของไต และ oxidative stress โดยใช้ doxorubicin (DOX) แบ่งหนูออกเป็น 4 กลุ่ม คือ กลุ่มควบคุม ฉีดน้ำเกลือ กลุ่มที่ 2 คือกลุ่มที่ฉีด DOX 7.5 มก./ กก. เข้าทางหลอดเลือดดำที่หางของหนู กลุ่มที่ 3 DOX ร่วมกับ L-carnitine 40 มก./ กก. และ กลุ่มที่ 4 DOX ร่วมกับ L-carnitine เข้าช่องท้อง 200 มก./กก. โดยให้ L-carnitine 1 ชม. ก่อน DOX และให้ตลอดการทดลองเป็นเวลา 15 วัน ผลการศึกษาพความเสียหายทางโครงสร้างของไต ในหนูที่ให้ DOX โดยเฉพาะอย่างยิ่งในที่กลุ่ม 2 พบโปรตีนอัลบูมินในพลาสม่าลดต่ำลง ไทรกลิสเซอไรด์ โคเล็สเทอรัลในพลาสม่าเพิ่มสูงขึ้น โปรตีนที่ขับทิ้งในปัสสาวะเพิ่มสูงขึ้น และพบการสูงขึ้นของ ลิพิดเพอร์เรอกไซด์ ครีอะทินนิน และ ยูเรียไนโตรเจนในพลาสม่า และพบไตของหนูกลุ่มที่ 2 การทำงานของ catalase ลดลงอย่างมีนัยสำคัญทางสถิติ นอกจากนี้DOX ยังทำให้ อัตราการกรองผ่านกลอเมรูลัส และ พลาสม่าที่ไปเลี้ยงไต ลดลงอย่างมีนัยสำคัญทางสถิติ และพบการสูงขึ้นของค่าความต้านทานของหลอดเลือดที่ไต ในกลุ่มที่ 3 และ 4 ซึ่งได้รับ L-carnitine สามารถช่วยให้ระดับ ไทรกลิสเซอไรด์ คลอเลสเตอรอล ครีเอตรินิน และยูเรียไนโตรเจนในพลาสม่าลดต่ำลง นอกจากนี้ L-carnitine ยังช่วยในการทำหน้าที่ของไต โดยเพิ่มอัตราการกรองผ่านกลอเมอรูลัสและพลาสม่าที่ไปเลี้ยงไต และยังพบว่า การทำงานของ catalase ในไตเพิ่มสูงขึ้นอย่างมีนัยสำคัญทางสถิติในกลุ่มที่ให้ L-carnitine เมื่อเทียบกับกลุ่มที่ให้ DOX เพียงอย่างเดียว ผลการศึกษาการเปลี่ยนแปลงทางโครงสร้างของไต พบการขยายใหญ่ของ glomerular capillary การขยายใหญ่ของท่อภายในไต และการลอกหลุดของเซลล์ในท่อภายในไตหนูกลุ่มที่ 2 ไตมีการเปลี่ยนแปลงโครงสร้างของกลอเมอรูลัส และท่อภายในไต จะลดต่ำลงเมื่อให้ L-carnitine ทั้งปริมาณต่ำและสูง เมื่อเทียบกับเมื่อให้ doxorubicin เพียงอย่างเดียว ดังนั้นจึงสรุปได้ว่า การให้ L-carnitine สามารถป้องกันความเสียหายจากการทำหน้าที่ของไตและการเปลี่ยนแปลงโครงสร้างของไตที่เกิดจาก DOX ซึ่งจะสัมพันธ์กับปริมาณของ oxidative stress ที่ลดลง

ภาควิชาสรีรวิทยา	ลายมือชื่อนิสิต.....
สาขาวิชาสรีรวิทยาการสัตว์	ลายมือชื่ออาจารย์ที่ปรึกษา.....
ปีการศึกษา 2547	ลายมืออาจารย์ที่ปรึกษาร่วม.....

4575556631 : MAJOR ANIMAL PHYSIOLOGY
KEY WORD: DOXORUBICIN / L-CARNITINE / OXIDATIVE STRESS / RENAL / RAT

DOLRUDEE BOONSANIT: EFFECTS OF L-CARNITINE ON RENAL FUNCTION AND OXIDATIVE STRESS IN RATS WITH DOXORUBICIN-INDUCED RENAL INJURY. THESIS ADVISOR: ASSOC. PROF. CHOLLADA BURANAKARL, Ph.D., THESIS COADVISOR: ASSOC. PROF. SUMOLAYA KANCHANAPANKA, Ph.D. 63 pp. ISBN 974-53-1906-6

Effects of L-carnitine on nephrotoxicity and oxidative stress induced by doxorubicin (DOX) in rats were investigated. Rats were divided into 4 groups; group 1 (control: NSS injection); group 2 DOX injection (7.5 mg/kg, i.v.); group 3 DOX plus low dose (40 mg/kg) L-carnitine and group 4 DOX plus high dose (200 mg/kg) L-carnitine. Treatment with L-carnitine (40 and 200 mg/kg, per day) was performed 1 hour before doxorubicin injection and daily thereafter for 15 days. Nephrotic syndrome was induced by a single intravenous injection of doxorubicin. The results showed that rats in group 2 were associated with hypoalbuminemia, hyperlipidemia, high urinary excretion of protein and elevated plasma lipid peroxide, creatinine and urea nitrogen. Kidneys from group 2 rats had significant decreases in catalase (CAT) activity and elevated lipid peroxides. Doxorubicin significantly decreased glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and increased renal vascular resistance (RVR). In group 3 an 4 which received L-carnitine, plasma triglyceride, cholesterol, creatinine and plasma urea nitrogen declined. L-carnitine improved renal functions by elevated GFR and ERPF. The CAT activity in the kidneys of L-carnitine treated rats were increased significantly compared with group 2. From histopathologic results, there were glomerular capillary dilation, tubular dilation and loss of tubular cell in kidneys of group 2 rats. The lesions in glomerulus and tubule were less in rat receiving L-carnitine both low and high doses (group 3 and 4) compared with doxorubicin alone. In conclusion, L-carnitine can protect renal impairment functionally, biochemically and histopathologically with a corresponding reduction of oxidative stress.

Department Physiology	Student's signature……..
Field of study Animal Physiology	Advisor's signature……..
Academic year 2004	Co-advisor's signature…….

ACKNOWLEDGEMENTS

I greatly thank to my parents for their support, carefulness, greatly taking care of me and giving me warmth all the time and forever.

I gratefully acknowledge to my advisor, Associate Professor Dr. Chollada Buranakarl and my co-advisor, Associate Professor Dr. Sumolya Kanchanapanka for their valuable advice, support, encouragement and extensive help in this work.

I am grateful to the thesis committee for their valuable suggestion.

I give grateful acknowledgment to Miss Siripen Komolvanish and Mr. Somchai Pohdeenana for their assistances in technically laboratory guidance.

This study was supported in part by fund from Graduate School and the Faculty of Veterinary Science, Chulalongkorn University.

Finally, I would like to thank everyone else who helped me in this study, and all my lovely friends for their kindness.

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CHAPTER I

INTRODUCTION AND AIMS

Doxorubicin (DOX, adriamycin) has been widely used as an antineoplastic agent in small animal medicine. It is useful in the treatment of a variety of carcinomas and sarcomas in both dogs and cats. However, the clinical usefulness is limited by a number of its side effects in cardiotoxicity, hematotoxicity and nephrotoxicity. Recently, in experimental animal model, DOX was also suspected to be nephrotoxic especially on glomerular epithelial cells. Doxorubicin was reported to induce changes in glomerular capillary permeability causing nephrotic syndrome without hypertension and without marked early renal functional loss (Bertani et al., 1986; Grond et al., 1988; Hall et al., 1986).

Clinical signs associated with nephrotic syndrome were characterized by heavy proteinuria, albuminuria, hypoalbuminemia and hyperlipidemia (Bertani et al., 1982; Milner et al., 1991) with progressive azotemia (Okasora et al., 1992). This experimental nephropathy resembles histologically and clinically nephropathy associated with focal and segmental glomerulosclerosis (Zima et al., 1997). There is an increasing evidence for the enhancing effect of free radicals involved in the primary pathogenic mechanism of DOX-induced nephropathy in rats (Bertani et al., 1986).

The mechanisms in which DOX induced renal injury are not clearly defined. Several mechanisms have been suggested for the induction of DOX associated nephrotic syndrome. The most possible mechanism for renal toxicity of DOX may be alterations of the permeability of the glomerular capillary wall (Weening and Rennkle, 1983) or a consequence of oxidative stress, such as oxidation and cross-linking of cellular thiols and membrane lipid peroxidation (Wu et al., 1990). In DOX induced nephropathy, the glomerular cells produce reactive oxygen species (ROS) which caused glomerular injury (Ginevri et al., 1990). ROS initiates free-radical which mediates

chain reactions resulting in the conversion of membrance unsaturated fatty acid into lipid peroxide and increased lipid peroxidase. In glomeruli, the generation of ROS initiates a cascade of degenerative events including the oxidation cellular thiols. DOX causes a reduction in renal content of antioxidant such as glutathione (GSH) and catalase activity (Montilla et al., 1997).

DOX may also cause ATP depletion by inhibition of carnitine palmitoyltransferase at both mitochondrial outer and inner membrance (Kaslfi et al., 1990). DOX causes a dose-dependent disruption of cardiac mitochondrial structure and bioenergetic function and also implicates in mitochondrial dysfunction in the pathogenesis of DOX induced cardiomyopathy. (Nicolay and De Kruijff, 1987; Papadopoulou et al., 1996; Praet and Ruysschaert, 1993; Solem et al., 1994). It inhibits both DNA and RNA synthesis and altering the function and structure of cellular and intracellular membranes (Calendi et al., 1965).

L-carnitine (L-trimethyl-3-hydroxy-ammoniabutanoate) is a small, water soluble molecule which is important in mammalian fat metabolism. It is naturally occurring compound that facilitates the transport of long-chain (>12 carbon molecules) fatty acids into the mitochondria in skeletal muscle and cardiomyocytes, where they undergo beta-oxidation (Brass, 1997). By this mechanism carnitine profoundly influences both skeletal muscle and myocardial fatty acid oxidation. It promotes fatty acid oxidation (Strohm et al., 1982).

Administration of L-carnitine was associated with a linear dose-dependent increase in myocardial ATP in rat model of doxorubicin myocardial injury (Shug, 1987). Carnitine can protect against doxorubicin-induced myocardial injury and mortality (Sayed-Ahmed et al., 1999). Carnitine may itself be an antioxidant. It appears to have a strong inhibitory effect on free radical production (Ben, 1994). L-carnitine can decrease severity of renal cortical proximal tubular necrosis and improve renal function (Kopple et al., 2002).

It seen that, some of the action of L-carnitine may antagonize the toxic effect of doxorubicin in nephrotic syndrone. The objective of the present study is

1. To study the effects of L-carnitine on renal function in DOX-induced rats.
2. To study the effects of DOX on renal damages by increasing lipid peroxidation.
3. To study the effects of L-carnitine in reducing oxidative stress.

Hypothesis

1. L-carnitine can protect renal injury in rats with doxorubicin administration.
2. Renal damage caused by doxorubicin can be detected by increasing in lipid peroxidation.
3. L-carnitine can reduce oxidative stress from doxorubicin administration.

CHARTER II

BACKGROUND INFORMATION

Pharmacological of doxorubicin

Doxorubicin (Adriamycin, DOX) is a glycoside and anthracycline antibiotic which has antineoplastic activity. The drug contains quinine structure as shown in figure 1. It can be isolated from cultures of Streptomyces peucetius var. caesius. DOX has to be administered intravenously as it is not absorbed through the gastrointestinal tract and is extremely irritate to tissue if administered subcutaneously or intramuscularly. It is rapidly and widely distributed in the body. High concentration of DOX is found in liver, lymph nodes, muscle, bone marrow, fat, and skin. It does not appreciably enter the cerebrospinal fluid (CSF). DOX is highly bound to tissues and plasma proteins; it probably crosses the placenta and is distributed into milk.

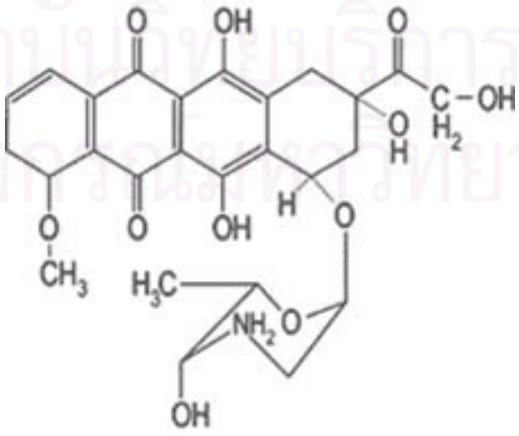


Figure 1. Structure of doxorubicin (adriamycin)

Doxorubicin is metabolized extensively by the liver and other tissues via aldo-keto reductase to doxorubicinol, which is reactive. Other inactive metabolites are also formed. Biliary excretion is a major excretory route whereas 40 to 50% is recovered in bile in seven days. Urinary excretion accounts only 4 to 5 % of the total dose in five days. Doxorubicin is eliminated in a triphasic manner. During the first phase (t1/2 =0.6 hours) doxorubicin is rapidly metabolized, followed by a second phase (t1/2=3.3 hours). The third phase has a much slower elimination half-life (17 hours for doxorubicin and 32 hours for metabolites), presumably due to the slow release of the drug from tissue proteins.

Clinical usage of doxorubicin

Doxorubicin has been used for treatment of a variety of malignancies. It has been shown to be effective in the treatment of several types of solid tumors affecting children and adults such as breast cancer, soft tissue sarcomas, endometrial cancer, osteosarcomas, tumors arising in bile ducts, esophagus and liver and non-Hodgkin's lymphoma (O'Bryan et al., 1973; Tan et al., 1973; Benjamin et al., 1974; Blum et al., 1974). In small animal, doxorubicin is widely used as an antineoplastic agent. It may be useful in the treatment of a variety of carcinomas and sarcomas.

Nephrotic syndrome

Nephrotic syndrome is not a single disease but a family of disorders that are characterized by increased glomerular permeability. The classical definition of nephrotic syndrome is massive proteinuria, hypoalbuminemia, hypercholesterolemia and peripheral edema. This syndrome may be present with or without azotemia but commonly progresses to chronic renal failure over a variable time period. Since the glomerular barrier is damaged with proteins leaking into the urine, the amount of

proteins in the circulation will be reduced. Edema is generally considered to be due to the decreased in plasma colloidal osmotic pressure, eventually leading to an enhanced fluid flow into the interstitial space. However, it has also been suggested that edematous is due to a generalized loss of permeability selectivity in the vascular beds. Dogs with this syndrome can lose several grams of protein each day (day1 to day4). Quantitation of urinary protein loss using a 24 hour urine collection is an essential diagnostic procedure.

Effects of doxorubicin on nephrotic syndrome

Application of this drug in chemotherapy is limited because of its cardiotoxicity, hepatotoxicity and nephrotoxicity. The side effect of doxorubicin was associated with nephrotic syndrome characterized by heavy proteinuria, albuminuria, hypoalbuminemia, hyperlipidemia and hypercholesterolemia (Manabe et al., 2001). Bertani and coworkers (1982) reported that DOX induced nephrotic syndrome in rats. Proteinuria started 4 to 5 days after a single intravenous injection of DOX (7.5 mg/kg of body weight). A similar result with a single intravenous injection of DOX (7.5 mg/kg) showed proteinuria in a few days (5-15) and persisted for several months (Weening and Rennke, 1983).

The various doses of doxorubicin were studied extensively. A single injection of DOX (20 mg/kg. i.p.) in rats resulted in hyperlipidemia and high grade proteinuria and marked increases in serum lipoperoxides, urea, and creatinine at 7 days after administration of DOX (Montilla et al., 1997). A single intravenous injection of doxorubicin (6 mg/kg) induced a severe nephrotic syndrome (after 5 weeks) associated with hypoalbuminemia, hypoproteinemia, elevated serum urea, hyperlipidemia and a high urinary excretion of protein and albumin (Badary et al., 2000). However, injection of with 5 mg/kg DOX, albuminuria was observed as early as on day 7 (Galli et al., 2001). The creatinine clearance was significantly reduced (Milner et al., 1991).

In long term administration of doxorubicin, rats injected with doxorubicin (2 mg/kg, i.v.) twice at a 20 day interval showed massive proteinuria, hypoalbuminemia, and hyperlipidemia at 4 weeks after the last doxorubicin injection. Both BUN and serum creatinine began to increase at 16 weeks of treatment and reached the uremic level at 28 weeks of treatment (Okuda et al., 1986).

Effects of doxorubicin on renal hemodynamics

A single dose of doxorubicin (7.5 mg/kg, i.v.) caused significant decrease in glomerular filtration rate (GFR) and renal plasma flow (RPF) in rats by 20% and 15%, respectively while the filtration fraction was unchanged (Weening and Rannke, 1983).

The progressive loss of renal function is associated with an altered hemodynamic pattern with glomerular capillary hypertension being frequently present. The experiment by Ballbi et al (1998) designed to analyze the impact of diminished renal perfusion pressure by renal clipping on the rat model of doxorubicin induced nephropathy. Male wistar rats divided into four groups: control(C), doxorubicin 3 mg/kg (DOX), saline with the left renal artery clipped (Rv) and doxorubicin with renal artery clipping (DOXRv). After 24 weeks, mean arterial pressure (MAP) was similar between Rv and DOXRv, but higher than the C and DOX groups. Inulin clearance (ml/min/100g) in DOX (0.2±0.05) was smaller than in C (0.53±0.17) and Rv (0.4±0.01), and was at an intermediate level in DOXRv(0.33±0.2). The level of PAH (ml/min/100g) was normal at 1.76 in C and diminished more in DOX (0.58) than in Rv (1.06) and DOXRv (1.18). Both DOX and the DOXRv had the higher degree of glomerulosclerosis (33% and 25%), compared with C and Rv (both 0%). The data suggests that diminished perfusion pressure of the clipped kidney, by decreasing the intraglomerular pressure protects the glomerulus and attenuates doxorubicin induced nephropathy.

Effects of doxorubicin on renal histopathology

Histopathologic results in doxorubicin treated rats showed enlarged kidneys with granular surface and pale color. Doxorubicin induced nephropathy characterized by early glomerular and late onset tubular lesion in rats. Doxorubicin rats had glomeruli with expanded mesangial area and capillary aneurysm (Manabe et al.,2001). The histologic pattern resembles that of focal and segmental glomerulosclerosis in human and the mechanisms influencing the progression of this nephropathy are still unknowns (Bertani et al., 1982; Bertani et al., 1986 and Okuda et al., 1986). The loss of glomerular polyanion, focal fusion of foot processes with swelling and vacuolation of epitherial cells were reported in rats treated with doxorubicin for short term experiments (Weening and Rennke, 1983). In long term studies, severe renal damages, extensive glomerular lesions, tubular dilatation and stromal fibrosis were also observed (Okuda et al., 1986).

Bertani et al (1982) reported that single intravenous injection of DOX (7.5 mg/kg of body weight) in rats was associated with many pathological changes which were developed 13 to 15 days later. Light microscopy showed minimal alterations with negative immunofluorescence and few focal fusion of foot processes by electron microscopy in the early phase after injection (28 hours). On day 13, loss of foot process architecture, and replacement by flattened epithelial cytoplasm was invariably found. The study of Bertani et al (1986) in rats induced neprotoxicity by injected doxorubicin 7.5 mg/kg showed that at 14 days of treatments, glomerular visceral epithelial cells had profound swelling of cytoplasm with increased number of reabsorptive vacuoles and lysosomes as well as segmental detachment of epitherial cells from the basement membrane. In the cytoplasm of many proximal tubules, many protein droplets were seen suggesting the intense reabsorption activity of proximal tubular cells consequent to a large scale proteinuria. Sixty days after DOX injecton, 5% of glomeruli with limited areas of focal glomerulosclerosis were found.

Bakker et al (1987) studied ATP-ase activity using the cerium-based method in kidneys from DOX-treated rats versus control. They found that ATP-ase activity was reduced in the endothelial cell membranes and in the glomerular basement membrane (GBM) of kidneys from DOX-treated rats.

Mechanisms of action of doxorubicin induced renal injury

The mechanisms responsible for the pathogenesis of DOX-induced renal injury are not clearly defined. Several mechanisms have been suggested for the induction of DOX-associated nephrotic syndrome.

1. Doxorubicin inhibited enzymes, decreased protein synthesis and disrupted extracellular matrix (Fukuda et al., 1992). Doxorubicin can derive quinine-semiquinone redox cycling. This semi-quinone can be taken up directly into the nucleus and react with DNA in the cell to cause damage (Gewirtz, 1999). This damage is preferred in the case of cancer cells but can cause damage to glomerular cells.
2. Doxorubicin caused mitochondria DNA (mtDNA) damage and dysfunction. Report of Lebrecht and coworker (2004) in rats treated with intravenous doxorubicin injection (1 mg/kg/wk) for 7 weeks (short-term) or 30 weeks (long-term) showed that the long-term group had significant depressed activities of mtDNA-encoded NADH dehydrogenase and cytochrome-*c* oxidase (COX) and increased citrate synthase activity. In addition, expression of the mtDNA-encoded COX subunit I was reduced. Kidneys from the ‘long-term’ group showed more mtDNA deletions than in ‘short-term’ animals.
3. The glomerular cells from doxorubicin induced nephropathy produced reactive oxygen species which cause glomerular injury (Ginevri et al., 1990).

Effects of doxorubicin on oxidative stress

Redox cycling of the quinine moiety of doxorubicin has been widely proposed as the key factor which mediates doxorubicin nephrotoxicity (Fukuda et al., 1992).The Doxorubicin semi-quinone also reduced O_2 to produce $O^{\bullet-}_2$. Superoxide dismutase (SOD) will then dismute the superoxide radicals ($O^{\bullet-}_2$) to hydrogen peroxide (H_2O_2). The doxorubicin semi-quinone can also react to reduce ferric iron complexes in a radical-driven Fenton reaction (Kalyanaraman et al., 1991). Doxorubicin semi-quinone reacts with H_2O_2 to produce hydroxyl radicals ($OH^{\bullet}$) in the presence of chelators. This suggests that iron is important to the doxorubicin semi-quinone catalyzed reduction of H_2O_2.These reaction of doxorubicin semi-quinone is showed in figure 2. Both iron and oxygen of these substances are found in body and easily accessible to the doxorubicin. These reactions are what lead to the toxic effect on cardiac cells and renal cells.

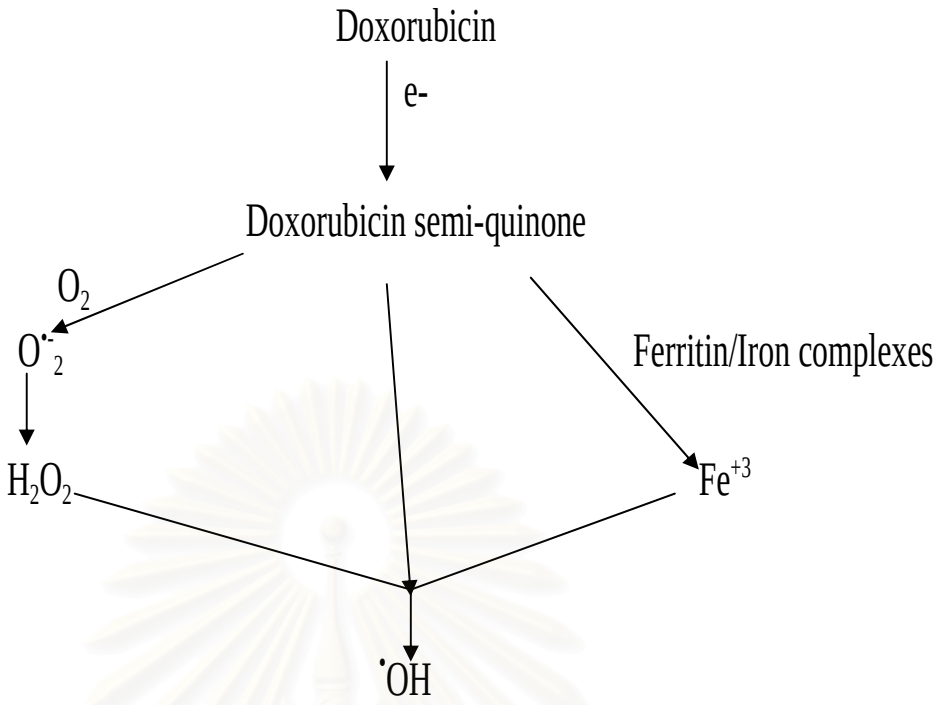


Figure 2. Doxorubicin semi-quinone driven radical reaction.

The hydroxyl free radical can react with polyunsaturated fatty acids initiating a lipid-radical chain reaction and oxidative damage to cell membrane. Increased levels of oxygen species due to doxorubicin have been detected directly by electron spin resonance spectroscopy and indirectly by increase in tissue malonedialdehyde (MDA) which is a breakdown product of lipid peroxidation (Singal et al., 1987; Myers et al., 1977).

Nomally, cell developes process to prevent reactive oxygen species formation in order to limit cell damage. Three antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GSHPX) and catalase) and antioxidant substrates (ascorbate, tocopherols, reduced glutathione etc.) play an important role in free radical-induced cell injury. In the kidney, single administration of DOX (7.5 mg/kg i.v.) increased lipoperoxides by significantly decreases the reduced glutathione (GSH) and catalase (CAT) (Montilla et al., 1997). DOX (6 mg/kg) also induced a significant increase of lipid peroxide but decrease the catalase (CAT) activity (Badary et al., 2000). At a lower

dose, DOX (5 mg/kg, i.v.) stimulated free radical production with a significant decrease in renal cortical glutathione content within 24 hours. (Milner et al., 1991)

A redox cycling of doxorubicin may cause cytotoxicity for renal injury. Morgan and coworkers (1998) compared the extent of the oxidative stress and cytotoxicity induced by doxorubicin and menadione (a model for redox cycling quione) in freshly isolated rat glomeruli. Doxorubicin and menadione decreased de novo protein synthesis. Doxorubicin and menadione increased ROS (reacting oxygen species) formation by 260 and 156% from controls after 30 min. Oxidative stress was assessed by measuring the intracellular level of reduced glutathione (GSH) and the decrease of the NADPH/$NADP^+$ ratio which stimulates the pentose phosphate pathway (PPP). Menadione reduced glomerular GSH approximately 10–20% from controls while doxorubicin had no effect. Menadione (10µM) increased PPP activity 6-fold while doxorubicin (125 µM) had only 2-fold effect. Although doxorubicin and menadione generate extensive ROS and decrease protein synthesis, there was no correlation between the extent of oxidative stress and cytotoxicity in glomeruli exposed to doxorubicin. These results suggest that oxidative stress may not be the primary mechanism by which doxorubicin induced selective glomerular toxicity.

L-carnitine

L-carnitine is found in foods from animal, but not from plant. Carnitine is synthesized endogenously from lysine, methionine**,** niacin, vitamin B6 and vitamin C. It is a small, water soluble molecule important in mammalian fat metabolism. L-carnitine is eliminated from the body mainly via urinary excretion. It is an important element in beta-oxidation of fatty acids. In human, L-carnitine decreased triglyceride and carnitine palmitoyl transferase activity in red bood cell. It reduced several complications seen in uremic patients (Bellinghieri et al., 2003).

Effects of L-carnitine on renal functions

Studies of L-carnitine given to rats with renal disease to improved renal function were demonstrated. In nephrectomized rats, L-carnitine (200 mg/kg) was administered daily for 3 days prior to 2/3 nephrectomy and given for 25 days thereafter. At the end of the experiment, plasma creatinine was lower in carnitine-treated rats. Plasma triglycerides and VLDL which were increased by nephrectomy were prevented by carnitine treatment. Thus, carnitine might protect against the development of renal failure in this experimental model (Palomba et al., 1996).

Effect of L-carnitine on cisplatin-induced renal injury was also studied. Cisplatin caused proximal tubular cell damage. Vacuolization, desquamation, degeneration, necrosis of proximal tubule cells and cast formation within tubules were apparent. Renal dysfunction induced by cisplatin was inhibited by carnitine almost completely. L-carnitine normalized plasma levels of BUN and creatinine in cisplatin-treated rats. Thus, L-carnitine improved renal dysfunction caused by cisplatin (Chang et al., 2002). In rats of ischemia/reperfusion model, histological study showed very mild post-ischemic lesion in kidney exposed to propionyl-L-carnitine as compared to untreated ischemic kidney (Mister et al., 2002). A major product of lipid peroxidation was very low in kidney infused with propionyl-L-carnitine as compared to untreated ischemic kidney. These data indicate that propionyl-L-carnitine is of value in preventing decline of renal function that occurred during ischemia-reperfusion.

Moreover, administration of L-carnitine ameliorates gentamicin-induced renal injury in rats. At day 12, the rats given gentamicin 80 mg/kg and L-carnitine 200 mg/kg/day, compared with rats given gentamicin 80 mg/kg and no carnitine, displayed lower serum urea and creatinine concentrations, and higher creatinine clearances. Rats given gentamicin 80 mg/kg/day and carnitine 200 mg/kg/day had less proximal tubular necrosis and greater mild proximal tubular necrosis compared with rats receiving L-carnitine 40 mg/kg/day or no carnitine. Thus, L-carnitine 200 mg/kg/day prevented

renal lesion and improved renal function in rats with gentamicin-induced renal injury (Kopple et al., 2002).

Effects of L-carnitine on oxidative stress

Carnitine may itself be an antioxidant. It appears to have a strong inhibitory effect on free radical. While cisplstin can induce mitochondrial dysfunction, DNA injury, lipid peroxidation and apoptosis of epithelial cells in the rat kidney (Chang et al., 2002). These impairments can be alleviated by L-carnitine. In streptozotocin-diabetic rats, treatment with L-carnitine (0.6g/kg/day, i.p.) 8 weeks after the induction of diabetes and continued for 2 weeks completely normalized plasma cholesterol, triglyceride and thiobarbituric acid reactive substance (TBARS) levels. In diabetic aorta, catalase (CAT) activity was increased but glutathione peroxidase (GSH-Px) activity was unchanged. These results suggested that L-carnitine partially improved antioxidant property and reduced plasma lipid (Irat et al., 2003). In hypercholesterolemia rabbits, daily administration of L-carnitine (250mg/kg/day) for 28 days completely prevented the progression of atherosclerotic lesion, reduced glutathione (GSH) levels and decreased malonedialdehyde (MDA) (Sayed-Ahmed et al., 2001)

Effects of L-carnitine on doxorubicin

There is no study on the on protective effect of L-carnitine in rats induced nephrotic syndrome with DOX. However, there was report of L-carnitine on doxorubicin induced cardiotoxicity, elevated lipid peroxidation and increased triglyceride and cholesterol. DOX inhibited the carnitine palmitoyltransferase system (CPT1) and consequently the transport of long chain fatty acids across mitochondrial membranes. L-carnitine decrease triglyceride and cholesterol in plasma on doxorubicin

rats. Hong et al (2002) studied effects of L-carnitine (200 mg/kg) in rats injected with DOX (5 mg/kg, i.p.) twice a week over a period of 2 week. Serum lipids (total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol) and fatty acid levels were determined on the 1, 8, and 15 day after injection of DOX. They increased in doxorubicin group compared with the control group. Striking reduction in these substances was observed when L-carnitine was added.

Luo et al (1999) studied the effect of L-carnitine on doxorubicin induced lipid peroxidation in rats. DOX was administered intraperitoneally in rats at a dose of 2.5 mg/kg doses over a 2 week period with and without L-carnitine (500 mg/kg, 5 week). The results indicated that free radical-mediated lipid peroxidation occurred early after DOX administration. The data also revealed that aldehyde production occurred in a cumulative fashion, parallel to the dose of DOX induced cardiotoxicity. The changes included increases of some of the most toxic aldehydes such as hexanal, HNE and MDA, which may be important mediators of DOX induced cardiac toxicity. Furthermore, the data provide direct evidence that exogenous L-carnitine can attenuate the extent of DOX induced lipid peroxidation.

CHAPTER III

MATERIALS AND METHODS

Experimental Animal

The experiment was performed in accordance with the institutional guidelines and conformed to the graduate school, Chulalongkorn University. Male Sprague-Dawley rats, weighing between 250-350 g were obtained from NLAC, Mahidol University and placed in Salaya District, Nakornpathom province. The animals were housed under standard condition of light and dark cycle (L:D = 12:12) with free access to food and water. The animals were adapted to the laboratory condition before use for seven days.

Experimental protocol

One day before drug injection, the animals were kept in metabolic cage with free access to water only for the measurements of urine volume, urinary protein concentration, electrolyte concentration (Na^+, K^+, Cl^-), osmolarity and malondialdehyde (MDA). Plasma (P0) was collected by clipping the rat's tail for the measurement of plasma urea nitrogen (PUN) and creatinine. Animals were divided into four groups, each group contained eight animals.

Group 1 - Control group (n=8), rats received intravenous injection of isotonic saline (0.1 ml/100g body weight). Another injection of isotonic saline was given intraperitoneally at the dose of (0.1 ml/100g body weight) to replace L-carnitine and daily thereafter throughout the study (14 days).

Goup 2 - Doxorubicin group (n=8), rats received intravenous injection of DOX (7.5 mg/kg body weight) on day 1. The intraperitoneal injection of isotonic saline (0.1 ml/100g body weight) was given 1 hr before the first DOX injection and daily thereafter throughout the study (14 days).

Group 3 - Doxorubicin + L-carnitine (low dose) group (n=8), rats received the same dose of DOX group with an intraperitoneal injection of L-carnitine (40 mg/kg bodyweight) 1 hr before the first DOX injection and daily thereafter throughout the study (14 days).

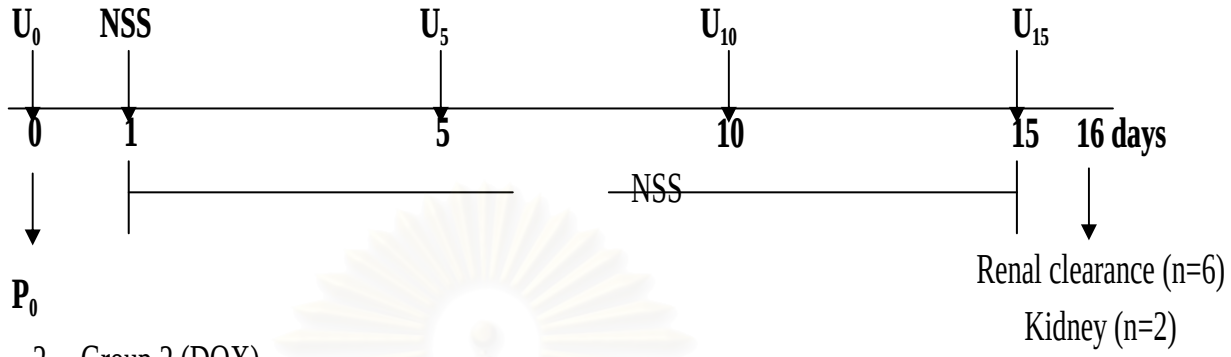
Group 4 - Doxorubicin + L-carnitine (high dose) group (n=8), rats received the same dose of DOX group with an intraperitoneal injection of L-carnitine (200 mg/kg bodyweight) 1 hr before the first DOX injection and daily thereafter throughout the study (14 days).

Body weight and food intake were recorded everyday throughout the experimental period. At day 5, 10 and 15 days urine sample were collected from rats for 24 hr in metabolic cages (U_5, U_{10}, U_{15}) 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 6 rats in each group on day 16. Each rat was anesthetized with pentobarbital (60 mg/kg b.w., i.p.). Before renal clearance study, blood was collected in heparinized tube. The plasma (Pb) was separated and kept at -20 C for measurements of total protein and albumin. Bovine serum albumin (6%) was administered after blood collection at the same volume to replace blood losses. Renal clearance was studied thereafter.

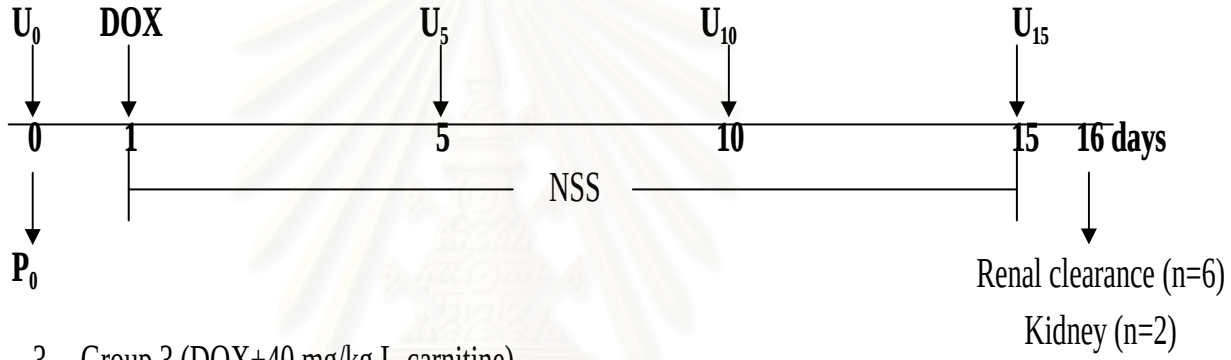
At the end of renal clearance study, 4 ml of blood (Pa) was collected to measure total cholesterol, creatinine, triglycerde, PUN, malondialdehyde (MDA), osmolality and eletrolyte (Na^+, K^+, Cl^-). Both kidneys were removed immediately. The cortex of kidney was homogenated and stored at -70 C for the determinations of MDA, reduced glutathione (GSH) and catalase (CAT) activities. In each group, two rats were not

performed the renal clearance but were anesthetized with pentobarbitone sodium and renal tissue was fixed in situ for histological studies.

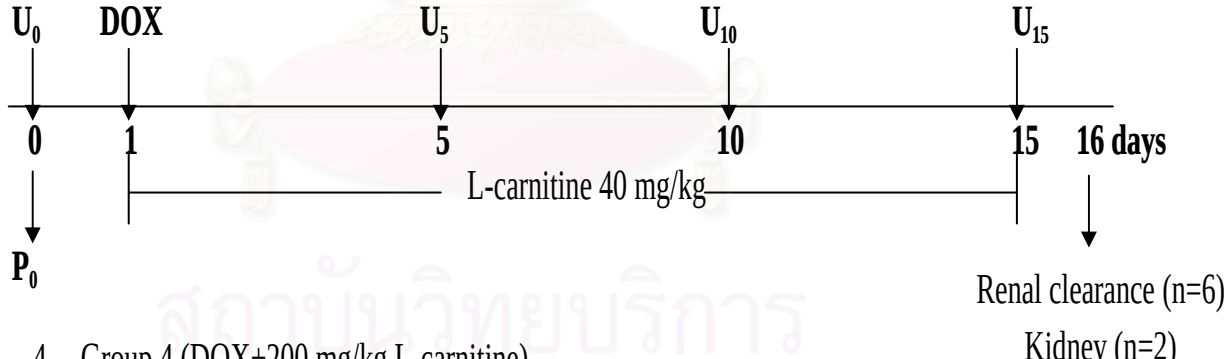
1. Group 1 (control)



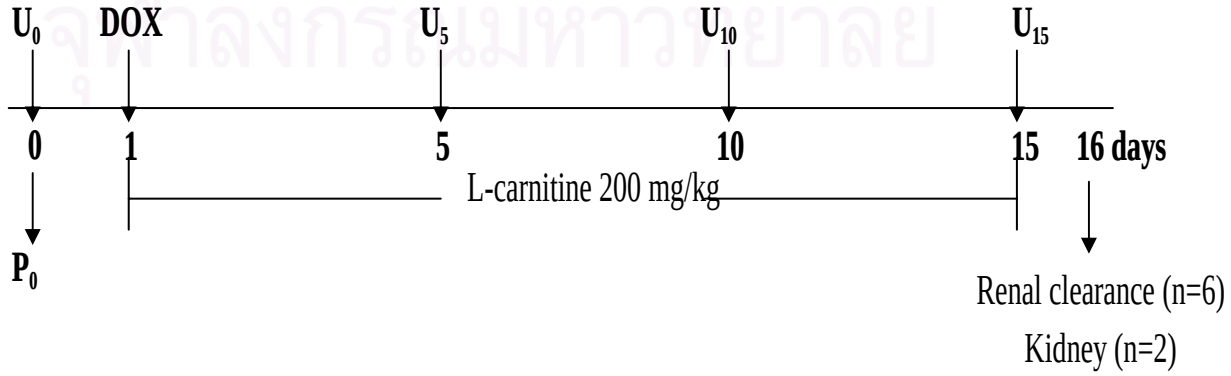
2. Group 2 (DOX)



3. Group 3 (DOX+40 mg/kg L-carnitine)



4. Group 4 (DOX+200 mg/kg L-carnitine)



U_0 U_5 U_{10} and U_{15}: Total protein, electrolyte (Na^+, K^+, Cl^-) Osmolarity and MDA
P_0: PUN and creatitine

Kidney: kidneys from renal clearance ⟶ MDA, GSH and CAT
2 rat kidneys ⟶ histopatology

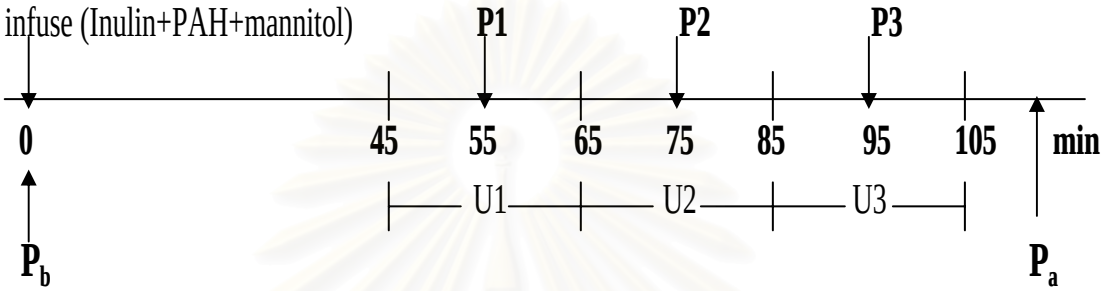
Operative procedure of renal clearance study

For renal clearance study, each rat was anesthetized with pentobarbitone sodium at a dose of 60 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 to monitor arterial blood pressure by connecting to a pressure transducer with a Glass polygraph recorder and also for blood collection. A polyethylene catheter was cannulated into the right femoral vein for infusion of inulin and para-aminohippurate (PAH) solution. The abdominal midline incision was performed and urinary bladder was located while the PE 250 catheter was inserted for urine collection. Urine samples were collected into a pre-weighed eppendorf.

Renal clearance study

Clearance study was started by infusing a mixture of 1% inulin, 0.1%PAH, 6% mannitol in normal saline at the rate of 1 ml/h per 100 g bodyweight continuously for 45 minutes to stabilize plasma inulin and PAH concentration. After equilibration period, three times of urine collections (U1, U2, U3) along with arterial blood sampling at midpoint of urine collections (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 was 20 minutes. Urine volume was measured from

the weight changes of pre-weighed eppendorf. Blood sample was collected for determinations of packed cell volume (PCV). Blood was separated and plasma and urine were kept at 4° C for analyses of inulin and PAH concentrations within 48 hr. Plasma samples for determinations of biochemical data were kept at -20° C for further analysis.



P1+P2+P3: inulin, PAH, PCV

U1+U2+U3: inulin, PAH

P_b: Total protein and albumin

P_a: cholesterol, triglyceride, creatinine, PUN, and MDA

Analytical procedures for determinations 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). Osmolality was measured by osmometer (Osmometer 3D3, Advance Instrument Inc., USA). The creatinine concentration was analyzed by picric acid. Plasma urea nitrogen concentration was measured by Urea Lipuicolor of Human Diagnostics. Total protein concentration and albumin concentration in plasma were measured by colorimetric method. Urine protein

concentration was measured by precipitating with sulfosalicylic while kidney protein concentration was measured according to Lowry et al (1951). Plasma cholesterol and plasma triglyceride were measured by colorimetric method. MDA from Plasma, urine and kidney were determined by method as descried by Ohkawa et al (1979). Kidney GSH content was measured by a modification of the method of Beutler et al (1963). Kidney catalase activity was determined following the method of Aebi et al (1983).

Determination of kidney urine and plasma MDA

The renal cortex were removed and immediately placed in iced phosphate buffered saline. Scar and surrounding capsule and fascia was excised, and the remaining tissue was homogenized with a polytron for 15 s in 3 ml 100 mM containing KCl plus 0.003M EDTA. The homogenized tissue was centrifuged at 600 g for 10 min. Supernatant (400µl) was added to a reaction mixture consisting of 0.2 ml 8.1% sodium dodecyl sulfate (SDS), 1.5 ml 20% acetic acid,1.5 ml 0.8% thiobarbituric acid, and 6.6 ml water. This solution was placed in a water bath kept at 95°C for 60 min. After removal from the water bath 1 ml water and 5 ml n-butanol-pyridine was added and samples was agitated and subsequently centrifuged at 2,000 g for 15 min. The upper organic layer was pipetted off and absorbance of this fraction was read at 532 nm in spectrophotometer. Malondialdehyde tetraethylactal was employed as the standard. MDA values will be expressed per milligram protein which was determined using Lowry method.

To determine urine MDA concentration, 500 µl of urine was employed with MDA assay describes above. Plasma MDA was obtained using 250 µl samples of plasma.

Determination of Kidney GSH

Renal GSH content was measured by a modification of Beutler et al. (1979). Renal cortex tissue (0.5 g) was suspended in 4.5 ml of 100 mM KCl plus 0.003 M EDTA and homogenized as described for the determination of MDA above. The homogenates was centrifuged at 600 g for 10 min. The 1 ml of supernatant was added to 1.5 ml methaphosphoric acid, and particulate debris was removed by centrifugation at 3,000 g for 10 min. 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. 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 (0.5 g) was transferred to tubes containing 1%Triton X100 following homogenization. The 100 µl of the supernatant was added to 1.9 ml of phosphate buffer. 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 sec for 1-2 min using UV-VIS spectrophotometer. The rate constant of a first-order reaction (k) was used: $k = (1/\Delta t) \times \ln (A1/A2)$, where Δt was a measure interval (30 s) and A1 and A2 were the absorbances at initial and final measurement, respectively. Catalase activity was expressed as sec^{-1} per milligram homogenate protein.

Structural studies

Two rats from each group were perfusing fixed in situ. Perfusion started with normal saline, followed by 18% glutaraldehyde in 0.02 M cacodylate buffer (pH=7.2) and 2% glutaraldehyde in 0.1 M cacodylate buffer (pH=7.2), afterward. Kidneys were collected and prepared for renal structural alteration studies at light microscopic level by fixing in 10% buffered formalin and processed for histological evaluation. Paraffin sections (3-4 µm) were stained with hematoxilin and eosin (H&E). Some sections were treated with periodic Acid Schiff reagent (PAS).

Severities of the lesions were graded as 0 to 4 according to 25, 50, 75 and 100 percent of tubular change in 10 visual fields at 10X.

Calculation

Mean arterial blood pressure (MAP) = $DP+1/3(PP)$

Glomerular filtration rate (GFR) = $\frac{UinV}{Pin}$

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

Effective renal blood flow (ERBF) = $\frac{ERPFx100}{(100-PCV)}$

Filtration fraction (FF) = $\frac{GFR\ x100}{ERPF}$

Renal vascular resistance (RVR) = $\frac{MAP}{ERBF}$

Urinary excretion of substance (x) = U_xV

$$\text{Fraction excretion of electrolyte (FEe)} = \frac{\text{UeV/Pe x 100}}{\text{GFR}}$$

$$\text{Cosm} = \frac{\text{UosmV}}{\text{Posm}}$$

$$\text{CH2O} = \text{V-Cosm}$$

Statistical analysis

Data were present as mean±SE. One way ANOVA with repeated measures design was used to compare data in the same group whereas the data between groups were compared with one-way ANOVA and post hoc analysis with student-Newman-keuls method were used to compare the data in all pairwise. Data of BUN and Creatinine before and after treatment (at day 16) were compared using paired-t-test. Non-parametric method was used to compare same data between groups in which normality failed by one way ANOVA on ranks. Differences between mean were considered significant at $P<0.05$. The Sigma-stat program was used for statistical analysis.

CHAPTER IV

RESULTS

Body weight and kidney weight

The body weight before and after treatment in eight rats of each group were showed in figure 3. Mean weight were similar before treatment. The average body weight of control (group 1), doxorubicin plus normal saline (group 2), doxorubicin plus low dose L-carnitine (group 3) and doxorubicin plus high dose L-carnitine (group 4) were 297.2 ± 4.6, 288.8 ± 5.7, 277.2 ± 7.7 and 281.3 ± 8.1 g, respectively. After 16 days of treatment, the body weight of group 2 was lower than group1 rats, but no significantly differed from group 3 or group 4 rats (table 1).

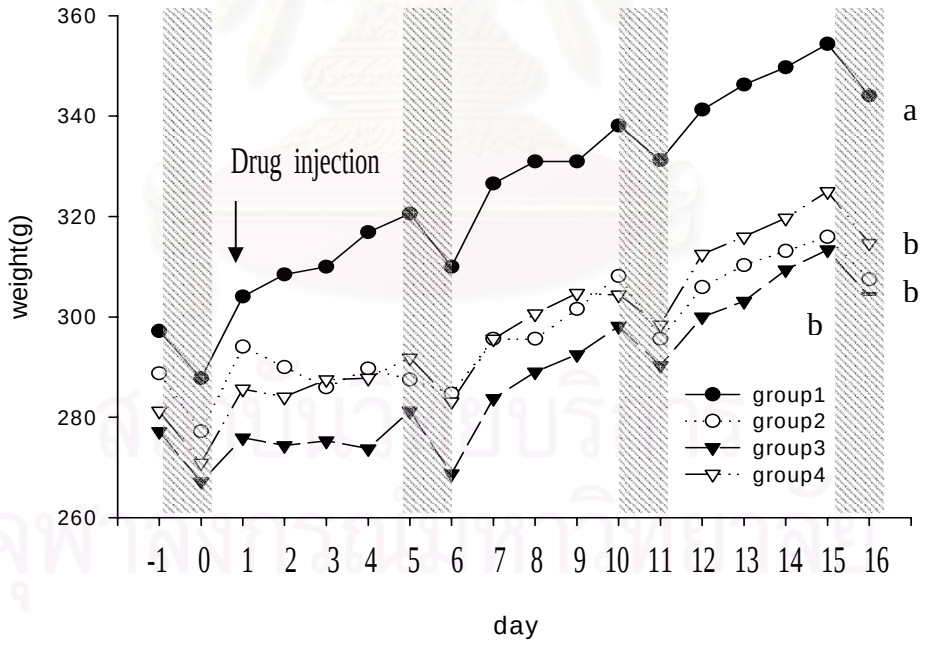


Figure 3. Daily mean values for body weight in four groups of rats throughout the experimental period.

[a, b] Means with different superscripts differ significantly ($p<0.05$) (at 16 days).

The shade area showed rats in metabolic cage.

Table 1. Body weight and kidney weight at 16 days of treatment in eight rats of each group.

	Body weight (g)	Kidney weight (g)
group 1	344.1 ± 8.5^{a}	2.51 ± 0.05^{a}
group 2	307.5 ± 5.5^{b}	3.95 ± 0.15^{b}
group 3	304.1 ± 6.0^{b}	3.75 ± 0.16^{b}
group 4	314.7 ± 8.2^{b}	3.52 ± 0.18^{b}

The data were show as mean±SE; n=8

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

group 1 = control group; group 2= doxorubicin plus normal saline

group 3 = doxorubicin plus L-carnitine 40 mg/kg/day

group 4= doxorubicin plus L-carnitine 200 mg/kg/day

At 16 days of treatment, the kidney weight obtained from both kidneys of rats in all group were presented in table 1. The kidney weights of group 2, 3 and 4 rats were increased significantly as compared with group 1 rats.

Food intake

Food intake before and after treatment in eight rats of each group were presented in figure 4. Mean food intake were similar to that before treatment. The average food intake of group 1, group 2, group 3 and group 4 were 17.40±2.33, 20.83±0.80, 21.33±0.74 and 20.06±0.97g, respectively. After injected doxorubicin, food intake of group 2, 3 and 4 were decreased and gradually increased in day 5 of treatment. At 15 days of treatment, the average food intake of group 1, group 2, group 3 and group 4 were 23.38±0.59, 20.21±0.90, 21.00±1.10 and 20.24±0.40g,

respectively. Food intake of group 1 was highest. Group 2, 3 and 4 were no significantly different among each other

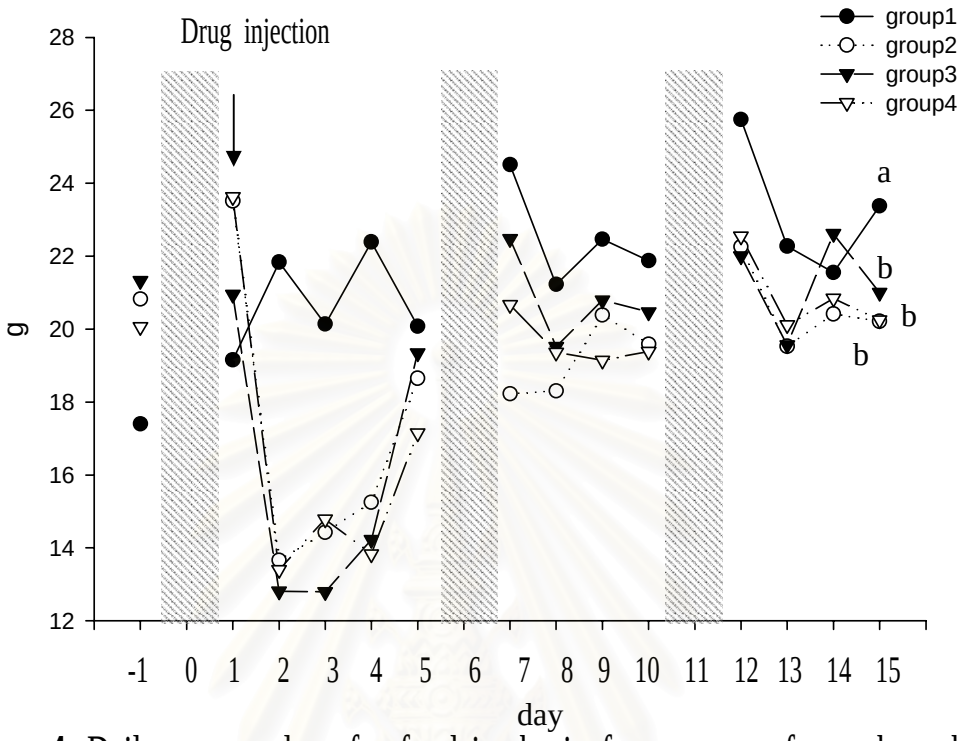


Figure 4. Daily mean values for food intake in four groups of rats throughout the experimental period.

[a, b] Means with different superscripts differ significantly ($p<0.05$) (at 16 days).

The shade area showed rats in metabolic cage.

Plasma creatinine and plasma urea nitrogen concentrations

Plasma creatinine and plasma urea nitrogen (PUN) before and after treatments in each group of rats were presented in table 2 and figure 5. Plasma creatinine and plasma urea nitrogen concentration in all groups were similar before treatment (day 0). After 16 days of treatment, the plasma creatinine concentrations were not significant difference although were slightly higher in group 2. The plasma urea nitrogen was higher in group 2 which received doxorubicin alone. However, group 3 and 4 had PUN less than group 2 and were not significant different from group 1 (control). In group 2,

plasma creatinine and PUN at day 16 after DOX treatment were higher significantly from before treatment (P<0.05).

Table2. Creatinine and PUN before and after treatment in each group.

	Creatinine (mg%)		PUN (mg%)	
	day0	day16	day 0	day16
group 1	0.40±0.00	0.45±0.02	18.23±1.28	16.56±0.90[a]
group 2	0.43±0.03	0.60±0.10[*]	18.28±0.59	33.42±4.09 [b,*]
group 3	0.40±0.03	0.47±0.03	19.17±1.49	21.58±2.04[a]
group 4	0.43±0.03	0.43±0.03	17.62±1.24	18.26±1.30[a]

The data were shown as mean ± SE, n=6

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

*=P<0.05Significantly compared with before treatment using paired t-test (p<0.05)

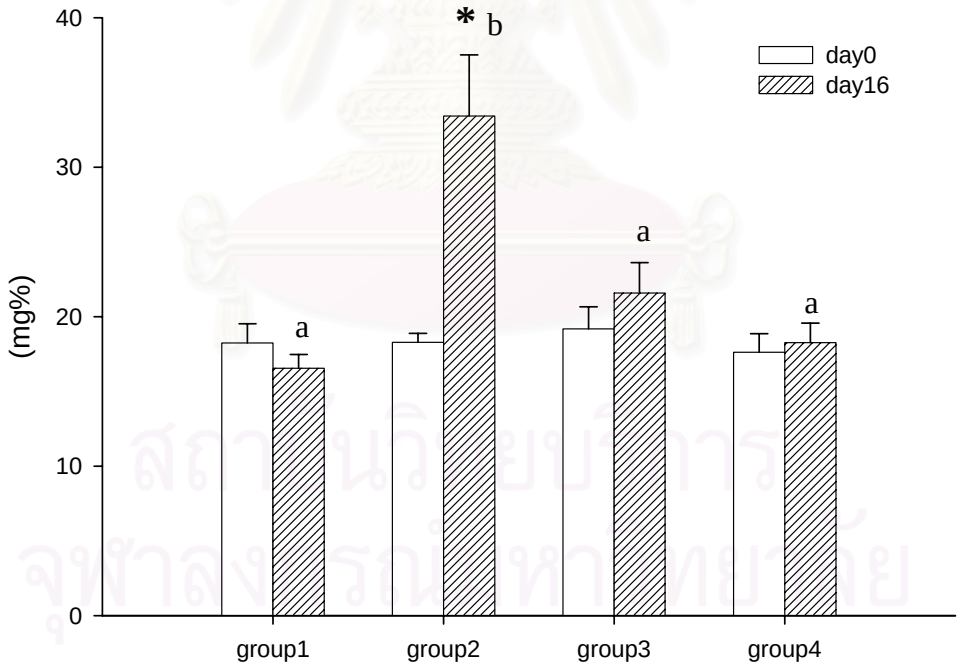


Figure 5. Mean values for PUN in four groups before and after treatment.

The data were show as mean±SE; n=6

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

*=P<0.05 significant different from PUN before treatment using paired t-test.

Effects of L-carnitine and doxorubicin on concentrations of total protein, albumin, triglyceride and cholesterol

Total protein, albumin, triglyceride and cholesterol in plasma at 16 day of treatment in each group of rats were presented in table 3. After 16 days of treatments, total protein concentrations in all groups were similar. Comparing albumin of all groups, concentration in group 2, 3 and 4 decreased significantly compared with control group 1. The lowest albumin concentration was found in group 2.

Triglyceride and cholesterol in group 2, 3 and 4 were higher than control group significantly (Table 3 and figure 6). There was no difference for triglyceride and cholesterol among groups 2, 3 and 4. However, the highest triglyceride and cholesterol concentrations were found in group 2.

Table 3. Total plasma protein, albumin, triglyceride and cholesterol at 16 days of treatment.

	Total protein (mg%)	Albumin (mg%)	Triglyceride (mg%)	Cholesterol (mg%)
group 1	4.95±0.10	2.66±0.14^{a}	32.37±8.34^{a}	57.73±7.64^{A}
group 2	5.00±0.29	1.85±0.10^{b}	247.25±50.73^{b}	258.72±51.52^{B}
group 3	4.98±0.10	2.23±0.13^{b}	228.10±50.82^{b}	174.15±35.60^{B}
group 4	5.27±0.15	2.17±0.15^{b}	161.20±37.04^{b}	167.16±22.71^{B}

The data were shown as mean±SE; n=6

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

^{A,B} Significant difference in non-parametric method ($P<0.05$)

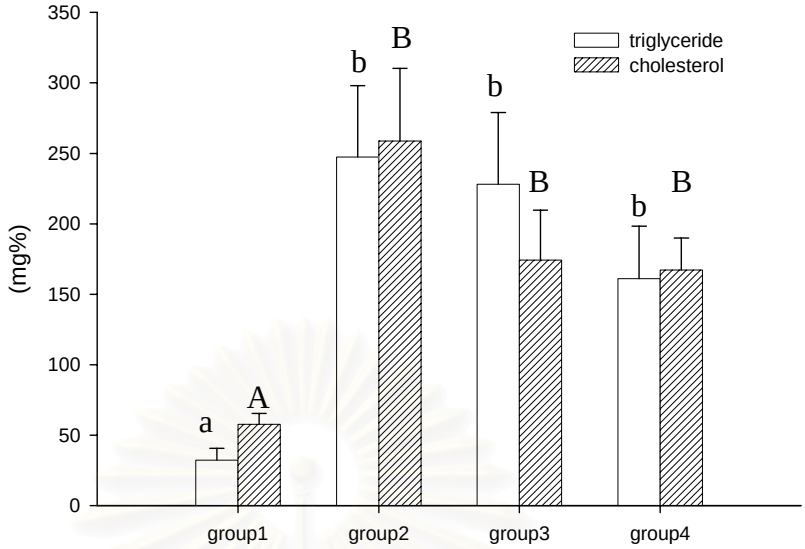


Figure 6. Mean values of triglyceride and cholesterol in four groups.
The data were shown as mean ± SE; n=6
[a, b] Means with different superscripts differ significantly ($p<0.05$)
[A,B] Significant difference in non-parametric method ($P<0.05$)

Effects on renal hemodynamics

Effects of L-carnitine and doxorubicin on mean arterial pressure (MAP), packed cell volume (PCV), heart rate (HR) and urine flow rate.

Mean arterial pressure, packed cell volume, heart rate (collected before renal clearance study) and urine flow rate at 16 days of treatment in each group of rats were presented in table 4. After 16 days of treatments, there were no significant changes in MAP and HR in all groups. Groups 2, 3 and 4 which were injected doxorubicin; PCV was lower significantly than control group. The lowest urine flow rate was found in group 2 and was significantly less than group 1, 3 and 4.

Table 4. Mean arterial pressure, packed cell volume, heart rate and urine flow rate at 16 days of treatment.

	MAP (mmHg)	PCV (%)	HR (beat/min)	urine flow rate (ml/kg/min)
group 1	142 ± 4	50.2 ± 1.0[a]	230±0.54	0.21±0.02[a]
group 2	139 ± 5	44.8 ± 1.3[b]	232±0.08	0.15±0.01[b]
group 3	132 ± 4	46.3 ± 1.2[b]	236±0.00	0.18±0.01[a]
group 4	147 ± 7	45.0 ± 1.9[b]	238±0.07	0.19±0.02[a]

The data were shown as mean±SE; n=6

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

Effects of L-carnitine and doxorubicin on glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF), renal vascular resistance (RVR) and filtration fraction (FF).

Glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF), renal vascular resistance (RVR) and filtration fraction (FF) in each group of rats were showed in table 5 and figure 7. Glomerular filtration rates (GFR) were decreased significantly in doxorubicin injection rats alone (Group 2) and DOX with low dose L-carnitine (group 3). Group 4 which received high dose L-carnitine, GFR was reversed nearly to the control group.

Table 5. Glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF) and renal vascular resistance (RVR) and filtration fraction (FF) in all group.

	GFR (µl/g/min)	ERPF (µl/g/min)	ERBF (µl/g/min)	RVR (mmHg/µl.g^{-1}.min^{-1})	FF (%)
group 1	5.63±0.23[a]	25.51±1.59[a]	46.58±3.73[a]	2.98±0.18[a]	22.31±1.05
group 2	3.67±0.38[b]	16.01±1.85[b]	25.66±2.59[b]	5.62±0.47[b]	24.32±3.56
group 3	3.80±0.37[b]	21.72±2.24[ab]	37.20±3.80[ab]	3.73±0.40[a]	18.97±3.49
group 4	4.99±0.42[a]	20.53±2.03[ab]	34.49±4.80[ab]	4.39±0.47[ab]	24.74±1.57

The data were show as mean±SE; n=6

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

There was no significant difference among the FF from all groups of rats. Group 3 (low dose L-carnitine) tended to have lower FF compared to other groups. The ERBF and ERPF were decreased significantly in group 2 which received doxorubicin alone. Group 3 and group 4 which treated (L-carnitine treated rats) tended to have higher ERPF and ERBF than group 2.

The RVR of group 2 appeared to be highest among all groups. The RVR of group 4 had tendency to increase, but it was not significant difference from those of the other groups (figure 7).

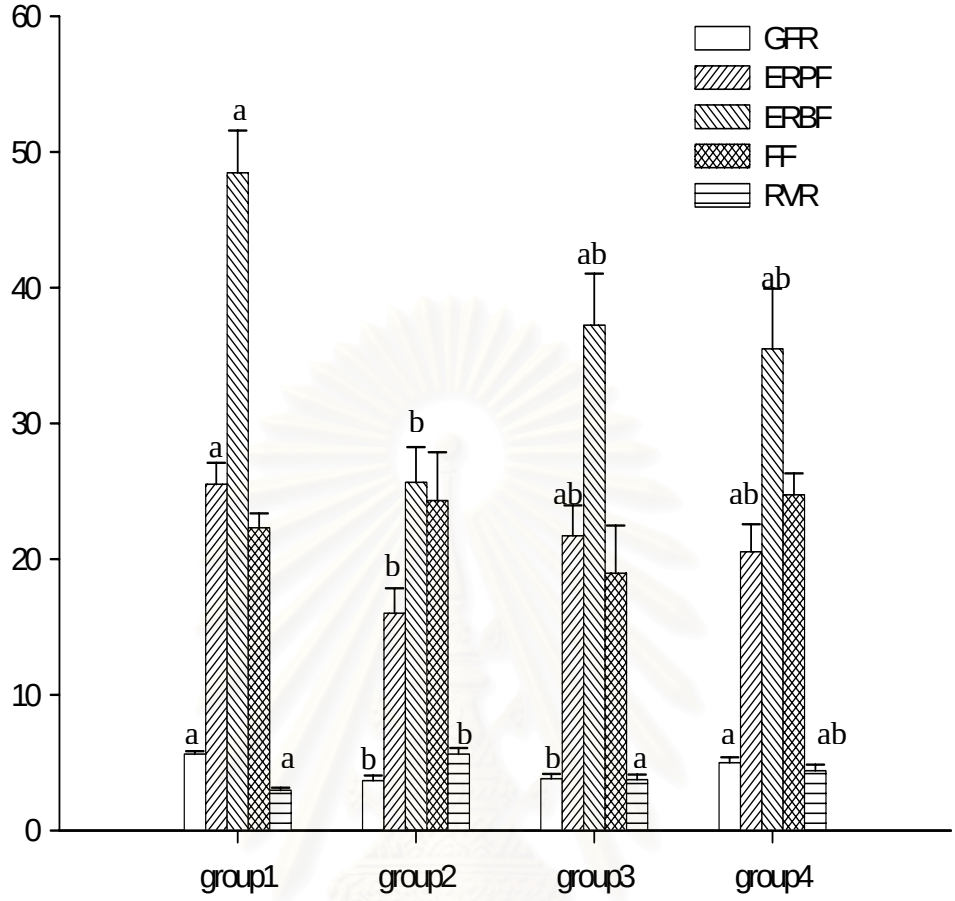


Figure 7. Mean values of glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF), renal vascular resistance (RVR) and filtration fraction (FF) in four group.

The data were shown as mean ± SE; n=6

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

Plasma electrolyte concentrations and fractional electrolyte excretions.

The plasma electrolyte concentrations (Na^+, K^+, Cl^-) and osmolarity at 16 day after treatment were presented in table 6. No difference was found in plasma Na^+, K^+, Cl^- and osmolarity in all groups.

Table 6. The plasma electrolyte concentrations in all groups at 16 days of treatment

Plasma	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)	Osm (mOsm/l)
group 1	108.7 ± 1.8	2.95± 0.14	120.0±1.7	317.5±6.3
group 2	108.2± 1.7	3.48± 0.16	120.8±2.1	350.7±10.2
group 3	110.5± 3.7	3.37± 0.16	120.7±1.6	332.7±11.1
group 4	110.2± 1.2	3.35± 0.19	123.7±1.7	322.0±7.7

The data were show as mean±SE; n=6

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

Fractional excretion (Na^+, K^+, Cl^-), osmolarity clearance (Cosm) and free water clearance (CH_2O) were presented in table 7. FEK^+ and $FECl^-$, Cosm and CH_2O were no significantly different among all groups. The fractional sodium excretion in group 2 was higher when compared to group 1 and group 3. In group 4 treated with high dose L-carnitine, FENa had a tendency to decrease without a difference from group 2.

Table 7. Fractional excretions (Na^+, K^+, Cl^-), osmolarity clearance (Cosm) and free water clearance (CH_2O)

Parameter	group 1	group 2	group 3	group 4
FE Na (%)	1.59 ± 0.12^{a}	3.56 ± 0.77^{b}	1.60 ± 0.36^{a}	2.18 ± 0.40^{ab}
FE K (%)	16.47± 1.04	31.67 ±5.57	14.77± 1.18	21.13± 4.21
FE Cl (%)	1.26±0.14	2.64±0.57	0.99±0.31	1.96±0.61
Cosm (ml/day)	48.02±5.85	49.26±6.50	35.94±5.24	52.65±8.67
CH2O (ml/day)	26.93±3.69	31.41±1.33	24.97±4.36	34.70±5.08

The data were shown as mean±SE; n=6

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

Effects of L-carnitine and doxorubicin on urinary excretion

Effects of L-carnitine and doxorubicin on urinary protein excretion

Protein in urine at 0, 5, 10 and 15 days of treatment in each group of rats were presented in table 8 and figure 8. Protein excretion started to increase on day 5 after DOX injection. At days 10 and 15, protein excretion was significantly increased in rats injected with doxorubicin (group 2, 3, and 4) compared with day 0. When comparing among groups, groups 2, 3 and 4 had higher protein excretion at day 10 and 15 compared with control group 1. On day 15, increased in protein excretion was more than 30 fold in group 2 compared with group 1. On day 15, group 3 and 4 rats which were received carnitine with DOX had slightly lower protein excretion compared with rats received only DOX.

Table 8. Urinary protein excretion (mg%/day) at 0, 5, 10 and 15 days of treatment in four groups.

	group 1	group 2	group 3	group 4
Day0	14.23±1.80	23.69±44.41^{x}	15.05±1.08^{x}	15.87±2.50^{x}
Day5	26.69±5.90	63.72±23.14^{x}	35.79±7.53^{x}	24.48±6.69^{x}
Day10	22.39±4.32^{a}	401.7±69.3^{b,y}	428.0±87.2^{b,y}	335.2±30.00^{b,y}
Day15	24.44±2.96^{a}	781.31±62.4^{b,y}	524.5±109.9^{b,y}	620.9±78.89^{b,y}

The data were show as mean±SE; n=8

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

$^{x, y}$ Means in the same column with different significantly ($p<0.05$)

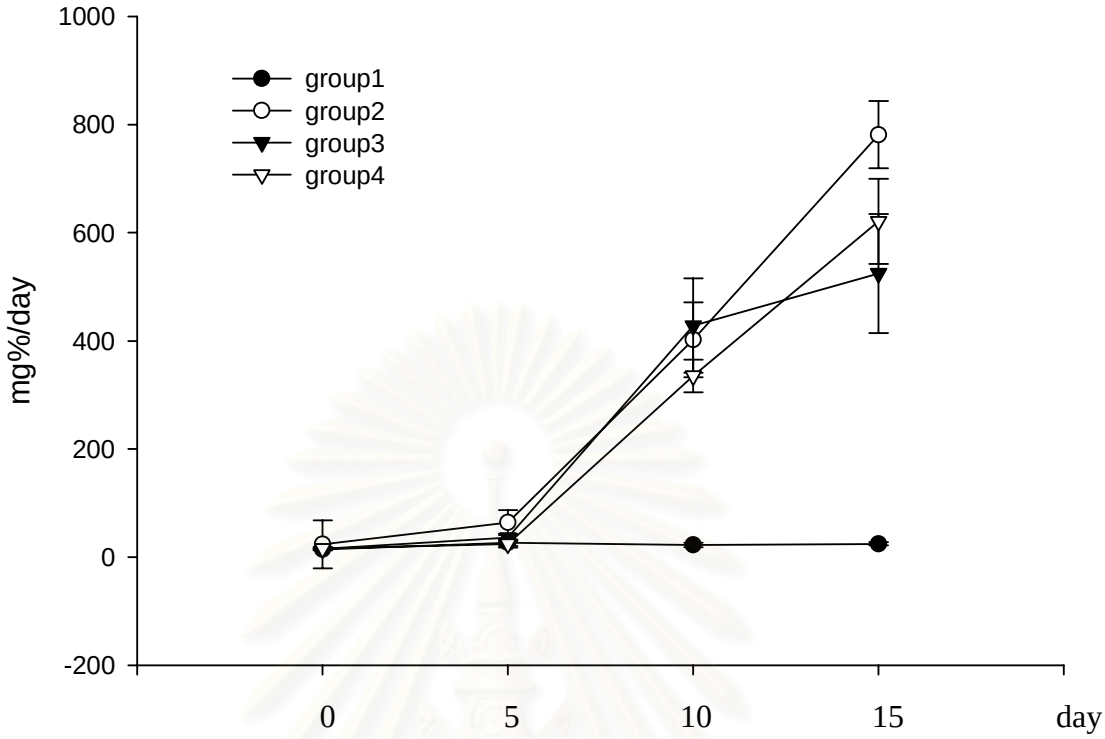


Figure 8. Mean values of urinary protein excretions in 8 rats on day 0, day 5, day 10 and day15 of treatment.
The data were show as mean±SE; n=8

Effects of L-carnitine and doxorubicin on urinary electrolyte excretion

Electrolytes in urine at 0, 5, 10 and 15 days of treatment in each group of rats were presented in table 9**.** The urinary excretion of K^+ was significantly lower in rats received both low and high dose of L-carnitine plus DOX (group 3 and 4) compared with group 1 only on day 5. In group 4, urinary excretions of both Na^+ and K^+ were significantly higher than on day 15 compared to day 0 and 5.

Table 9. Urinary excretion of the electrolytes, sodium, potassium, chloride of four groups.

Day	group 1 (mEq/day)			group 2 (mEq/day)			group 3 (mEq/day)			group 4 (mEq/day)		
	UNa*V	UK*V	UCl*V	UNa*V	UK*V	UCl*V	UNa*V	UK*V	UCl*V	UNa*V	UK*V	UCl*V
0	3.21±0.38	0.82±0.12	2.72±0.25	3.16±0.25	1.04±0.21	2.75±0.22	2.74±0.21	0.71±0.04	2.10±0.08	2.85±0.24^{x}	0.69±0.10^{x}	2.13±0.25
5	3.92±0.29	1.18±0.13^{a}	3.55±0.33	3.24±0.50	0.93±0.15^{ab}	2.73±0.27	2.53±0.42	0.64±0.10^{b}	2.15±0.41	2.88±0.28^{x}	0.70±0.10^{x,b}	2.81±0.45
10	3.59±0.48	1.12±0.20	2.94±0.28	3.29±0.56	0.98±0.18	3.28±0.37	3.58±0.58	0.88±0.13	2.75±0.40	3.62±0.05^{xy}	1.02±0.15^{XY}	3.30±0.41
15	3.00±0.37	1.18±0.12	3.53±0.32	4.62±0.73	1.36±0.25	4.39±1.07	3.55±0.67	0.89±0.11	2.29±0.56	4.38±0.57^{y}	1.29±0.18^{y}	4.19±1.07

The data were show as mean±SE; n=8

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

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

Effects of L-carnitine and doxorubicin on urinary malondialdehyde (MDA) excretion

MDA excretion in urine at day 0,5,10 and 15 after treatment in each group of rat were presented in table 10 and figure 9. Urinary malondialdehyde excretion of all group showed no significance in every periods of experiment.

Table 10. Urinary malondialdehyde excretion (nmol/day) at day 0,5,10 and 15 after treatment.

	group 1	group 2	group 3	group 4
day0	120.8±10.9	156.0±22.8	135.7±14.7	168.7±49.9
day5	194.6±20.2	224.2±40.9	167.4±25.9	150.7±17.9
day10	155.7±28.4	203.4±18.5	170.9±30.9	204.6±34.7
day15	198.3±20.5	266.4±55.0	190.1±18.8	185.5±13.6

The data were show as mean±SE, n=8

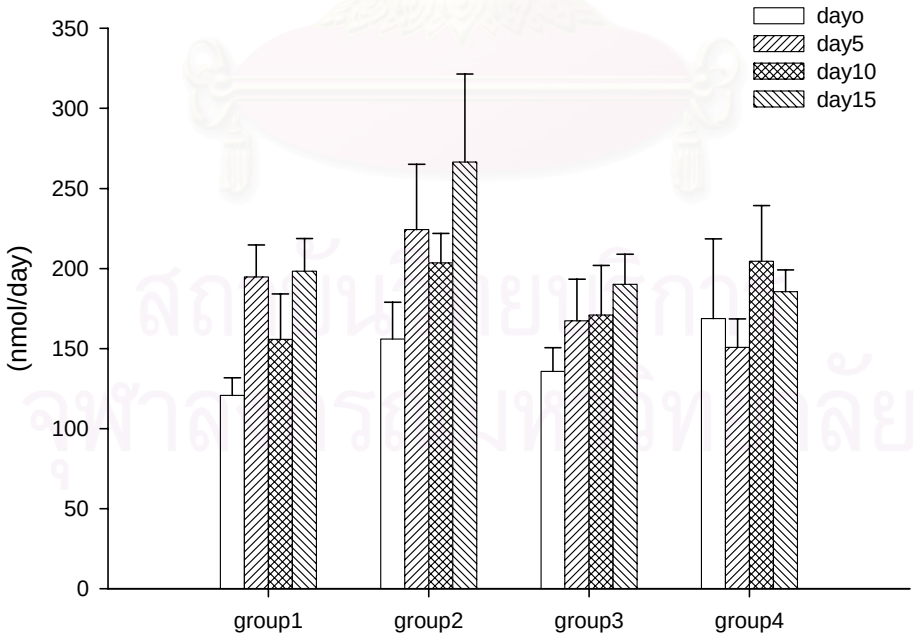


Figure 9. Mean values of urinary MDA excretion at day 0, 5, 10 and 15
The data were show as mean±SE, n=8

Effects of L-carnitine and doxorubicin on malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT) in kidney and MDA in plasma.

There were no significant changes of GSH, MDA in kidney and MDA in plasma in four groups. However, group 2, 3 and 4 which received doxorubicin with and without carnitine produced increase in MDA of both kidney and plasma, compared with group 1. The highest values of plasma MDA were found in group 2 (table 11.). Catalase (CAT) activities collected from kidney in all groups were significantly different from each other. The CAT activity was lowest in group 2 and highest in group 4 (table 11. and figure 10.).

Table 11. Reduced glutathione, catalase activity, malondialdehyde in kidney and malondialdehyde in plasma at 16 days of treatment

	Kidney			Plasma
	MDA (nmol/mg protein)	GSH (nmol/mgprotein)	CAT (S^{-1}/mg protein)	MDA (nmol/ml)
group 1	5.00 ± 0.90	460.5± 63.0	0.74 ± 0.03^{A}	6.92 ± 1.16
group 2	6.27 ± 1.08	468.2± 48.9	0.35 ± 0.03^{B}	16.81 ± 6.50
group 3	6.74 ± 1.05	528.0± 58.9	0.56 ± 0.03^{C}	11.32 ± 3.25
group 4	5.30 ± 0.58	513.9± 22.6	1.24 ± 0.14^{D}	10.26 ± 2.43

The data were show as mean±SE, n=6

^{A,B,C,D} Significant difference in non-parametric method ($P<0.05$)

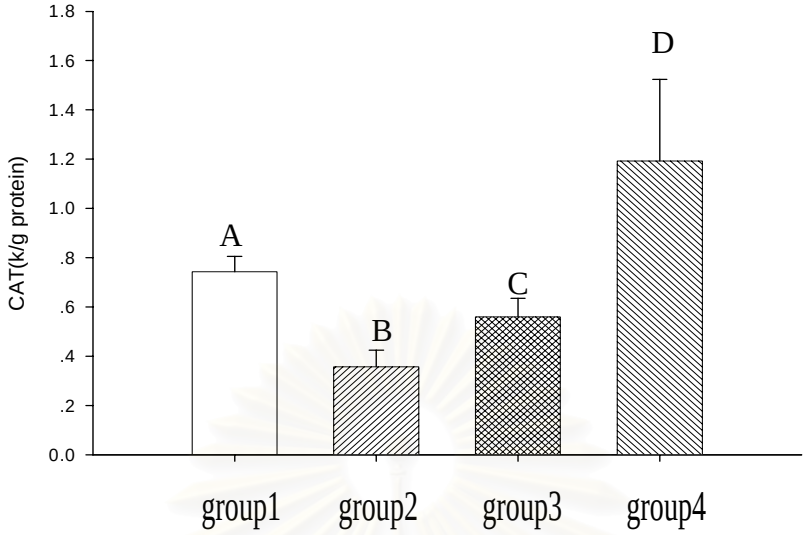


Figure10. Mean values of CAT activity in kidney.
The data were show as mean±SE, n=6
[A,B,C,D] Significant difference in non-parametric method ($P<0.05$)

Structural alteration

The kidney in doxorubicin group was enlarged and swollen, so as those in low and high dose L-carnitine groups. However, the kidney in doxorubicin (group 2) was very pale and enlarged.

- **Glomerular alterations**

After 16 days of treatment, glomerulus from the control group was generally normal (figure 11). Glomerular changes were observed in the kidney of the doxorubicin group. Hyaline droplets were found in the glomerular tuft and urinary space (figure 12 & 13). Furthermore, small hyaline droplets were scattered in the proximal tubular epithelium as well (figure 12). Thickening of the Bowman's capsule basement membrane (figure 14) can be readily observed with the PAS staining. Some of the glomerular capillary of the doxorubicin group developed severe dilation (figure

15). Shrinkage of glomeruli and widening of glomerular spaces were also observed (figure 16)

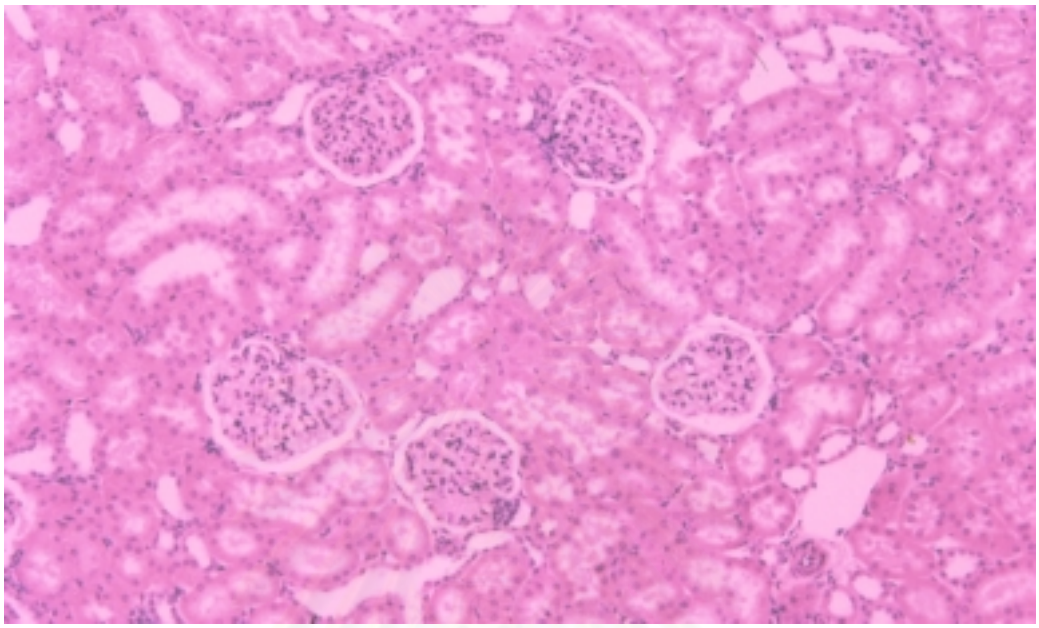


Figure 11. Glomerulus of the control group appeared normal. H&E, 10X

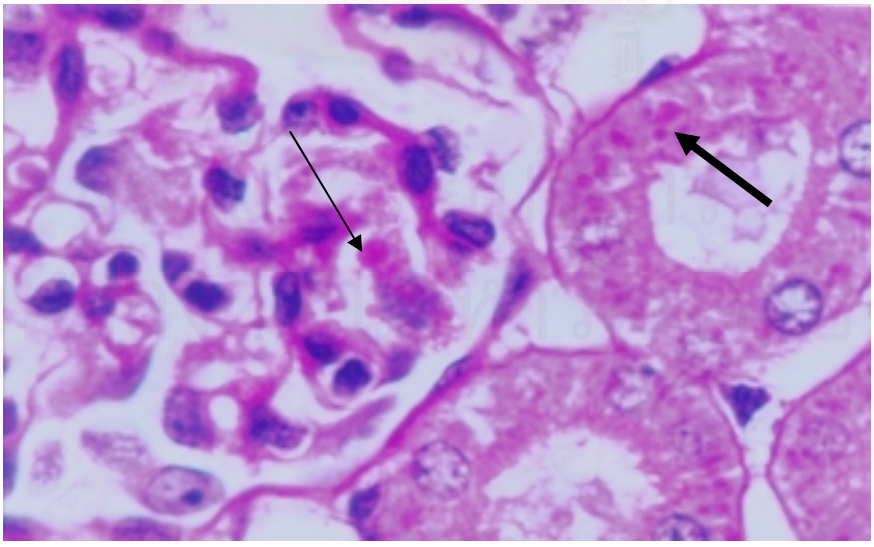


Figure 12. Rat kidney (doxorubicin). Glomerulus had hyaline droplets in the visceral epithelium (arrow) and proximal tubules (thick arrow). PAS, 100X

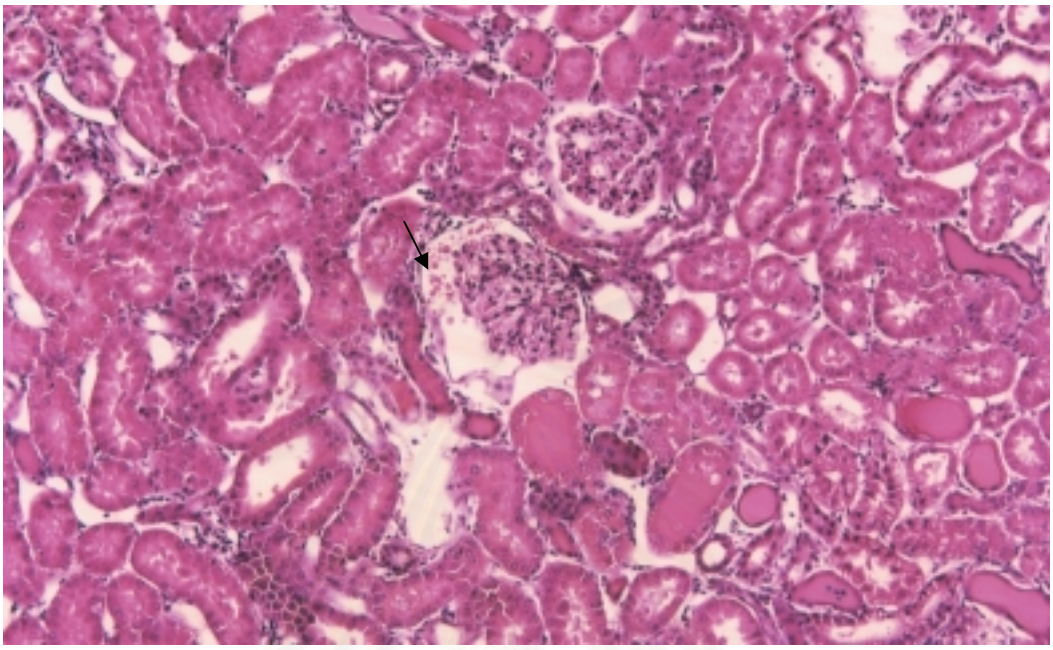


Figure 13. Rat kidney (doxorubicin). Glomerulus with numerous hyaline droplets in the urinary space (arrow). PAS, 10X

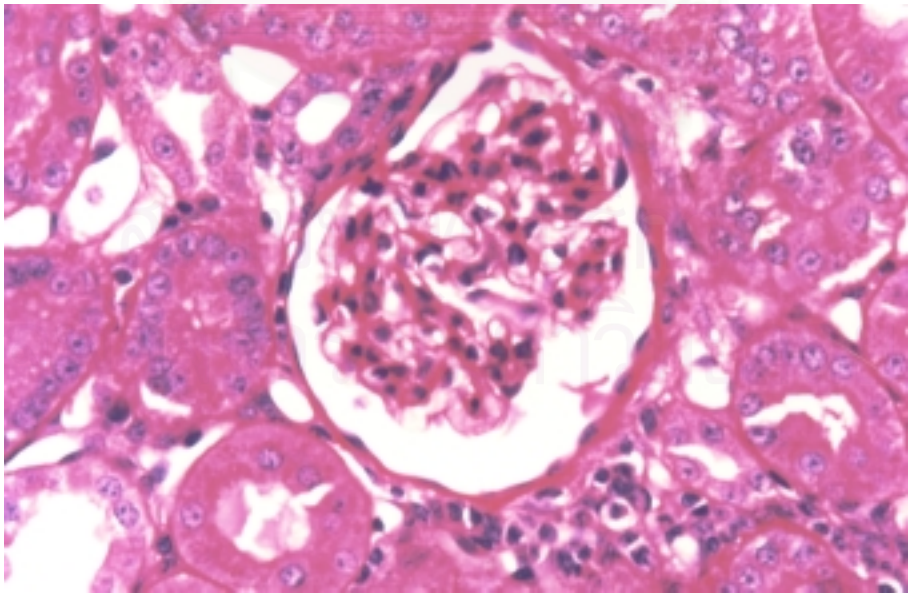


Figure 14. Rat kidney (doxorubicin). Glomerulus with thickening of Bowman's capsule. PAS, 40X

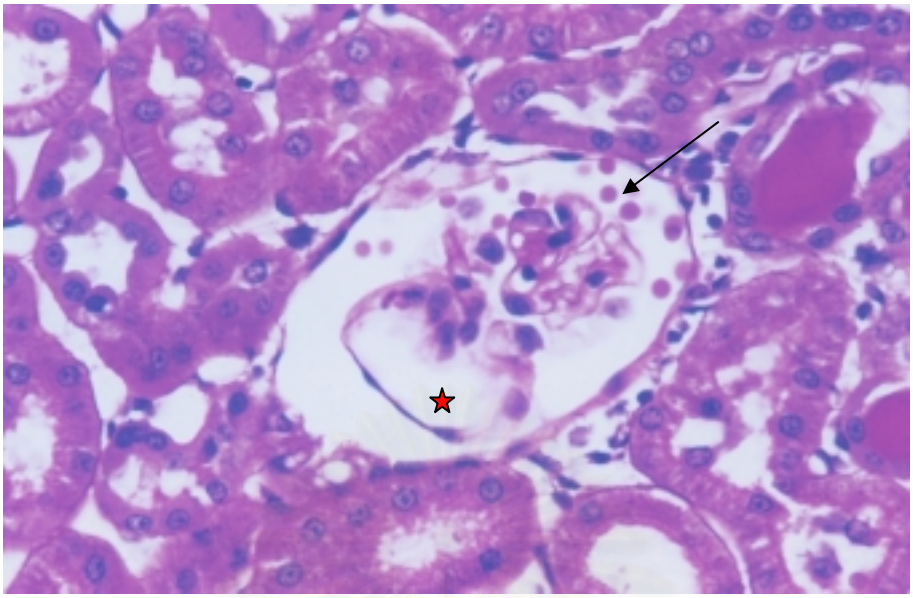


Figure 15. Rat kidney (doxorubicin). Glomerulus with hyaline droplets in the urinary space (arrow) and severe capillary dilatation (star). PAS, 40X

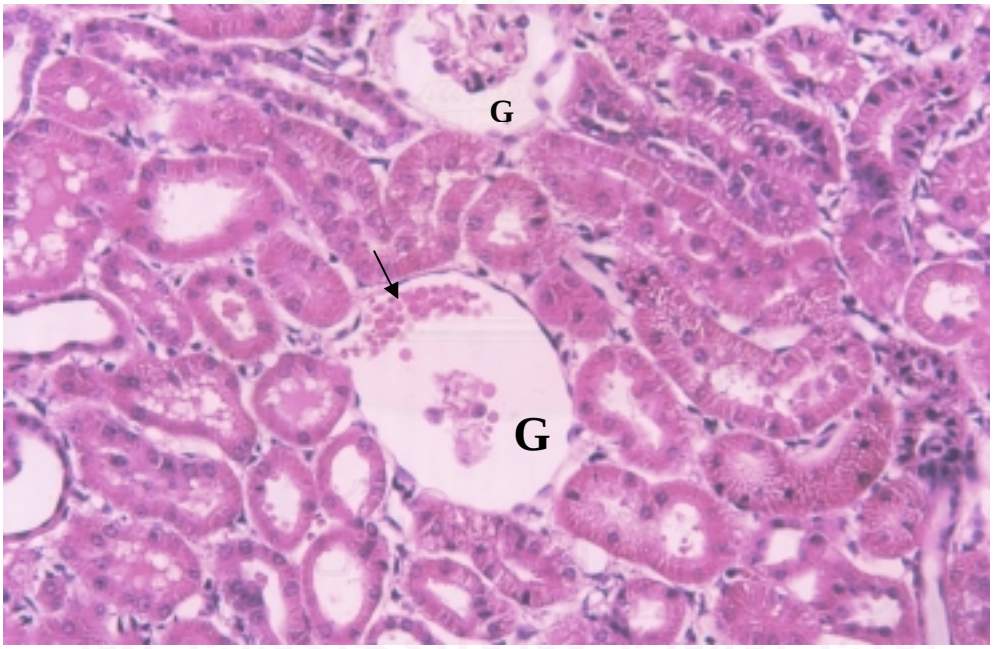


Figure 16. Rat kidney (doxorubicin). Shrinkage of glomeruli and widening of glomerular spaces (G) with numerous hyaline droplets (arrow). H&E, 20X

The kidney in doxorubicin plus low dose L-carnitine group had modulate hyaline droplets in the glomerular space. Capillary dilation was also observed (Figure 17). Most of the glomeruli in the doxorubicin plus high dose L-carnitine group showed

mild alterations with capillary dilation with abundant hyaline cast was found in the tubular space. Shrinkage of glomeruli and widening of glomerular space were also observed (figure 18).

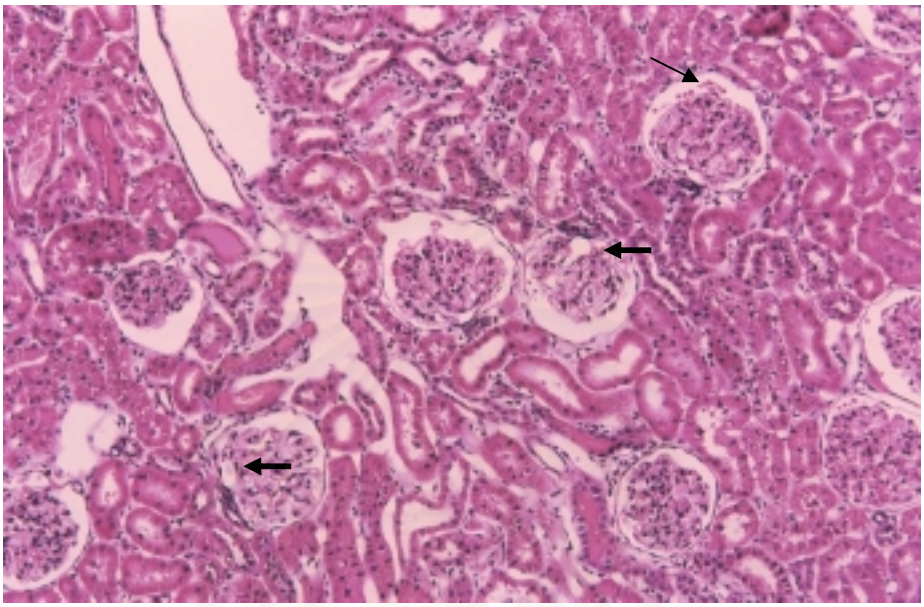


Figure 17. Rat kidney (doxorubicin plus low dose L-carnitine). Glomeruli showed hyaline droplets (arrow) and capillary dilation (thick arrow). H&E, 10X

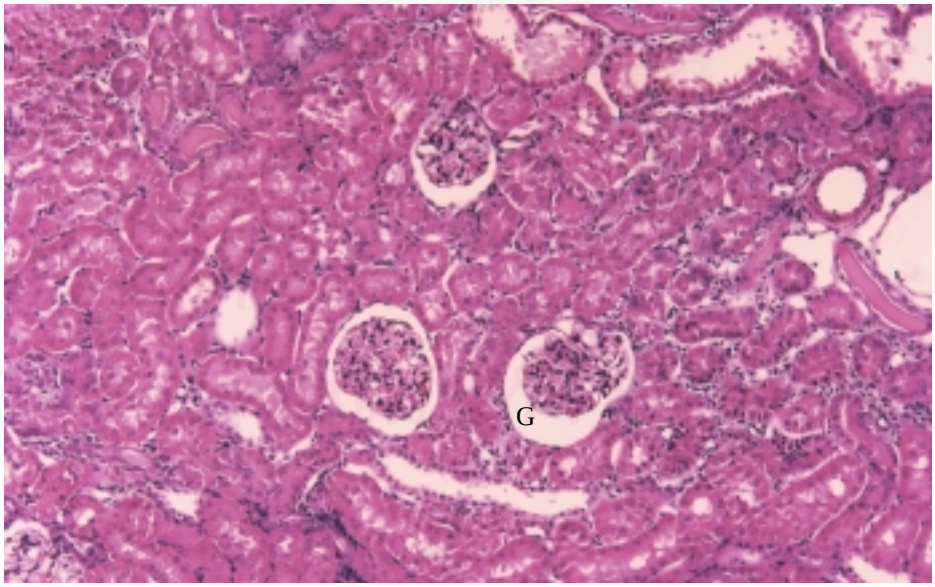


Figure 18. Rat kidney (doxorubicin plus high dose L-carnitine). Shrinkage of glomeruli and widening of glomerular spaces were in the picture (G) H&E, 10X

- **Tubular and interstitial alterations**

Tubular epithelial from the control group appeared normal (figure 19).

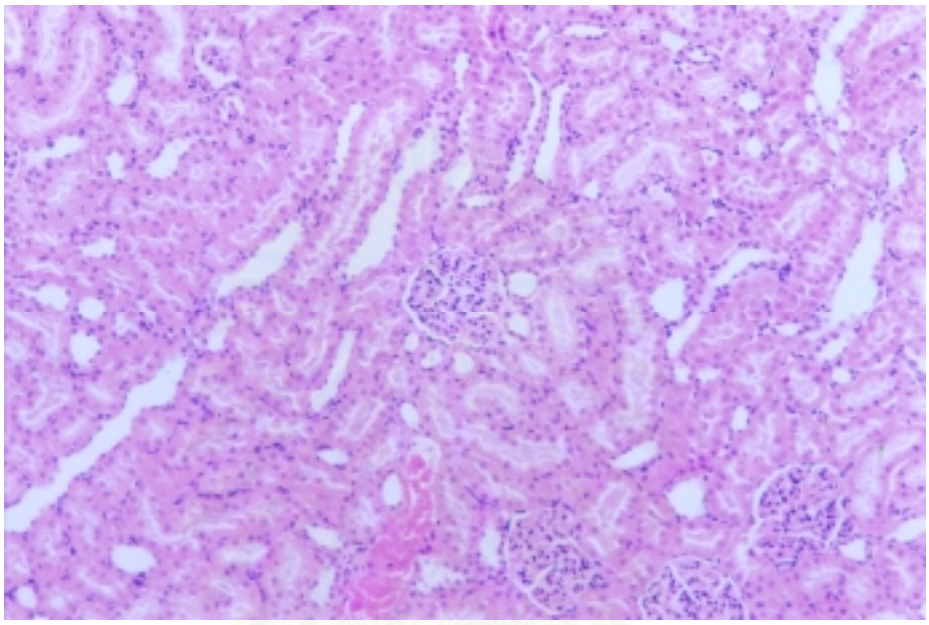


Figure 19. Control group, tubular epithelium showed no alteration. H&E, 10X

Kidneys of the doxorubicin group developed tubular dilation with hyaline cast in the tubular lumen (figures 20&23). Interstitial edema was generally localized in the kidney treated with doxorubicin (figure 20). Proximal tubular epithelium contained numerous hyaline droplets while some epithelial cells were in mitotic figures (figure 21). Severe interstial swelling with devoid of tubular structure was eminent in some areas (figure 22). Interstitial leukocytic infiltration was evident in the doxorubicin group (figure 23). Desquamated tubular epithelial were found in the lumen (figure 24).

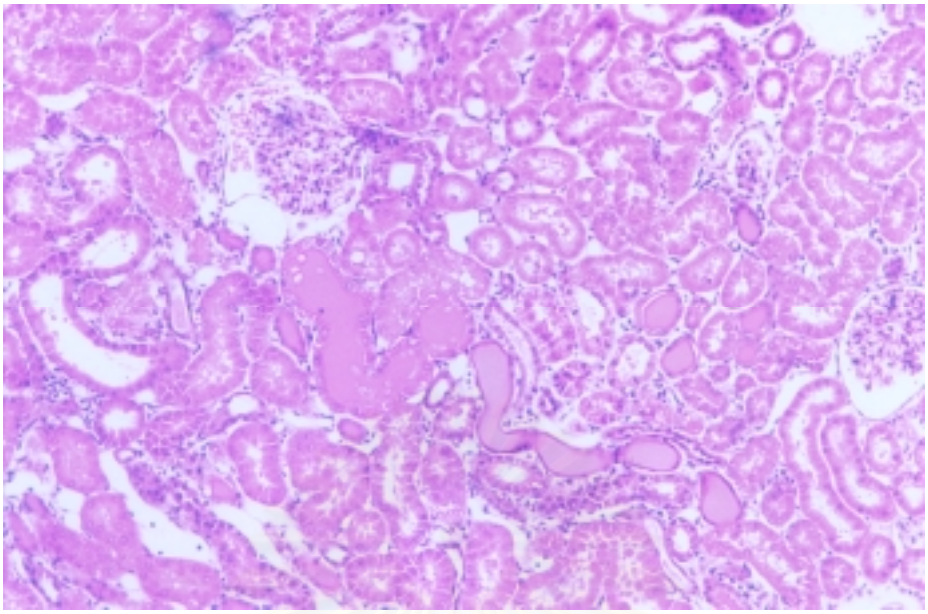


Figure 20.Rat kidney (doxorubicin). The kidney tubules were dilated with voluminous hyaline cast inside. The interstitium appeared edematous. H&E, 10X

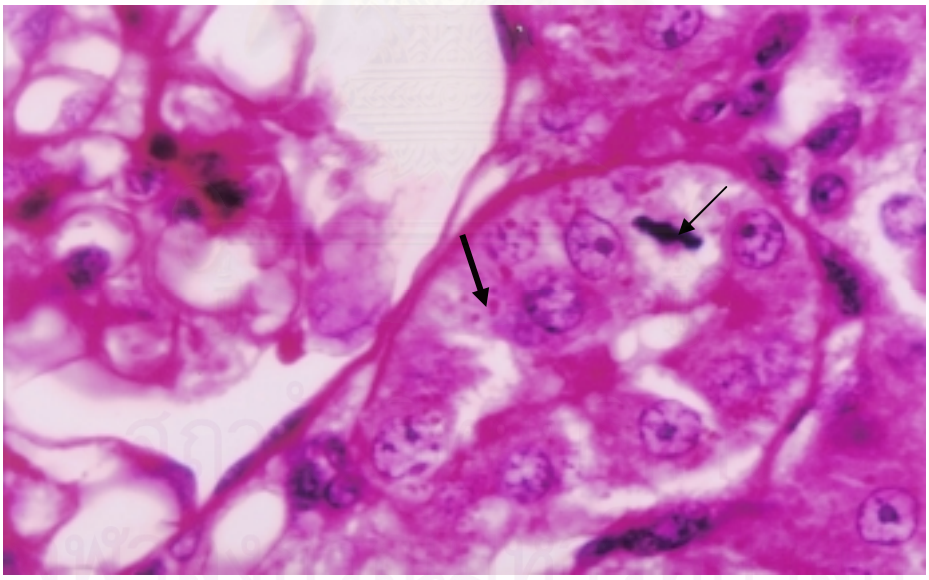


Figure 21. Rat Kidney (doxorubicin). Proximal tubular cell with mitotic figure (arrow) and hyaline droplets in the cytoplasm (thick arrow). PAS, 100X

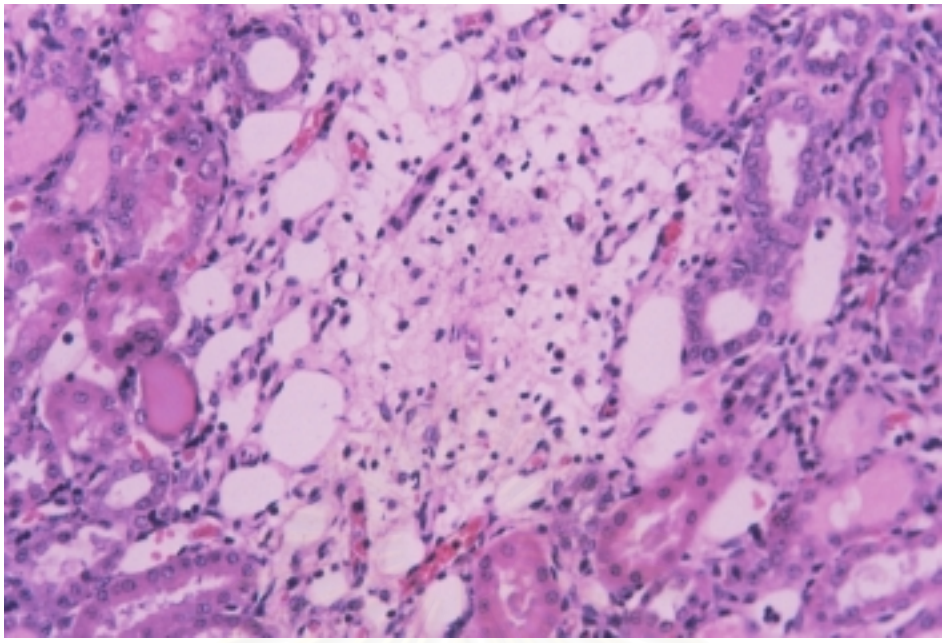


Figure 22. Rat kidney (doxorubicin) with severe interstitial swelling. PAS, 10X

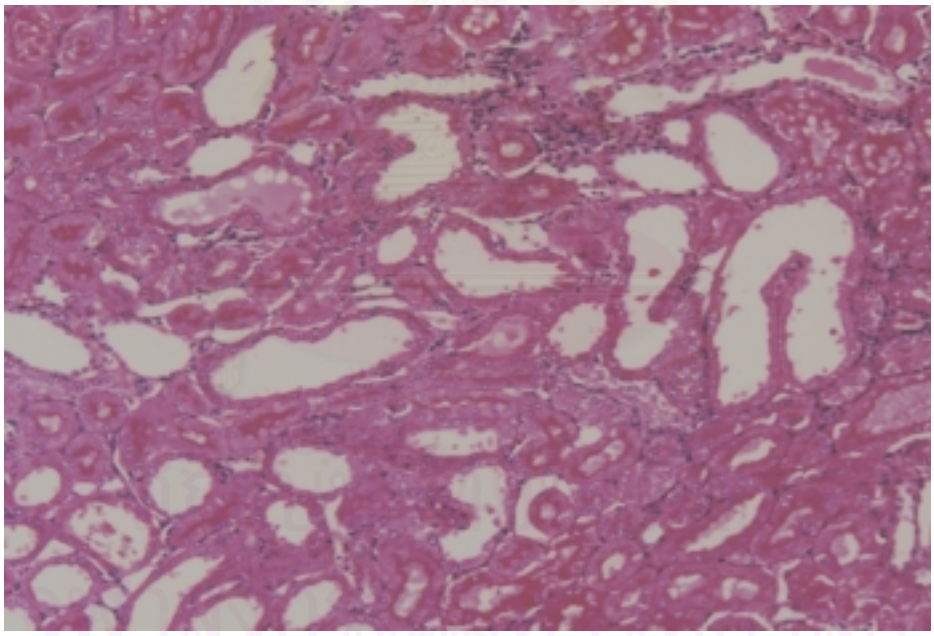


Figure 23. Rat kidney (doxorubicin). Tubular dilatation with interstitial leukocytic infiltration. PAS, 10X

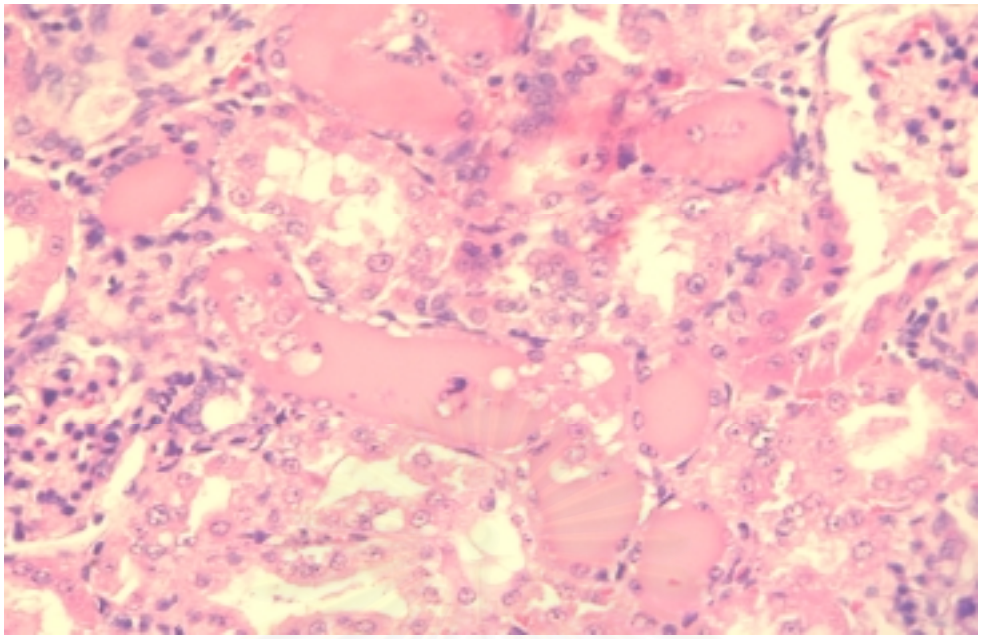


Figure 24. Rat Kidney (doxorubicin). Tubules were distended with hyaline cast and cellular debris. H&E, 20X

The kidney of the doxorubicin plus low dose L-carnitine had moderate tubular dilation with hyaline cast in the lumen. Epithelial desquamation was also prominent (figure 25). The kidneys of the doxorubicin plus high dose L-carnitine showed mild alteration with modulate tubular dilation and hyaline cast in some tubules. Protein droplets in the proximal tubular epithelium were still observed with desquamated cellular debris in the tubular lumen (figure 26). Kidneys treated with doxorubicin plus low L-carnitine or high L-carnitine had comparable degree of edema (figures 25&26).

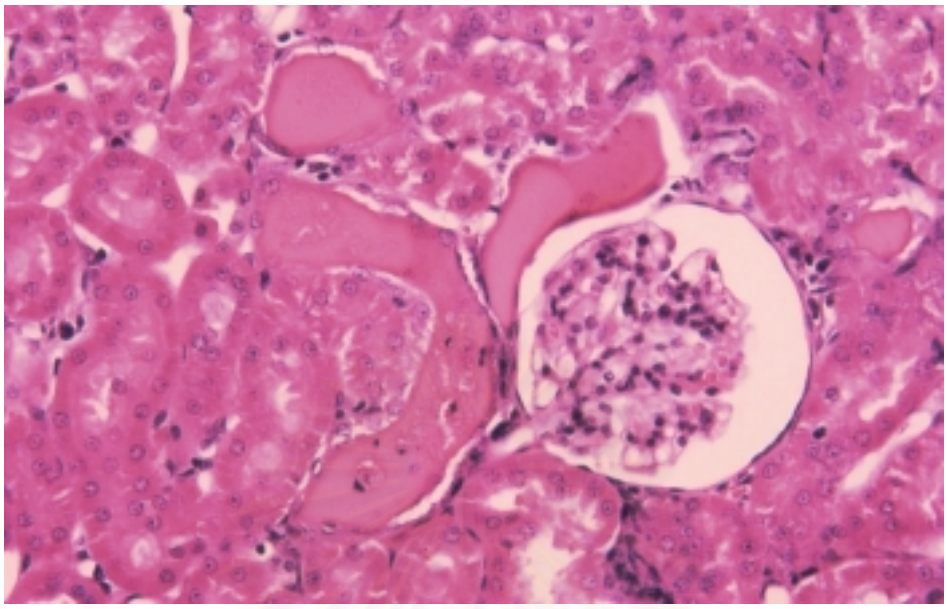


Figure 25. Kidney (doxorubicin plus low dose L-carnitine). Modulate tubular dilation, hyaline cast in tubule and cellular debris in lumen. H&E, 10X

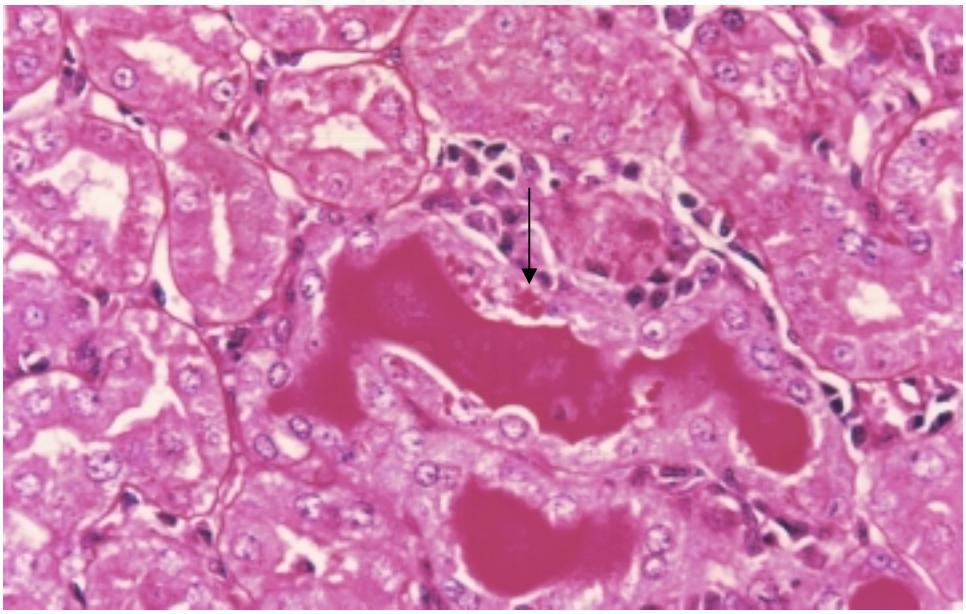


Figure 26. Kidney (doxorubicin plus high dose L-carnitine) Modulate tubular dilation, few hyaline cast in the tubule or lumen with protein droplets in the proximal tubular cell (arrow) were observed. Interstitial edematous were scattered. PAS, 10X

In summary, renal structural changes in the DOX groups were the most severe. The prominent alterations in the DOX group were glomerular capillary dilation with hyaline droplets were founded in the glomerular visceral epithelium and proximal tubular cells. In addition, numerous hyaline droplets were founded in the glomerular space. Glomerular alterations of the low dose L-carnitine were similar to those in the high dose L-carnitine. However, lesions of the glomeruli were more pronounce in the low dose group. Tubular and interstitial changes in the DOX group were severe. Kidneys from group 2, 3, 4 developed tubular dilation and epithelial desquamation. Interstitial alterations of the kidney groups 2, 3 and 4 displayed inflammatory cells infiltration with edematous swelling. L-carnitine treatment, both low and high doses refluxed a decrease in tubular and interstitial alterations (table12).

Table 12. Semiquantitative assessment of structural changes.

	Hyaline droplet in glomerular space*	Tubular cast**	Tubular** dilation	interstitial** changes
Group1	-	-	-	-
Group2	++	+++	+++	+++
Group3	+	++	++	++
Group4	+	+	++	++

* Counts from 500 glomeruli per groups

** 10 visual fields at low power (10X)

CHARTER V

DISCUSSION

Anticancer therapy usually demolishes the physiological homoeostasis during treatment of cancer in many organs. Effective anticancer therapy with anthracyclines is limited by toxicity to various organs including kidneys (Hertzan-levy et al., 2000).

It is known that doxorubicin causes weight loss (Dorek et al., 1998). In the present study, a single dose of doxorubicin (7.5 mg/kg, i.v.) did affect the growth of rats. After doxorubicin injection, significant reductions in daily food intake and body weight with or without L-carnitine as compared to the control group were found. The decrease in body weight gain may be due to mobilization and utilization of fat deposits for the synthesis of glucose (Malarkodi and Varalakshmi, 2003). Venkatesan et al (2000) observed similar results in rats administered doxorubicin. The effect on growth rate may also be due to inhibition of Krebs' cycle and other enzyme systems by doxorubicin (Malarkodi and Varalakshmi, 2003).

In the present study, doxorubicin induced nephrotoxicity manifested by increased in kidney weight with significant increase in plasma concentrations of creatinine and urea nitrogen. L-carnitine (40, 200 mg/kg, i.p.) given 1 hr before doxorubicin administration and for 15 days thereafter may protect the kidney from damages caused by doxorubicin. This protection was clearly reflected by decreases significant of plasma concentrations of creatinine and urea nitrogen.

Doxorubicin induced nephrotic syndrome in rats characterized by heavy proteinuria, hypoalbuminemia, hypercholesterolemia and increased triglyceride (Montilla et al., 1997). In the present experiment, DOX significantly increased triglyceride and cholesterol but decreased albumin. Both glomerular and tubular injury occurred 5 days after DOX injection as evidenced by proteinuria. At the end of the experiment, losing protein in urine was so severe in consistent with the results from

histopathology in which hyaline cast was present in the glomerlar space and tubular lumen. The most possible mechanisms for protein losing by of doxorubicin may be alterations of the permeability of the glomerular capillary wall (Weening and Rennkle, 1983).

Doxorubicin induced cholesterolemia and triglyceremia in group 2 rats. Doxorubicin inhibited carnitine palmitoloytransferase system (CPT I) (Hong et al., 2002). Doxorubicin may lower the level of cytochrome P450 which may in turn depress cholesterol 7œ hydroxylase activities, the key enzyme in conversion of cholesterol to bile acids (Malarkodi and Varalakshmi, 2003). Administration of L-carnitine (group 3 and 4) rats caused reduction of plasma cholesterol and triglyceride. L-carnitine inhibits the effect of doxorubicin on CPT I. Carnitine function is to carry long chain fatty acid into the mitochondria (Hong et al., 2002).

The renal function, as determined by GFR and ERPF decreased after doxorubicin injection. The GFR and ERPF significantly decreased by 35% and 37% respectively leaving filtration fraction unchanged. The results were in agreement with a study in rats after injection of doxorubicin (7.5 mg/kg, i.v.) in which GFR and ERPF decreased by 20 and 15%, respectively while filtration fraction was unchanged (Weening and Rennke, 1983).

Decreased GFR was due to either systemic changes or directly affect kidney circulation. Bristow and coworker (1979) reported that intravenous doxorubicin caused cardiac and peripheral vascular hemodynamic abnormalities associated with increased histamine and catecholamine released into the circulation. Giving histamine antagonists and adrenergic blockers could prevent the renal lesion. Since, RVR increased, thus, DOX affected the renal arteriolar resistance both efferent and afferent arteriole. Using the micropuncture technique in doxorubicin treated rats resulting in the decrease of GFR, RPF and ultrafiltration coefficients, however, an increase in glomerular capillary pressure (GCP) accompanying the augmented afferent arteriolar resistance was reported (Ballbi et al.,1998).

In low dose L-carnitine rats (group 3), GFR was similar to those received doxorubicin alone (group 2). However, renal plasma flow was slightly higher than group 2 causing lower filtration fraction. Renal vascular resistance was only slightly higher than the control group after DOX administration. The results indicate that vasoconstriction may play a role in the kidney of group 3 rats.

In high dose L-carnitine GFR was higher than group 2 and 3. The degree of increase in GFR was much greater than ERPF compared with doxorubicin alone causing increase in filtration fraction. The renal vascular resistance was also less than doxorubicin group, improved renal function may partly due to effect of L-carnitine on cardiac function since L-carnitine improved both cardiac and renal function, as in the previous study showed that L-carnitine improved cardiac failure caused by doxorubicin (Luo et al., 1999)

Administration of doxorubicin alone caused decrease in urine flow rate but increase in fractional excretion of sodium. The urinary sodium excretion (detected from metabolic cage) was also elevated. This result showed that doxorubicin impaired renal tubular reabsorption of sodium.

Group 3 and 4 rats had lower FENa compared to group 2 indicating the improvement of tubular reabsorption of Na^+. Higher urine flow was also found. In group 4, 15 days after DOX treatment with high dose L-carnitine caused the higher increase of urinary sodium excretion from day 0 compared with the rate of increase in group 2. These results may suggest the evidence of hyperfiltration. In nephrectomized rats which L-carnitine 200 mg/kg were administered daily for 3 days prior to 2/3 nephrectomized and for 25 days thereafter, urinary sodium excretion was high in carnitine treated rats suggesting stage of renal hyperfilltration (Palomba et al., 1996).

In the present study, doxorubicin caused severe nephrotic syndrome. These changes were associated with a marked change in the antioxidant defense system measured from plasma and kidney. An increase in lipid peroxides and a significant decrease in CAT activity in kidney occurred. These changes may reflect the

involvement of oxidative stress and renal dysfunction. The characteristic features of an increased lipid peroxidation by doxorubicin were similar to those previously reported by other investigation (Desassis et al., 1997; Montilla et al., 1997; Venkatesan et al., 1997; Zima et al., 1997) The mechanism related to renal injury may be a consequence of oxidative stress, such as oxidation and cross-linking of cellular thiols and membrane lipid peroxidation (Wu et al., 1990)

Although GSH in renal tissue was unchanged following DOX treatment, many studies showed variable results. The change may occur early by decreased (4 and 8 h after doxorubicin), then increased within 24 h, while GSSG levels were reduced at 1h, and significantly elevated 4-24 h after doxorubicin treatment, suggesting an early depletion of GSH and later over compensation (Luo et al., 1997)

On basis present study, we were hypothesized that L-carnitine might play an important role as an antioxidant agent in DOX model of nephrosis. Treatment with L-carnitine produces a significant reduction in doxorubicin induced loss of renal function and oxidative stress.

It was demonstrated that L-carnitine treatment improved cardiac energy metabolism and reduced lipid peroxidation in doxorubicin rats (Luo et al., 1999).

In addition to antioxidant effects of L-carnitine, L-carnitine was effective in attenuating cisplatin induced nephrotoxicity by metabolic effect. Six hours after administration of 200 mg/kg of carnitine, its level in kidney increased by 1.5 fold (Chang et al., 2002). Thus, the administered L-carnitine preferentially accumulates in the kidney and facilitates the ß-oxidation to generate ATP, thereby minimizing the toxic effects of free form of long-chain fatty acids in and around the mitochondria. In fact, mitochondrial dysfunction caused by free fatty acid could be inhibited by carnitine.

Finally, kidney structural alterations indicated a protective effect of L-carnitine. Rats given doxorubicin without carnitine had abundant hyaline cast (3+) in tubular lumen with tubular dilation occurred which was consistent with the high protein

excretion. However, L-carnitine at the dosages of 40 and 200 mg/kg showed the protective effect against the kidney lesion caused by doxorubicin. Rats given low and high dose L-carnitine developed less (+1 to +2) hyaline cast and tubular dilation in the renal tubule. There was no significant difference between rats given low and high L-carnitine doses.

In conclusion, the present study demonstrates that 7.5 mg/kg single injection of DOX in Sprague Dawley rats caused renal injury by reducing GFR and ERPF with glomerular and tubular lesion at 16 days of treatment. The oxidative stress was enhanced by detectable higher MDA and significantly lower catalase activity in the kidney. Administration of L-carnitine markedly attenuated DOX induced nephrotic syndrome by the improved GFR, reduced plasma lipids level, reduced oxidative stress and alleviated glomerular and tubular structural alterations. However, the protective effects of L-carnitine on DOX induced renal injury were more dramatic in high dose L-carnitine group.

REFERENCES

Aebi, H 1983. Catalase. In : Methods of enzymatic analysis. Bergmeyer, H.U. (ed) Academic Press, New York. 3: 237-282.

Badary, O.A., Abdel-Naim, A.B., Abdel-Wahab, M.H. and Hamada, F.M. 2000. The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats. Toxicology 143 (3): 219-226.

Bakker, W.W., Kalicharan, D., Donga, J., Hulstaert, C.E. and Hardonk M.J. 1987. Decreased ATPase activity in adriamycin nephrosis is independent of proteinuria. Kidney Int. 31 (3): 704-709.

Ballbi, A.L., Franco, R.J.S., Barretti, P., Gavras, I. and Gavras, H. 1998. Renal Artery Clipping Attenuates the Progression of Adriamycin Nephropthy. Am. J. hypertens. 11(9): 1124-1128.

Bellinghieri, G., Santoro, D., Calvani, M., Mallamace, A. and Savica, V. 2003. Carnitine and hemodialysis. Am. J. Kidney Dis. 41: S116-122.

Ben Ismail, T.H., Ali, B.H. and Bashir, A.A. 1994. Influence of iron, deferoxamine and ascorbic acid on gentamicin induced nephrotxicity in rat. Gen. Pharmacol. 25:1249-1252.

Benjamin, R.S., Wiernik, P.H. and Bachur, N.R. 1974. Adriamycin chemotherapy efficacy, safety and pharmacologic basis of an intermittent single high dosage schedule. Cancer. 33: 19-27.

Bertani, T., Poggi, A., Pozzni, R. and Delaini, F. 1982. Adriamycin-induced nephritic syndrome in rat : sequence of pathology rat. Lab. Invest. 46(1):16-23.

Bertani, T., Cutillo, F., Zoja, C., Broggini, M. and Remuzzi, G. 1986. Tubulo-interstitial lesions mediate renal damage in adriamycin glomerulopathy. Kidney. Int. 24: 152-159.

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

Billingham, M.E., Minobe, W.A., Masek, M.A. and Daniels, J.R. 1979. Histamine and catecholamines mediate chronic cardiac and renal damage associated with adriamycin administration. Clin. Res. 27: 156 (Abstract).

Blum, R.H. and Carter, S.K. 1974. Adriamycin: a new anticancer drug with significant clinical activity. Ann. Intern. Med. 80: 249-259.

Brass, I.P. 1997. Carnitine in renal failure. In: Nutritional Management of Renal Disease. Kopple, J.D. and Massary, S.G. (eds.) Williams and Wilkins, Baltimore, MD. 191-201.

Bristow, M.R., Billingham, M.E., Minobe, W.A., Masek, M.A. and Daniels, J.R. 1979. Histamine and catecholamines mediate chronic cardiac and renal damage associated with adriamycin administration. Clin. Res. 27: 156.

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

Calendi, E., Dimacro, A. and Regiani, M. 1965. On physiochemical interactions between davnomytocin and nucleic acid. Biochem. Biophys. Acta. 103: 25-49.

Chang, B., Nishikawa, M., Sato, E., Utsumi, K. and Inouc, M. 2002. L-carnitine inhibits cisplatin-induced injury of the kidney and small intestine. Arch. Biochem. Biophys. 405 (1): 55-64.

Desassis, J.F., Raats, C.J.I., Bakker, M.A.H., Van den Born, J. and Berden, J.H.M. 1997. Antiproteinuric effect of cyclosporine A in adriamycin nephropathy in rats. Nephron. 75: 336-341.

Durek, I., Ozturk,H.S., Kavutcu, M., Birey, M., Yel, M., Guven, T., Olcay, E., Kacmaz, M. and Canbolat, O. 1998. Protective role of antioxidant vitamins on adriamycin-induced free radical production and cardiotoxicity in guinea pigs. Cancer Res. Ther. Cont. 5: 133-141.

Fukuda, F., Kitada, M., Horie, T. and Awazu, S. 1992. Evaluation of adriamycin-induced lipid peroxidation. Biochem. Pharmacol. 44: 755-760.

Galli, L.G., Volpini, R.A., Costa, R.S., da Silva, C.G. and Coimbra, T.M. 2001. Tubular cell lesion, albuminuria and renal albumin handling in rats treated with Adriamycin. Ren. Fail. 23(5): 693-703.

Gewirtz, D.A. 1999. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochem. Pharmacol. 57: 727-741.

Ginevri, F., Gusmano, R.,Allegrini, S., D'Allegri, F. and Ghiggeri, G. 1990. Renal purine efflux and xanthine oxidase activity during experiment nephrosis in rats : difference between puromycin amnionucleoside and adriamycin nephrosis. Clin. Sci. (Colch) 78: 283-293.

Ground, J., Weening, J.J., Vangoor, H. and Elema, J.D. 1998. Application of pyromycin aminonucleoside and adriamycin to induce chronic renal failure in the rat. Contrib. Nephrol. 60: 83-93.

Gutteridge, J.M. and Halliwell, B. 1989. Iron toxicity and oxygen radicals. Baillieres. Clin. Haematol. 2(2): 195-256.

Hall, R.L., Wilke, W.L. and Fettman, M.J. 1986. The progression of adriamycin induced nephritic syndrome in rats and effect of captopril. Toxicol. Appl. Pharmacol. 82 (1): 164-174.

Hertzan-Levy, S., Fish, R., Skutelsky, E., Wollman, Y., Chernichovsky, T., and Polak-Charcon, S. 2000. Glomerular basement membrane anionic sites in adriamycin nephropathy: effect of saline loading and nitric oxide modulation. Nephron. 84(4): 354-361.

Hong, Y.M., Kim, H.S. and Yoon, H. 2002. Serum lipid and fatty acid profiles in adriamycin-treated rats after administration of L-carnitine. Pediatr. Res. 51(2): 249-255.

Irat, A.M., Aktah, F. and Ozansoy, G. 2003. Effect of L-carnitine treatment on oxidant/antioxidant state and vascular reactivity of streptozotocin-diabetic rat aorta. J. Pharm. Pharmacol. 55(10): 1389-1395.

Kalyanaraman, B., Morehouse, K.M. and Mason, R.P. 1991. An electron paramagnetic resonance study of the interactions between the adriamycin semiquinone, hydrogen peroxide, iron-chelators and radical scavengers. Arch. Biochem. Biophys. 286: 164-167.

Kaslfi, K., Israel, M., Sweatman, T.W., Seshadri, R. and Cook, G.A. 1990. Inhibition of mitochondrial carnitine palmitoyltransferases by adriamycin and adriamycin analogues. Biochem. Pharmacol. 40(7): 1441-1448.

Kopple, J.D., Ding, H., Letoha, A., Jvanyi, B., Qing, D.P., Dox, L., Wang, H.Y. and Sonkodi, S. 2002. L-carnitine ameliorates gentamicin-induced renal injury in rats. Nephrol. Dail. Transplant. 17(12): 2122-2131.

Lebrecht, D., Setzer, B., Rohrbach, R. and Walker, U.A. 2004. Mitochondrial DNA and its respiratory chain products are defective in doxorubicin nephrosis. Nephrol. Dial. Transplant. 19(2): 329-336.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, K.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193(1): 265-275.

Luo, X.P., Evrovsky, Y., Cole, D., Trines, J., Benson, L.N. and Lechotay, D.C. 1997. Doxorubicin-induced acute changes in cytotoxic aldehydes, antioxidant status and cardiac function in the rat. Biochim. Biophy. Acta. 1360: 45-52.

Luo, X., Reichetzer, b., Trines, J., Benson, T.L. and Lehotay, D.C. 1999. L-carnitine attenuates doxorubicin-induced lipid peroxidation in rats. Free Radic. Biol. & Med. 26: 1158-1165.

Malarkodi, K.P. and Varalakshmi, P. 2003. Lipoic acid as a rescue agent for adriamycin-induced hyperlipidemic nephropathy in rats. Nutrition Res. 23: 539-548.

Manabe, N., Kinoshita, A., YAmagushi, M., Furuya, N., Nagowa, N., Yamada-Uchio, K. and Akaski, W. 2001. Change in quantitative profile of extracellular matrix components in kidneys of rats with adriamycin-induced nephropathy. J. Vet. Med. Sci. 63(2): 125-133.

Milner, L.S. Wei, S.H. and Houser, M.T. 1991. Amelioration of glomerular injury in doxorubicin hydrochloride nephrosis by dimethythiourea. J. Lab. Clin. Med. 118(5): 427-434.

Mimnaugh, E.G., Trush, M.A. and Gram, T.E. 1986. A possible role for membrane lipid peroxidation in anthracycline nephrotoxicity. Biochem. Pharmacol. 35(23): 4327-4335.

Mister, M., Noris, M., Szymczu, K.J., Azzollini, N., Aiello, S., Abbate, M. and Trochimowicz, L. 2002. Propionyl-L-carnitine prevents renal function deterioration due to ischemia repertusion. Kidney Int. 61(3): 1064-1078.

Montilla, P., Tunez, I., Munoz, M.C., Lopez, A. and Soria, J.V. 1997. Hyperlipidemic Nephropathy Induced by Adriamicin : Effect of Melatonin Administration. Nephron. 76: 345-350.

Morgan, W.A., Kaler, B. and Bach, P.H. 1998. The role of reactive oxygen species in adriamycin and menadione-induced glomerular toxicity. Toxicol. Lett. 94(3): 209-215.

Myers, C.E., McGuire, W.P., Liss, R.H., Ifrim, I., Grotzinger, K. and Young, R.C. 1977. Adriamycin : the role of lipid peroxidation in cardiac toxicity and tumor respone. Science. 197: 165.

Nicolay, K. and De Kruij fl B. 1987. Effect of adramycin on respiratory chain activity in mitochondria from rat liver, rat heart and bovine heart. Evidence for a preferential inhibition of complex III and IV. Biochim. Biophys. Acta. 892: 320-330.

O' Bryan, R.M., Luce, L.K., Talley, R.W., Gottlieb, J.A., Baker, L.H. and Bonadonna, G. 1973. Phase II evaluation of adriamycin in human neoplasia. Cancer. 32:1-8.

Okasora, T, Takikawa, T., Utsunomiya, Y.,Hayashibara, H, Shiraki, K, Kasagi, T. and Shimizu, F. 1992. Suppressive effect of superoxide dismutace on adriamycin nephropathy. Nephron. 60: 199-203.

Ohkawa, H., Ohisi, N. and Yagi, K. 1979. Assay for lipid peroxidation in animal tissue thiobarbiture acid reaction. Anal. Biochem. 95: 531-538.

Okuda, S., Oh, Y., Ysuruda, H., Onoyamo, K., Fijimi, S. and Fujishima, S. 1986. Adriamycin-induced nephropathy as acidosis model of chronic progressive glomerular disease. Kidney Int. 29: 502-510.

Palomba, D., Pes, G.M., Demontis, M.P., Varoni, M.V., Deiana, L. and Ananior, V. 1996. Metabolic and renal effect of Laevo-carnitine and propinyl-carnitine in rats with subtotal nephrectomy. Phamacol. Res. 34(3-4): 161-165.

Papadopoulou, L.C. and Tsiftsoglou, A.S. 1996. Effects of hemin on apoptosis, suppression of cytochrome c oxidase gene expression, and bone-marrow toxicity induced by doxorubicin(adriamycin). Biochem. Pharmoacol. 52: 713-722.

Praet, M. and Ruysschaert, J.M. 1993. In vivo and in-vitro mitochondrial membrane damages induced in mice by adriamycin and derivatives. Biochem. Biophys. Acta. 1149: 79-85.

Sayed-Ahmed, M.M., Khattab, M.M., Gad, M.Z. and Mostafa, N. 2001. L-carnitine prevents the progression of atherosclerotic lesions in hypercholesterolaemic rabbits. Pharmacol. Res. 44 (3): 235-242.

Sayed-Ahmed, M.M., Shaarawy, S., Shouman, S.A. and Osman, A.M. 1999. Reversal of doxorubicin-induced cardiac metabolic damage by L-carnitine. Pharmacol. Res. 39 (4): 289-295.

Shug, A.L. 1987. Protetion from adriamycin-induced cardiomyopathy in rats. Z. Kardiol. 76 (5): 46-52.

Single, P.K., Delly, C.M.R. and Weinberg, L.E. 1987. Subcellular effects of adriamycin in the heart : a concise review. J. Mol. Cell. Cardiol. 19: 817-828.

Solem, L.E., Heller, L.J. and Wallace, K.B. 1994. Dose-dependent increase in sensitivity to calcium-induced mitochondrial dysfunction and cardiomyocyte cell injury by doxorubicin. J. Mol.Cell Cardiol. 28: 1023-1032.

Strohm, G.H., Payne, C.M. and Alberts, P.S. 1982. Cardiotoxic effects of doxorubicin with and without carnitine. Arch. Pathol. Lab. Med. 106: 181-185.

Tan, C., Etcubanas, E., Wollner, N., Rosen, G., Gilladoga, A., Showel, J., Murphy, M.L. and Krakoff, I.H. 1973. Adriamycin-an antitumor antibiotic in the treatment of neoplastic diseases. Cancer. 52: 9-17.

Venkatesan, N., Venkatesan, P., Karthikeyan, J. and Venkatesan, A. 1997. Protection of taurine against adriamycin-induced proteinuria and hyperlipidemia in rats. Proc. Soc. Exp. Med. 215: 158-164.

Venkatesn, N., Punithavathy, D. and Arumugam, V. 2000. Curcumin prevents adriamycin nephrotoxicity in rats. Br. J. Phamacol. 129: 231-234.

Weening, J.J. and Rennke, H.G. 1983. Glomerular permeability and polynion in adriamycin nephrosis in the rats. Kidney Int. 24: 152-159.

Wu, S.H., Yang, Y.C. and Wang, Z.M. 1990. Role of oxygen radicals in adriamycin induced nephrosis. Clin. Med. J. Engl. 103: 283-289.

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.

Zima, T., Tesar, V., Stipek, S., Crkovska, J., Poledme, R., Teminova, J., Platenik, J., Rychlik, I., Merta, M. and Nemecek, K. 1997. The influence cycosporin on lipid peroxidation and superoxide dismutate adriamycin nephropathy in rats. Nephron. 75: 464-468.

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