จุลพยาธิวิทยาของตับและไตปลานิล *Oreochromis niloticus* ภายหลังได้รับสุราขาว 30 ดีกรีระดับความเข้มข้นต่ำเป็นระยะเวลานาน

นางสาวศิรินทร์นภา พุ่มแจ้

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสัตววิทยา ภาควิชาชีววิทยา
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2547

ISBN 974-53-1164-2

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

HISTOPATHOLOGY OF NILE TILAPIA *Oreochromis niloticus* LIVER AND KIDNEY AFTER LONG-TERM LOW LEVEL EXPOSURE TO DISTILLED LIQUORS 30 DEGREE

Miss Sirinnapa Pumchae

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Zoology
Department of Biology
Faculty of Science
Chulalongkorn University
Academic Year 2004

ISBN 974-53-1164-2

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Thesis Title	Histopathology of Nile tilapia *Oreochromis niloticus* liver and kidney after long-term low level exposure to distilled liquors 30 degree
By	Miss Sirinnapa Pumchae
Filed of study	Zoology
Thesis Advisor	Associate Professor Kingkaew Wattanasirmkit, Ph. D.

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

…….Dean of the Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)

THESIS COMMITTEE

…….Chairman
(Professor Siriwat Wongsiri, Ph.D.)

…….……Thesis Advisor
(Associate Professor Kingkaew Wattanasirmkit, Ph.D.)

……..Member
(Assistant Professor Patchanee Singh-asa, Ph.D.)

……..Member
(Assistant Professor Apichai Daorai, Ph.D.)

…….Member
(Duangkhae Sitthicharoenchai, Ph.D.)

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

ศิรินทร์นภา พุ่มแจ้ : จุลพยาธิวิทยาของตับและไตปลานิล *Oreochromis niloticus* ภายหลังได้รับสุราขาว 30 ดีกรีระดับความเข้มข้นต่ำเป็นระยะเวลานาน. (HISTOPATHOLOGY OF NILE TILAPIA *Oreochromis niloticus* LIVER AND KIDNEY AFTER LONG-TERM LOW LEVEL EXPOSURE TO DISTILLED LIQUORS 30 DEGREE อ. ที่ปรึกษา : รศ. ดร. กิ่งแก้ว วัฒนเสริมกิจ, 105 หน้า. ISBN 974-53-1164-2.

การทดสอบความเป็นพิษของสารเคมีต่างๆนั้น นอกจากทำการทดสอบในสัตว์เลี้ยงลูกด้วยน้ำนมแล้วยังมีการทดสอบในสัตว์น้ำ แต่ยังไม่เคยมีรายงานการศึกษาความเป็นพิษของสุราขาวในสัตว์น้ำพวกปลา ดังนั้นการศึกษาในครั้งนี้จึงมีวัตถุประสงค์ที่จะศึกษาความเป็นพิษเฉียบพลันและพิษกึ่งเรื้อรังของสุราขาว 30 ดีกรี ต่อเนื้อเยื่อตับและไตของปลานิล *Oreochromis niloticus* ได้ทำการศึกษาความเป็นพิษเฉียบพลัน เพื่อหาค่า LC_{50} ที่ 96 ชั่วโมง โดยใช้วิธี acute static toxicity test ได้ค่า LC_{50} ที่ 96 ชั่วโมง เท่ากับ 15,751.21 ppm และคำนวณค่าความเข้มข้นที่ใช้ในการศึกษาความเป็นพิษกึ่งเรื้อรัง ได้เท่ากับ 964.30 ppm นำปลานิลอายุ 1 เดือน มาเลี้ยงในน้ำที่มีสุราขาวตามความเข้มข้นข้างต้นเป็นเวลานาน 6 เดือน ทำการเก็บตัวอย่างตับและไตปลานิลทุกๆ เดือน นำมาชั่งน้ำหนักตัว น้ำหนักตับ และนำเนื้อเยื่อไปทำสไลด์ถาวร เพื่อศึกษาพยาธิสภาพของเนื้อเยื่อตับและไตภายใต้กล้องจุลทรรศ์แบบใช้แสง

ผลการศึกษาพบว่า ปลาที่เลี้ยงในน้ำที่มีสุราขาว 30 ดีกรี เข้มข้น 964.30 ไมโครลิตรต่อลิตร ตั้งแต่เดือนที่ 4 ถึงเดือนที่ 6 มีค่าดรรชนีความสัมพันธ์ระหว่างน้ำหนักตับและน้ำหนักตัวสูงกว่าค่าปกติและแตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) และไม่พบความแตกต่างในระหว่างกลุ่มทดลองของทุกเดือน จากการศึกษาเนื้อเยื่อพบว่าตับปลากลุ่มทดลองมีการเกิดพยาธิสภาพ ดังนี้ เซลล์ตับบวมน้ำ มีการสะสมของไฮยาลินกรานูลและไขมัน พบการตายของเซลล์ตับเป็นกลุ่มๆ และกระจายทั่วไป ช่องไซนูซอยด์ขยายตัว และผนังหลอดเลือดชั้นในหลุดลอก เนื้อเยื่อตับอักเสบ มีเซลล์เม็ดเลือดขาวชนิดลิมโฟไซท์และกรานูลโลไซท์แทรกเข้ามาภายในเนื้อเยื่อ พบเซลล์ใหม่เกิดขึ้นในบริเวณเนื้อเยื่อที่ได้รับความเสียหาย จากการศึกษาพยาธิสภาพเนื้อเยื่อไตปลากลุ่มทดลองพบการหดตัวของโกลเมอรูลัส เซลล์บุท่อไตส่วนต้นบวมน้ำ และมีการสะสมไฮยาลินกรานูล นิวเคลียสหดตัวและติดสีเข้ม บางเซลล์มีการสลายตัวของนิวเคลียส ภายในท่อไตส่วนต้นมีเศษเซลล์และสารโปรตีน ผลการศึกษาครั้งนี้สรุปได้ว่า สุราขาว 30 ดีกรี มีความเป็นพิษต่อเนื้อเยื่อตับและไตของปลานิลและพยาธิสภาพที่เกิดขึ้นมีความรุนแรงเพิ่มขึ้นตามระยะเวลาที่ได้รับสาร

ภาควิชา.....ชีววิทยา…… ลายมือชื่อนิสิต.....

สาขาวิชา…….....สัตววิทยา..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2547……. ลายมือชื่ออาจารย์ที่ปรึกษาร่วม…….....…….....

4472424823 : MAJOR ZOOLOGY

KEY WORD: DISTILLED LIQUORS / *Oreochromis niloticus* / TOXICITY/ LIVER / KIDNEY

SIRINNAPA PUMCHAE : HISTOPATHOLOGY OF NILE TILAPIA *Oreochromis niloticus* LIVER AND KIDNEY AFTER LONG-TERM LOW LEVEL EXPOSURE TO DISTILLED LIQUORS 30 DEGREE. THESIS ADVISOR : ASSOC. PROF. KINGKAEW WATTANASIRMKIT, Ph.D. 105 pp. ISBN 974-53-1164-2.

In general, the toxicity testing of various chemicals is not the studies only in mammals but also in aquatic organisms. From the review, most of research on toxicity of distilled liquors was studied in mammals and a few recorded in aquatic organisms especially the fish. Therefore, the aim of this study was to investigate acute toxic and subchronic toxic of distilled liquors 30 degree on liver and kidney of Nile tilapia *Oreochromis niloticus*. Acute toxicity bioassay was performed by acute static toxicity test type to obtained the LC_{50} at 96 hours. The median lethal concentration of distilled liquors was 15,751.21 ppm. From the calculation, sublethal concentration of subchonic toxicity studies was 964.30 ppm. One-month-old Nile tilapias were exposed to distilled liquors at sublethal concentration for 6 months. Liver and kidney of control group and treated group were sampled every month. Body weight and liver weight were recorded. Liver and kidney were processed for permanent slide and studied under light microscope.

The results showed that the liver somatic indices of all treated fish in every month from 4^{th} months to 6^{th} months of experiment were higher than normal value and significantly different from the control group ($p<0.05$) and not significant different among treated groups of all experimental months. From histological studies, liver of treated fish exhibited histological changes that consisted of hydropic swelling, hyaline granules and lipid droplets accumulation, diffuse and focal necrosis, sinusoid dilatation and sloughing of blood vessels endothelium. Lymphocyte and granulocyte infiltration were seen in the inflammatory areas. Some damage area showed the regeneration of hepatocytes. Histological alterations of kidney consisted of glomerulus shrinkage, hydropic swelling of proximal tubular cells and hyaline granules accumulation, pyknotic nuclei, and karyolysis of proximal tubular cells. Tubular lumen showed the accumulation of cellular debris and protein cast. In conclusion, this research showed that distilled liquors 30 degree caused toxic effects on Nile tilapia liver and kidney and the severity of histopathological changes depended on the exposure time.

Department…….....Biology…… Student's……

Field of study…….....Zoology……... Advisor's……

Academic year…….....2004…… Co-advisor's…….-…….

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to my thesis advisor, Associate Professor Dr. Kingkaew Wattanasirmkit for her precious suggestions, guidances, patiences and encouragements throughout the study

I wish to express my grateful thank to Professor Dr. Siriwat Wongsiri, chairman of thesis committee for his valuable advice. I also would like to express my appreciation to Assistant Professor Dr. Patchanee Singh-asa, Assistant Professor Dr. Apichai Daorai and Dr. Duangkhae Sitthicharoenchai, thesis committee members for their invaluable suggestions and guidances.

I am grateful to Assistant Professor Kanokthorn Piyathumrongrat for her kind suggestions and understanding.

I would like to thank Pathumtani Breeding Station for their kindly supplied the juvenile fish in this thesis.

I wish to thank the Department of Biology, Chulalongkorn University for facilities.

Finally and most importantly, I would like to express my appreciation to my family for their support, believing and understanding during my study.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CONTENTS

Page

THAI ABSTRACT.....iv

ENGLISH ABSTRACT.....v

ACKNOWLEDGEMENTS.....vi

CONTENTS.....vii

LIST OF TABLES.....x

LIST OF FIGURES.....xi

CHAPTER I INTRODUCTION.....1

CHAPTER II LITERATURE REVIEW

2.1 Meaning and types of alcoholic beverage.....4

2.2 Pharmacokinetics of ethanol

2.2.1 Absorption…….5

2.2.2 Distribution…….....5

2.2.3 Metabolism and excretion…….....6

2.3 Physical and chemical properties of ethanol……...6

2.4 Fate in the environment……...7

2.5 Ethanol toxicology

2.5.1 Ingestion……8

2.5.2 Aquatic toxicity……...13

2.6 Nile tilapia *Oreochromis niloticus*

2.6.1 Histopathological alteration of fish liver……16

2.6.2 Histopathological alteration of fish kidney.....20

Page

CHAPTER III MATERIALS AND METHODS

3.1 Subjects……..25

3.2 Test material……..25

3.3 Acute toxicity test

3.3.1 Range finding test……26

3.3.2 Definitive test……..26

3.3.3 Analysis for LC_{50} value……...26

3.4 Determination of application factor (AF)……..26

3.5 Subchronic exposure…….27

3.6 Sampling……27

3.7 Determination of Liver somatic index (LSI)…….28

3.8 Data analysis……..28

3.9 Light microscopic study

3.9.1 Standard paraffin technique……29

3.9.2 Frozen technique…….30

CHAPTER IV RESULTS AND DISCUSSION

4.1 Acute toxicity of distilled liquors 30 degree on Nile tilapia *O. niloticus*

4.1.1 Range finding test……31

4.1.2 Definitive test…….....32

4.1.3 Analysis for LC_{50} value……32

4.2 Subchronic toxicity of distilled liquors on Nile tilapia *O. niloticus*

4.2.1 Liver somatic index (LSI)……...34

4.2.2 Histopathology of liver……36

4.2.3 Histopathology of kidney……....55

Page

CHAPTER V CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions.....67

5.2 Recommendations.....67

REFERENCES.....68

APPENDICES

APPENDIX I.....80

APPENDIX II…….....89

BIOGRAPHY.....105

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF TABLES

Page

Table 2-1 Concentration of alcohol in alcoholic beverage.....4

Table 2-2 Relative concentration of ethanol at balancing stage.....5

Table 2-3 A summary of physical and chemical properties of ethanol.....7

Table 2-4 Estimated half-life of ethanol in the environment.....7

Table 2-5 Lethal dose of ethanol in rats and mice.....8

Table 2-6 Reported median lethal and median effective concentration of ethanol.....14

Table 4-1 Percentage of mean mortality of *O. niloticus* at various distilled liquors concentration in range-finding test.....31

Table 4-2 Percentage of mean mortality of *O. niloticus* at various distilled liquors concentration in definitive test.....32

Table 4-3 Median lethal concentration (LC_{50}) of distilled liquors for Nile tilapia.....33

Table 4-4 Mean body weight and LSI for Nile tilapia of control and liquors treated group at 4 months - 6 months.....35

Table 4-5 Incidence of histopathological findings in Nile tilapia liver after exposure to distilled liquors 30 degree at 964.30 ppm from first through sixth months of experiment.....37

Table 4-6 Incidence of histopathological findings in Nile tilapia kidney after exposure to distilled liquors 30 degree at 964.30 ppm from first through sixth months of experiment.....56

LIST OF FIGURES

Page

Figure 4-1 Median lethal concentration (LC_{50}) at 24, 48, 72 and 96 hours after exposure to various concentration of distilled liquors……33

Figure 4-2 Photomicrograph of *O. niloticus* liver of control group (H&E stain).....42

Figure 4-3 Photomicrograph of *O. niloticus* liver at 1 month experimental period. (H&E stain).....43

Figure 4-4 Photomicrograph of *O. niloticus* liver at 1 month experimental period. (H&E, Oil red O stain).....44

Figure 4-5 Photomicrograph of *O. niloticus* liver at 2 months experimental period. (H&E stain).....45

Figure 4-6 Photomicrograph of *O. niloticus* liver at 2 months experimental period. (H&E, Oil red O stain).....46

Figure 4-7 Photomicrograph of *O. niloticus* liver at 3 months experimental period. (H&E stain).....47

Figure 4-8 Photomicrograph of *O. niloticus* liver at 3 months experimental period. (H&E, Oil red O stain).....48

Figure 4-9 Photomicrograph of *O. niloticus* liver at 4 months experimental period. (H&E stain).....49

Figure 4-10 Photomicrograph of *O. niloticus* liver at 4 months experimental period. (H&E, Oil red O stain).....50

Figure 4-11 Photomicrograph of *O. niloticus* liver at 5 months experimental period. (H&E stain).....51

Figure 4-12 Photomicrograph of *O. niloticus* liver at 5 months experimental period. (H&E, Oil red O stain).....52

Page

Figure 4-13 Photomicrograph of *O. niloticus* liver at 6 months experimental period. (H&E stain).....53

Figure 4-14 Photomicrograph of *O. niloticus* liver at 6 months experimental period. (H&E, Oil red O stain).....54

Figure 4-15 Photomicrograph of *O. niloticus* kidney of control group. (H&E stain).....60

Figure 4-16 Photomicrograph of *O. niloticus* kidney at 1 month experimental period. (H&E stain).....61

Figure 4-17 Photomicrograph of *O. niloticus* kidney at 2 months experimental period. (H&E stain).....62

Figure 4-18 Photomicrograph of *O. niloticus* kidney at 3 months experimental period. (H&E stain).....63

Figure 4-19 Photomicrograph of *O. niloticus* kidney at 4 months experimental period. (H&E stain).....64

Figure 4-20 Photomicrograph of *O. niloticus* kidney at 5 months experimental period. (H&E stain).....65

Figure 4-21 Photomicrograph of *O. niloticus* kidney at 6 months experimental period. (H&E stain).....66

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER I

INTRODUCTION

The excessive intake of alcohol for a long time has been the causes of several diseases. The toxic effects were brought about by acetaldehyde, the first product of ethanol metabolism (Vamvakas *et al*., 1998). Many researchers reported that chronic alcoholism affected the gastrointestinal system (West *et al*.,1984 cited in Woods *et al*., 1993; Hasumura, 1997; Vamvakas *et al*., 1998) and the cardiovascular system (West *et al*., 1984 cited in Woods *et al*., 1993; Vamvakas *et al*., 1998; Lorimier, 2000). Alcoholism has been reported to increase risk of cancer at tonque, mouth, pharynx, larynx, esophagus and liver (West *et al*., 1984 cited in Woods *et al*., 1993; Vamvakas *et al*., 1998; Lorimier, 2000). Furthermore, chronic alcoholism might damage both of the central and peripheral nervous system (West *et al*., 1984 cited in Woods *et al*., 1993; Vamvakas *et al*., 1998). Liver disease had been consistently reported that being the major consequence of alcohol abuse (West *et al*., 1984 cited in Woods *et al*., 1993). Alcoholic liver disease was characterized by steatosis, inflammation, necrosis, fibrosis and finally cirrhosis (Nakano *et al*., 1982; Takahashi *et al*., 1987; Ishak *et al*., 1991; Woods *et al*., 1993; Levinthal *et al*., 1996; Iimuro *et al*., 1997a, 1997b). However, the histopathological changes were depended on the amount of alcohol consumption and duration of intake (Woods *et al*., 1993).

Alcoholic beverages or liquor is the beverage which contains the amount of ethanol not more than 60 degree (% v/v) and are produced from primary agricultural product origin, for examples, fruit, wheat grain and molasses. It is divided into 2 types such as fermented liquors and distilled liquors. The amount of ethanol in fermented liquors is not more than 15 degree while it is more than 15 degree in distilled liquors (Kiatisevi, 2001).

Most of acute or subchronic toxicity studies of ethanol are on mammals, for examples, rats (Adachi *et al*., 1994; Yamada *et al*., 1991; Qu *et al*., 1996; Bradford *et al*., 1993; Iimuro *et al*., 1997a, 1997b; Yin *et al*., 2000; Iimuro *et al*.,1996), mice (American Methanol Institute, 1998), baboons (Davison, 1980; Hasamura, 1997) and human (Davison, 1980; Hasamura, 1997; Hill, 1998). On the contrary, subchronic toxicity information of ethanol has been limited on aquatic organisms. Only the LC_{50} values of ethanol were reported for brine shrimp (Cogwill *et al*., 1991; American Methanol Institute, 1998), shrimp (Foss and Ray, 1997), mussel (Lagderspetz *et al*., 1993), fish and frog (American Methanol Institute, 1998). The LC_{50} values of various distilled liquors that produced in Thailand had also been observed on brine shrimp (Sam-angsri, 2002) and it was reported that the distilled liquors 30 degree from Surattani province had the highest acute toxicity on this species. In fish species, the LC_{50} values were reported only on some species such as blue gill, fathead minnow, rainbow trout and salmon (American Methanol Institute, 1998).

Therefore, the aim of this study was to investigate the acute and subchronic toxicity of distilled liquors 30 degree on Nile tilapia *Oreochromis niloticus*. Nile tilapia was the target organism in this study for their commercially importance and widely abundant in Thailand. Subchronic toxicity was conducted to evaluate effects of this liquors on liver and kidney of tilapia. Liver has been chosen as a target organ for histopathological observed since it is a major site of accumulation, biotransformation and excretion of xenobiotic compound (Phillips *et al*., 1987). Kidney has also been chosen because of the functions of osmoregulation, elimination of waste (Larsen and Perkins, 2001) and also the biotransformed organ. Thus, histopathological changes of these organs may occur after long-term exposure to distilled liquors 30 degree.

Objectives

1. To study acute toxicity of distilled liquors 30 degree on Nile tilapia *O. niloticus*.
2. To study subchronic toxicity of distilled liquors 30 degree on liver and kidney of Nile tilapia *O. niloticus*.

Anticipated benefits

1. To provide histopathological data on subchronic effects of distilled liquors 30 degree on liver and kidney of Nile tilapia.
2. The toxicological data of subchronic effects of distilled liquors 30 degree on Nile tilapia can be used as basic data for the other study which involved in the toxicity of liquors.
3. To obtain basic knowledge about common histology of liver and kidney of Nile tilapia.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER II

LITERATURE REVIEW

2.1 Meaning and types of alcoholic beverage

The alcoholic beverage is divided into 2 types according to Kiatisevi (2001) as presented below.

Type 1 Distilled liquors such as whisky, brandy, gin and rum.

Type 2 Fermented liquors such as beer, wine, champagne, sake and satho

Both types of alcoholic beverages have contained the mixture of alcohol in the different ratio as shown in Table 2-1.

Table 2-1 Concentration of alcohol in alcoholic beverage (Kiatisevi, 2001).

Alcoholic beverage	Concentration (% v/v, degree)
Beer	3-6
White/Red wine	8-10
Champagne	8-10
Brandy	30-32
Whisky, Gin	35-40
Rum	50-60

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

2.2 Pharmacokinetics of ethanol

The pharmacokinetics process of ethanol consists of 3 steps as presented below.

2.2.1 Absorption

Alcohol was moderately soluble in fat and highly soluble in water because it was a very small molecule in liquid form and non-ionic. So that, it was easily absorbed through the gastrointestinal tract once it was ingested without the need of any digestion. About 20 percent of alcohol was directly absorbed into the bloodstream from the stomach, while the remaining 80 percent was absorbed from the upper portion of the small intestine (Levinthal, 1996).

2.2.2 Distribution

Its solubility in water helps ethanol to be distributed to all body tissues that have high water content in a relatively greater proportion such as blood, plasma, serum, spinal fluid and urine (Table 2-2).

Table 2-2 Relative concentration of ethanol at balancing stage
(Di Maio and Di Maio, 1989 cited in Thongra-ar, 1991)

Biological matter	Relative concentration
Brain	0.85
Liver	0.85
Blood	1
Spinal fluid	1.10-1.27
Plasma and serum	1.12-1.20
Urine	1.3

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

2.2.3 Metabolism and excretion

Alcohol was absorbed from the stomach and the duodenum, and was transported to the liver. About 95-98% of the alcohol absorbed were metabolized by the hepatocytes. The metabolism was responsible by three pathways. The first pathway utilizes the enzyme alcohol dehydrogenase (ADH) and was responsible for majority of the metabolism. The minority of the metabolism was responsible by enzyme microsomal ethanol oxidizing system (MEOS) and catalase. The remaining 2-5% of the alcoholic beverage will be eliminated through exhalation, urine, sweat, saliva, or another secretion (Ammon *et al*., 1996 cited in Thongra-ar, 1991).

Alcohol was oxidized to acetaldehyde by ADH, MEOS and catalase. After that, it is metabolized to acetic acid by aldehyde dehydrogenase. Next, acetic acid enters the citric acid cycle and undergoes decomposition to CO_2 and water. About 40% of acetate will be into the lipid synthesis. ADH was active at low blood alcohol concentration, but the MEOS and catalase become active when the blood alcohol concentration was elevated (Hasumura, 1997).

2.3 Physical and chemical properties of ethanol

Ethanol is a highly small chain alcohol with an infinite aqueous solubility. It is hygroscopic. It has a tendency to absorb moisture when in contact with the air. Ethanol has high oxygen content. The physical and chemical properties of ethanol are summarized in Table 2-3.

Table 2-3 A summary of physical and chemical properties of ethanol
(American Methanol Institute, 1998)

Physical and chemical properties	
Physical state: Clear liquid	Specific gravity/density: 0.99 at 20 ^{o}C
Appearance: Colourless liquid	Vapour density: (Air = 1), 1.59
Odour: Mild, rather pleasant	Vapour pressure: 59 mmHg at 20 ^{o}C
Solubility in water: Complete	Viscosity: 1.2 mPa at 20 ^{o}C
Boiling point: 78 ^{o}C	Molecular formular: C_2H_5OH
Melting point: -114.1^{o}C	Molecular weight: 46.0414

2.4 Fate in the environment

In the atmosphere, ethanol will be quickly oxidized, the half-life ranges between 12 hours to 5 days. In the soil or groundwater, it was also rapidly biodegraded with a half-life ranging from 2.4 hours to 2.1 days. Finally, in surface water following a pure ethanol spill, ethanol was predicted to quickly biodegraded, estimated half-life range from 6 hours to 1 day (American Methanol Institute, 1998).

Table 2-4 Estimated half-life of ethanol in the environment
(American Methanol Institute, 1998)

Environment medium	Half-life (hours)
Soil	2.6-24
Air	12.2-122
Surface water	6.5-26
Groundwater	13-52

2.5 Ethanol toxicology

The toxicological studies of ethanol have been reported on the health effects from ingestion in mammals and human, and acute toxicity in aquatic system.

2.5.1 Ingestion

The toxicity and health effects from ingestion of moderate to large quantities of ethanol had been extensively investigated. Ethanol could increased the risks of cancer, adversely affected on embryo development, produce neurotoxicity and cause various other types of damage (American Methanol Institute, 1998).

Adverse effects on the liver have been noted in both animals and humans chronically exposed to ethanol. Symptoms initially include fatty infiltration, inflammation and may progress to focal necrosis and/or fibrosis. Chronic oral doses to animals are typically 8-15 g/kg/day (rats, dogs). The oral LD_{50} for adult rats has been reported to range from 11.5 to 17.8 g/kg. Younger animals are more sensitive (LD_{50}= 6.2 g/kg). Lethal effects of ethanol are presented in Table 2-5.

Table 2-5 Lethal dose of ethanol in rats and mice (American Methanol Institute, 1998).

Test animal	Route of administration	LD_{50} (mg/kg)
Rat	Intravenular injection	1,400
Rat	Intraperitoneal injection	4,070
Rat	Oral	7,060
Mouse	Oral	7,800

Effects of ethanol on the liver

Three histologically different stages of alcoholic liver disease had been identified: hepatic steatosis or alcoholic fatty liver; alcoholic hepatitis or steatonecrosis and alcoholic cirrhosis (Ishak *et al*., 1991).

The steatosis was resulted from an abnormal concentration of fatty deposits inside liver cells. As a result, fat accumulated and ultimately interfered with liver function. Without consumption, the accumulated fat was gradually biodegraded and the liver returns to normal (Levinthal *et al*., 1996). In addition, a mild increase of connective tissue of the central veins and centrilobular sinusoids, minute foci of hyaline necrosis and sinusoidal sclerosis might occurred (Anderson and Kissane, 1977).

Alcoholic hepatitis was characterized by swollen hepatocytes with pale granular cytoplasm. Mallory bodies, intracellular eosinophilic aggregates of dense proteinaceous material, were frequently presented within these cells. It possesses strong chemotaxic properties. Neutrophils were migrated into liver cells containing Mallory bodies, contributing to ethanol induced hepatocellular damage (Takahashi *et al*., 1987). In addition, a mild thicken of sinusoidal and central vein walls was commoned (Anderson and Kissane, 1977). This stage was reversible with abstinence though some residual scar might remained. Less numerous, lymphocytes was also invaded typically in portal areas (Woods *et al*., 1993).

Although deposition of fibrous tissues in the liver could occurred almost any where, early fibrosis typically surround central veins. This pericentral fibrosis was often associated with sclerosis hyaline necrosis. Central vein obliteration could occurred with severe fibrosis, leading to post sinusoidal portal hypertension. However, pericentral fibrosis could be seen in the absence of inflammation and necrosis (Nakano *et al*., 1982).

Cirrhosis was characterized by extensive fibrosis and regeneration nodules (Woods *et al*., 1993). Though obstinence helps to prevent further liver degeneration when cirrhosis was diagnosed, the condition was not reversibled except by liver transplantation surgery (Levinthal, 1996).

Kupffer cells in ethanol-induced liver injury in vivo

Adachi *et al*. (1994) demonstrated that when Kupffer cells in rats treated with enteral ethanol were inactivated by gadolinium chloride ($GdCl_3$); serum enzyme levels, fatty changes, inflammation and necrosis were decreased significantly. Additionally, ethanol affected Kupffer cell functions such as phagocytosis bactericidal activity and cytokine production (Yamada *et al*., 1991). Next, serum tumor necrosis factor-α (TNF-α) was increased in alcoholics (Stahnke *et al*., 1991 cited in Thurman, 1998), supporting the idea that Kupffer cells were activated in patients with alcohol liver disease. TNF-α was produced exclusively by the monocyte macrophage lineage and Kupffer cells were the major population of this lineage (Decker *et al*., 1989).

TNF-α plays a role in alcoholic injury

It was hypothesized that activated Kupffer cells release mediators were toxic to liver cells or serve as chemoattractant for cytotoxic neutrophils that invaded the liver. TNF-α, and oxygen radicals were released from activated Kupffer cells (Martinez *et al*., 1992). Monden *et al*. (1991) reported that TNF-α and superoxide inhibited protein synthesis in cultured rat hepatocytes and this effect could be observed in the supernatant of cultures of activated Kupffer cells. Iimuro *et al*. (1997b) showed that rats administered ethanol enterally and injected with antibody to TNF-α were protected from ethanol-induced hepatic injury. Moreover, TNF-α could stimulated neutrophil migration and activation and also induced protease and oxygen radical release (Theile, 1989).

Kupffer cells play a role in hypermetabolism induced by ethanol

During the ethanol metabolism, the reduction in both glycolysis and glycogen reserves had occured (Yuki and Thurman, 1980 cited in Thurman, 1998). Basal rates of oxygen and ethanol uptake were almost doubled after ethanol treatment (Casteleijn *et al*., 1988 cited in Thurman, 1998), but were blocked by $GdCl_3$ (Bradford *et al*., 1993). Thus increases in respiration and ethanol metabolism were blocked by inactivation of Kupffer cells. Importantly, condition medium from isolated Kupffer cells which contained elevated levels of prostaglandins, were stimulated oxygen consumption of parenchymal cell (Qu *et al*., 1996).

Ethanol causes hypoxia in parenchymal cells

In addition to hypermetabolism, high doses of ethanol could altered hepatic microcirculation by stimulated endothelial-1 production (Hijioka *et al*., 1991). Arteel *et al*. (1997) used the pimonidazole, a 2-nitroimidazole hypoxia marker, to assess hypoxia in ethanol fed rats. Pimonidazole was reductively activated by nitroreductases and was binded to thiol residues on proteins and macromolecules in the absence of oxygen and adducts. Results of these experiments confirmed that pericentral hypoxia could occured during alcoholic metabolism and had been blocked when Kupffer cells were destroyed with $GdCl_3$ (Bradford *et al*., 1993). It also demonstrated that chronic ethanol treatment caused hypoxia (Adachi *et al*., 1994).

Involvement of free radicals in the mechanism of ethanol induced liver injury

Adachi *et al*. (1994) detected a free radical in bile from rats exposed to ethanol. This free radical signal was diminished over 50% when Kupffer cells were destroyed after treatment with $GdCl_3$.

Role of gender

Iimuro *et al*. (1997a) reported that ethanol caused greater hepatic injury in females than males. This study measured parameters including serum aspartate aminotransferase, pathological score, neutrophil infiltration, levels of circulating endotoxin and expression of intercellular adhesion molecule-1. All parameters assessed were increased by ethanol about twofold more in females than in males. For the histological change, fatty liver was panlobular deposited in livers of female rats, whereas it was pericentral localized in males. Furthermore, significantly more hepatic infiltration of inflammatory cells was observed after ethanol administration in females. Yin *et al*. (2000) demonstrated that the sensitivity of rat liver to alcohol-induced injury was directly related to estrogen, which increased endotoxin in the blood and CD14 expression in the liver, leading to increased TNF-α production.

Iimuro *et al*. (1996) quantitated tissue hypoxia in ethanol fed male and female rats. In this study, hypoxia marker binding was two to three times more intensed in females than in males after 4 wks of ethanol treatment. Furthermore, nuclear factor-kB (NF-kB) was sensitive to oxidants and was elevated seven to eight times more in females than in males.

Effect of ethanol on the kidney

The high concentrations of alcohol did not interfere with tubular cell function. In contrast, acetaldehyde exerted could disturbed the electrical membrane potential and intracellular ion concentrations (Deetjen, 1985 cited in Vamvakas *et al*., 1998). In *in vitro* studies of Parenti *et al*. (1991), ethanol interfered with Na^+/K^+- ATPase of rat renal brush border membrane vesicle. In contrast, Na^+-ATPase was impaired by ethanol in the basolateral membrane (Rothman *et al*., 1992). Since Na^+-ATPase was involved in the

active regulation of cell volume, its inhibition could contribute to the alcohol-induced cellular hypertrophy.

In most investigations acute alcohol administration did not alter glomerular filtration rate (GFR) and renal blood flow (RPF) (Kalbfleisch *et al*., 1963). After chronic alcohol ingestion, an impairment of GFR associated with renal hypertrophy in rats was found. Histopathological examination revealed interstitial edema and tubular dilatation with flattening of epithelial lining cells (Van Thiel *et al*., 1979).

2.5.2 Aquatic toxicity

Most of the acute toxicity studies on aquatic organism have been observed. Cogwill and Milazzo (1991) reported the sensitivity to ethanol for two cladocerans, *Ceriodaphnia dubia* and *Daphnia magna.* This study reported values of median lethal concentration (LC_{50}) and median effective concentrations (EC_{50}) for ethanol (Table 2-6). LC_{50} is the estimated concentration that is expected to be lethal to 50% of the test animal; and EC_{50} is the estimated concentration that is expected to cause an effect other than death to 50% of the test animals (non-lethal effects include changes in behavior, growth, immobilization and reproduction).

Using the USEPA classificatory scheme, the LC_{50} for ethanol was found to be slightly toxic. This study also presented comparative response data which included the two cladocerans and fathead minnow *(Pimephales promelas).* The results of this study indicated that ethanol was essentially non-toxic to the species tested (American Methanol Institute, 1998). In another study, Lagderspetz *et al*. (1993) provided a comparison of the toxicity of ethanol to *Anodonta cygnea* (mussel) and *Daphnia magna* (water flea). The authors reported EC_{min} value of 2 mg/L (EC_{min} is defined as the lowest concentration of ethanol which in one hour significantly affected the particles transport rate by frontal gill cilia of mussels).

Table 2-6 Reported median lethal and median effective concentration of ethanol.

Test animal	LC_{50}	EC_{50}	References
Daphnia			
Daphnia magna	9,248 mg/L (48 h)	14 mg/L (216 h)	Cogwill and Milazzo, 1991.
Daphnia pulex	-	24 μg/L (48 h)	AMI, 1998.
Ceriodaphnia dubis	-	26 mg/L (216 h)	Cogwill and Milazzo, 1991.
Shrimp			
Palaemonetes pugio	0.53 % v/v (12 d)	-	Foss and Ray, 1997.
Fish			
Acipenser transmontanus	1,000 μg/L (42 d)	-	AMI, 1998.
Lepomis macrochirus	93 μg/L (96 h)	-	AMI, 1998.
Leuciscus idus	8,000 mg/L (48 h)	-	AMI, 1998.
Oncorhynchus mykiss	12,000-15,000 mg/L (96 h)	-	AMI, 1998.
Pimephales promelas	14,740 mg/L (96 h)	-	AMI, 1998.
Salmo gairdneri	13,000 mg/L (96 h)	-	AMI, 1998.
Frog			
Rana arvalis (embryo)	-	0.89-187.40 μg/L (3 h)	AMI, 1998.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

2.6 Nile tilapia *Oreochromis niloticus*

General classification of *O. niloticus* is:

Kingdom Animalia

Phylum Chordata

Subphylum Vertebrata (Craniata)

Superclass Gnathostomata

Class Osteichthyes

Order Perciformes

Suborder Percoidei

Family Cichlidae

Genus Oreochromis

Species *Oreochromis niloticus*

Common name: Nile tilapia, Nile mouth-brooder

Thai name: Pla Nil

The family Cichlidae is widely distributed in Africa and Palestine, South and Central America, Southern India and Sri Lanka. The tilapias originated exclusively from the Africa continent and from Palestine. The original distribution of *O. niloticus* is the Africa continent. The species, originating from upper Nile in Uganda evidently moved southwards, colonized in all the western Rift lakes down to Lake Tanganyika. It also colonized in central and western Africa via the Chad and Niger basins. Introduction of tilapias outside Africa began in 1939. Tilapias occur in natural waters throughout the tropic, even in Australia (Philipart and Ruwet, 1982). They are fishes of economic importance in tropic and subtropic countries.

O. niloticus was first introduced to Thailand in March 1965 by His Royal Highness Akihito, the Prince of Japan. Consequently, they were given to the Department of Fisheries for further development of culturing by His Majesty the King of Thailand (Phumipat, 1981). Nowsday, Nile tilapia is an essential food fish widely cultured in many areas throughout Thailand.

Nile tilapia is a well known freshwater fish of the big order Perciformes. The species is distinguished from other perch-like fishes in having one nostril on each side of the snout. Its body is fairly elongate, moderately deep and greatly compressed. Dorsal and ventral profiles are about equally convex. It has a dorsal fin with long base, spinous dorsal fin with 16-17 spinous finrays, followed by 11-15 soft finrays. Its anal fin is pretty short, consisting of 3 spinous finrays and 8-11 soft finrays. Scale is fairly large, cycloid, 2-3 series on cheek. Caudal peduncle broadly short. Mouth slightly oblique, portractile, broad with swollen lips. The variation of color is influenced by breeding season and its wide habitats or distribution. The upper posterior margin of fins is coverd by broden dark crossed bands (Wongratana, 1996 cited in Srijunngam, 1998).

All the tilapias, in the broad sense, have in common a mainly herbivorous diet. Structural adaptations to this diet are the long, coiled intestine, the bicuspid and tricuspid teeth of the jaws and the small, sharp pharyngeal teeth (Trewavas, 1982).

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

2.6.1 Histopathological alteration of fish liver

A wide variety of histopathological changes have been investigated in the livers of various fish species exposed to different xenobiotic chemicals.

Effect of insecticides

Jonsson and Toledo (1993) demonstrated the acute toxicity of endosulfan to zebra fish *Brachydanio rerio* and yellow tetra *Hyphessobrycon bifasciatus* was determined by 24-h LC_{50}. The LC_{50} value were 2.6 and 1.6 ug/L for *H. bifasciatus* and *B. rerio*, respectively. The liver of both species showed zonal necrosis and lipid accumulation. In some fish, mononuclear inflammatory infiltrates were also observed. For catfish *Tandanus tandanus*, after the 24 h exposure to endosulfan, the histological studies of liver showed atrophied hepatocytes with pyknotic nuclei and vacuolation in cytoplasm. Moreover, dilation of sinusoids and lysis of cell membranes were presented (Nowak, 1996). Patwardhan and Gaikwad (1991) showed the histopathological alterations in the liver of *Gambusia affinis* exposed to sumithion. The alterations included necrosis of hepatocytes, vacuolation in cytoplasm and destruction of sinusoidal wall. Patil *et al*. (1992) demonstrated fatty accumulation and lysis of cell membrane in the hepatocytes of *Boleophthalmus dussumieri* exposed to the monocrotophos. Radhaiah and Jayantha-Rao (1992) studied on *Tilapia mossambica* that had exposed to fenvalerate, this insecticide caused histological changes in the liver. The alterations were observed in vacuolation with necrosis of hepatocyte, pyknotic and eccentric nucleus and degeneration in the cytoplasm. Wattanasirmkit and Patamastan (1997) showed the acute toxicity of *Derris trifoliate* root extract to Nile tilapia *Oreochromis niloticus* . The LC_{50} at 96 hours value were 6.5 ppm. The histopathological alterations in the liver showed fat accumulation, foci and diffused necrosis in nearby blood vessels. In addition, dilatation of blood vessels and congestion were seen in both central vein and sinusoids.

Effect of fungicides

Gerundo *et al*. (1991) showed the effect of malachite green on the liver of salmon *Salmo gairdneri*. The histopathological findings included sinusoidal congestion and focal

necrosis. The rainbow trout *Oncorhynchus mykiss* exposed to the triphenyltin acetate, the histological changes showed an irregular vacuolation of hepatocytes and slight inflammation (Schwaiger *et al*., 1996).

Effect of herbicides

Histopathological observed in the liver of carp *Cyprinus carpio* exposed to glyphosate herbicide at 620 mg/L, congestion of few sinusoid and focal fibrosis were recorded (Neskovic *et al*., 1996).

Effect of water pollution

Folmar *et al*. (1993) reported that the brown bullheads *Ameiurus nebulosus* collected from the Buffalo and Niagara rivers in New York had a high incidence of liver cancer. These locations contained a variety of chlorinated pesticides, PCBs, dioxin, aromatic amines, polynuclear aromatic hydrocarbons and metals. Wu *et al*. (1999) reported histological changes of the crucial carp *Carassius auratus* exposed to dioxin and related compounds. These lesions were characterized by severe enlargement of hepatocytes. Alterations in the cell included formation of condensed and irregular cell nucleus, polynuclei, dispersed heterochromatin, enlargement of the nucleolus and degeneration of the nucleus. Effluent originating from pulp and paper mills had been reported to induce a variety of histological changes in fish including the winter flounder *Pleuronectes americanus* (Khan *et al*., 1994; Khan, 1997, 2003). These effects included focal vacuolation, pyknotic hepatocytes, lipid accumulation and macrophage aggregation in the liver. In addition, bile duct hyperplasia with increased wall thickness and granulomas (pericholangiolar fibrosis) were also noticed. Khan (1998) demostrated histological abnormalities in the liver of winter flounder *Pleuronectes americanus*, captured from the petroleum refinery in New foundland. Examination of tissues revealed

in the liver basophilic staining hepatocytes, pericholangitis and macrophage aggregates with hemosiderin. Additionally, bile duct hyperplasia were also presented.

Rudolph *et al*. (2001) studied the effects the water-accommodated fraction (WAF) of petroleum hydrocarbons on the rainbow trout *Oncorhynchus mykiss*. Hepatic alteration in fishes exposed to WAF showed cellular changes related to loss of cellular shape in hepatocytes and differences in quantities of cytoplasmic components. Khan (2000) reported the histopathological lesions in the liver of 4 species of benthic fish, celpout *Lycodes lavalei*, celpout *L.vahli*, striped wolffish *Anarhichas lupus* and American plaice *Hippoglossoides platessoides* on the Grand Banks of Newfoundland. The lesions were characterized by hypervacuolation with microvesicles in hepatocytes and hypertrophy. Furthermore, the bile duct hyperplasia and vesicular nuclei were noticed. Myers *et al*. (1994) found the necrosis and proliferation of hepatocytes in the English sole *Pleuronectes vetulus*, starry flounder *Platichthys stellatus* and white croaker *Genyonemus lineatus*. Kumari and Kumari (1995) presented the vacuolation in cytoplasm, necrosis and degeneration of hepatocytes in the *Channa striatus* captured from Hyderabod, India. Moreover, the abnormality of nucleus and cell membrane were also observed.

Effect of metals

Heteropneustes fossilis had been exposed to cadmium at concentration of 57 mg/L for 30 days, the cell membrane showed fragmention, vacuolation in the cytoplasm and modification in the nucleus (Ghosh and Chakrabarti, 1993). Kotsanis and Greorgudaki (1999) who investigated the role of arsenic in fish carcinogenesis, the 6 month bioassay was conducted and utilized the rainbow trout *Oncorhynchus mykiss* at the early life stage of development. The histological analysis showed the degeneration and inflammatory lesions (necrosis, granulomas and fibrosis) and nonneoplastis proliferative lesions (liver hyperplasia). Histopathological changes were observed in the liver of lake whitefish

Coregonus clupeaformis fed arsenic contaminated diets included hepatocytes degeneration and disruption of cord structure as well as the areas of eosinophilic hepatocytes. The inflammation, characterized by the aggregation of lymphocytes, and focal necrotic lesions were also noticed (Pedlar *et al*., 2002). The lake whitefish that were fed uranium contaminated diets showed necrosis, hydropic swelling of hepatocytes, abnormal architecture, clear foci of liver parenchyma and alterations of the bile ductule epithelium (Cooley *et al*., 2000). In lake whitefish fed nickle contaminated diets, areas of normal parenchyma were interspersed with larger area of focal necrosis. These areas were most commonly characterized by swollen and ruptured hepatocytes, altered positioning of nuclei, pyknotic nuclei and granular cytoplasm. Furthermore, the presence of debris in sinusoids, altered cellular architecture, cellular dissolution, inflammation, and altered stainning were also observed (Ptashynski *et al*., 2002).

2.6.2 Histopathological alteration of fish kidney

Several studies on histopathological alterations of kidney have been documented in many fish species exposed to various toxicants.

Effect of metals

Gill *et al*. (1987) reported the histopathological changes of kidney in *Puntius conchonius* after exposure to cadmium. These alterations included necrosis of epithelial cell in proximal tubules and destruction of glomerulus wherease the histological lesions of kidney in stickle back *Gasterosteus aculeatus* presented the vacuolation and granulation in the epithelial cell of proximal tubules (Oronsaye, 1989). Singhal and Jain (1997) exposed common carp *Cyprinus carpio* to cadmium for 4 weeks and found pyknosis, karyorhexis and karyolysis of proximal tubules. The lumen of the Bowman's capsule was reduced. Furthermore, diffusively thickened glomerular basement membrane was separated from

the basal lamina. Other changes, such as disappearance of brush border in proximal tubules, reduction in the number of collecting ducts, extreme swelling and simultaneous disintegration of the renal tubules and displacement of the nucleus in the renal tubules were also observed. Georgudaki and Kotsanis (2001) observed the histopathological changes in the rainbow trout injected with cadmium in the sac-fry stage of development. The lesions showed the fibromas and necrosis, in addition to these lesions, granulomas were also found. They were basically multifocal lesions, consisting of several cell types, but primarily histocyte-like cells with large, foamy cytoplasm and a small vesicular nucleus distributed among a network of fibrocytes and capillaries. In addition, Forlin *et al*. (1986) found hyaline droplets and lysosome in the proximal tubules of rainbow trout.

The fish exposed to mercury showed granulation and hyperplasia of proximal tubules. Moreover, the pyknosis of epithelial cell of proximal tubules, shrinkage of glomerulus with enlargement of Bowman's space were observed (Pandey, 1994; Reimschuessel and Gonzalez, 1998). Mercury was found mainly in lysosome and in the basal lamina of proximal tubules (Bautrup *et al*., 1986). Cooley *et al*. (2000) reported the kidneys alterations of lake whitefish fed with uranium. The alterations were observed in all segments of the nephron, the interstitial tissue, the vascular system and immune system. The most consistently observed histopathologies were proximal tubules necrosis, foci of dilated tubules, increased abundance of pigmented macrophages, haemorrhaging, inflammation and depletion of haematopoietic tissue. Kidneys of whitefish fed with dietary nickel exhibited the increase of the presence of swollen, ruptured and necrotic epithelial cell in proximal tubules, distal tubules and collecting ducts, disintegrating tubules, appearance of debris in Bowman's space of glomeruli, debris in the lumen of proximal tubules, distal tubules, collecting ducts and hematopoietic tissue. In addition, depletion of hematopoietic tissues, increased presence of pigmented macrophages and altered staining were also found (Ptashynski *et al*., 2002). After the single injection of arsenic in the sac-fry stage of rainbow trout, the kidneys lesions observed were large areas

of fibrosis. Moreover, an extensive proliferation of fibrotic tissues and a regenerative proliferation of glomerular cells were also noticed (Kotsanis and Georgudaki, 1999).

The lusitanian toadfish *Halobatrachus didactylus* exposed to vanadium by intraperitoneal administration, showed the alterations in the renal tissue. These alterations included damaged renal tubules showing disorganized epithelial cells in different state of necrosis. Reabsorbed renal tubules and hyperchromatic intersititial tissues were also observed (Borges *et al*., 2003). Yang and Chen (2003) exposed juvenile common carp *Cyprinus carpio* to gallium and found the degenerative changes of tubular epithelial cells and ectasis of Bowman's capsules. Severe renal lesions were also found in fish exposed to the highest concentration. Similarly, damage to tubular epithelial cells and degeneration of glomeruli became more prominent.

Effect of insecticides

Kumar and Pant (1984) exposed rosy bard *Barbus conchonius* to aldicarb and found renal epithelial necrosis, blood congestion in glomerulus and destruction of lymphoid tissue. Mophatra and Noble (1992) reported the renal epithelial necrosis, increase in amount of vacuole, and renal lumen dilation in gray mullet *Liza parsia* exposed to dichlorvos. *Ophiocephalus punctatus* exposed to diazinon showed renal epithelial necrosis and accumulation of necrotic debris within the tubule lumen. In addition, the shrinkage of glomerulus was also found (Sastry and Sharma, 1981). Banerjee and Bhattacharya (1994) found distal tubules degeneration in *Channa punctatus* exposed the elsan.

Effect of herbicides

The 2,4-dichlorophenoxyacetic acid (2,4-D) poisioning prompts a series of pathological processes in the excretory renal parenchyma of tench *Tinca tinca*, these included mesengial proliferative glomerulonephritis and tubular nephrosis (Gomez *et al*., 1999).

Effect of antibiotics

Trout *Oncorhychus kisutch* given intraperitoneal injection of tobramycin also showed renal epithelial necrosis, sloughing of the epithelium and accumulation of necrotic debris within the tubule lumen (Schneider *et al*., 1980). Intramuscular injection of gentamycin sulfate resulted in thickening and sloughing of the glomerular epithelium in channel catfish *Ictalurus punctatus* (Rolf *et al*., 1986). On the other hand, goldfish *Carassius auratus* and tilapia *Oreochromis niloticus* showed pyknotic nuclei and karyolysis of proximal tubules but renal epithelial proliferation had occurred (Reimschuessel and Williums, 1995; Augusto *et al*., 1996). Toadfish *Opsanus tau* showed renal epithelial necrosis after being exposed to gentamycin (Jones *et al*., 1997). Lauren *et al*. (1989) found tubular degeneration and eosinophilic, proteinaceous, intratubular casts and hyaline droplets, and an increase in amount of melanin-like intertubular deposits in rainbow trout fed with the antibiotic fumagillin.

Effect of water pollution

Brown bullheads *Ameiurus nebulosus* were collected from the Black river and Old Woman Creek in Ohio State, receiving effluent from a steel plant and an associated coking facility. Histopathological evaluations showed that the incidence of cholangiomas, cholangiocellular carcinomas and hepatocellular carcinomas (Folmar *et al*., 1995).

Reimschuessel *et al*. (1989) reported the renal epithelial necrosis, vacuolization and shrinkage of glomerulus in goldfish exposed to haxachlorobutadiene (HCBD) but proliferation of epithelium had happened. Rainbow trout showed proximal tubules degeneration after being exposed to the terachloroethylene and the proliferation of epithelium was also observed (Reimschuessel *et al*.,1993).

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER III

MATERIALS AND METHODS

3.1 Subjects

Nile tilapia *Oreochromis niloticus* at the age of 3 weeks, were purchased from the Pathumtani Breeding Station. Fish were transported to a glass aquarium in laboratory. For acclimatization purposes, they were held in 325-L glass aquarium with well aerated and dechlorinated water for one week. They were fed commercial pellets (CP company) twice daily at approximate 3-5% body weight. Water was changed every 2 days.

3.2 Test material

Distilled liquors 30 degree (% v/v) from Surattani Province was used. Because this liquors had the highest acute toxicity in brine shrimp test when compared with other distilled liquor (Sam-angsri, 2002).

3.3 Acute toxicity test

The acute static toxicity bioassay was carried out to determine the LC_{50} value at 95% confidence intervals of the ethanol after 96 hours (ASTM, 1980). Fish at the age of 4 weeks were divided into experimental and control groups, and they were fasted 24 hours prior to the exposure. Acute toxicity test consisted of 2 protocols, range finding test and definitive test were presented below.

3.3.1 Range finding test

The test was conducted in 14-L glass jars containing 10 litres of different concentrations of the 30 degree alcoholic solution. Six jars were filled with the tested solution at concentration of 1, 10, 100, 1000, 10,000 and 100,000 ppm while one jar was filled with holding water and left as control. Of all experimental conditions each was done for 3 replicated with 10 fish used for each replicates. Mortality was observed and recorded every 24 hours throughout the test period of 96 hours.

3.3.2 Definitive test

The definitive test was carried out with concentrations of 15,000, 20,000, 25,000, 30,000, 35,000 and 40,000 ppm and the test protocol was as described in the previous test.

3.3.3 Analysis for LC_{50} value

The median lethal concentration (LC_{50}) value and its corresponding 95% confidence intervals of each exposure time were calculated by Probit analysis program (SPSS 10).

3.4 Determination of application factor (AF)

The maximum acceptable toxicant concentration (MATC) is hypothetical concentration and is in range of no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) (Rand and Petrocelli, 1985; Sprague, 1990), the range of these concentration was selected from the previous calculation of LC_{50} value. The LC_3 was chosen as a maximum acceptable toxicant concentration (MATC). So the MATC was 3857.18 ppm. The test concentration was estimated by use of the application factor (AF) concept. Based on these values, the application factor (AF) was calculated as presented below.

$AF = MATC/LC_{50}$ 96 hours

AF = 3857.18/15751.21

AF = 0.25

From the AF, the sublethal concentration of the solution used for subchronic toxicity test was determined at $0.25xLC_3$. Therefore, the concentration of solution for subchronic exposure was 964.30 ppm.

3.5 Subchronic exposure

New *O. niloticus* brood stock was obtained from the same source and was acclimatized for 1 week in 325-L glass aquaria, provided with aerated water supply prior to and during subchronic exposure to ethanol solution. The treated aquarium was filled with 150 litres of 964.30 ppm ethanol while the control aquarium was filled with 150 litres of clear water. The exposure was carried out continuously for six months.

The static renewal system was used throughout the test. Holding water of both treatment and control groups was renewed every two days. The fish (aged 4 weeks) were divided into control and treatment groups. Each aquarium contained approximatly 250 fish which were consequently separated into two aquaria when the fish grew up to the age of 4 months.

3.6 Sampling

During the exposure period, fish of both control and exposed groups were sampled (n=20) every month for 6 months. They were fasted for 24 hours prior to sampling during exposure period.

Sampling was initiated by dipnetting randomly selected fish from each aquarium. The weight (g) of each fish was measured. Then, five fish were cool shocked at 0 ^{o}C. The abdomen of each fish was opened. Liver and kidney were removed. Livers were measured for weight, fixed in 10% neutral buffered formalin for 48 hours and divided into 2 parts. First part was preserved in 70% ethanol, the remaining was embedded in frozen media in order to provide the histochemical study. Kidneys were also fixed in 10% neutral buffered formalin for 48 hours and preserved in 70% ethanol for histopathological study.

3.7 Determination of Liver somatic index (LSI)

Liver somatic index (LSI) of fish was calculated by dividing the liver weight by the body weight and multiplying by 100.

$$LSI = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100$$

3.8 Data analysis

The LC_{50} values at 95% confidence interval of each exposure time were determined by Probit analysis (Finney, 1971). General calculations of body weight, liver weight, and LSI were performed on computer by Microsoft Excel for Windows 2000. Statistical analyses of the data was performed on computer by SPSS 10. Student's t-test was used to compare effects on LSI. Significant difference was considered from probability of $p<0.05$.

3.9 Light microscopic study

There are two categories of light microscopic study technique; the standard paraffin technique and the frozen technique.

3.9.1 Standard paraffin technique

The liver and kidney samples were fixed in 10% buffered formalin for 48 hours and preserved in 70% ethanol. Five livers and kidneys from each experimental group were sampled for histological study. Liver and kidney tissues were processed according to standard histological techniques (Humason, 1979). All paraffin blocks of tissues were sectioned at 7 μm and stained with hematoxylin and eosin; and then studied under light microscopy. The standard histological method is presented below.

Routine paraffin method for liver and kidney of *O. niloticus*

70% ethanol (several days)

↓

90% ethanol (6 hours)

↓

95% ethanol (2 changes at overnight)

↓

N-butanol (1 hour)

↓

Xylene (1 hour)

↓

Xylene in paraplast 1:1 (30 min)

↓

Paraplast 1 (30 min)

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Routine paraffin method for liver and kidney of *O. niloticus* (cont.)

Paraplast 2 (1 hour)

↓

Embed in paraplast

3.9.2 Frozen technique

The frozen blocks of liver tissue were sectioned at 7 μm in a cryostat and stained with oil red O for lipid staining (Culling, 1963).

The oil red O staining

Air dry slide

↓

Immerse in oil red O (1 min)

↓

Rinse in distilled water

↓

Immerse in hematoxylin (1 min)

↓

Wash in running water

↓

Mount with glycerine jelly

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Acute toxicity of distilled liquors 30 degree on Nile tilapia *O. niloticus*

4.1.1 Range finding test

The mortal fish was not observed in the control and treated group at 1 ppm. The concentration of liquors at 100,000 ppm were killed all fish within 24 hours, the percentages of mortality at 10,000, 1,000, 100 and 10 ppm were 6.67, 6.67, 0 and 0, respectively. The concentration at 100,000 ppm was the lethal concentration which caused 100% mortality in young tilapia within 96 hours while the concentration of 10,000 ppm solution caused 26.67% mortality in the fish (Table 4-1).

Table 4-1 Percentage of mean mortality of *O. niloticus* at various distilled liquors concentrations in range-finding test (n=30).

Exposure time	Mean mortality (%) at distilled liquors concentrations (ppm)						
	1	10	100	1,000	10,000	100,000	Control
24-hours	0	0	0	6.67	6.67	100	0
48-hours	0	0	6.67	6.67	13.33	100	0
72-hours	0	6.67	13.33	13.33	20.00	100	0
96-hours	0	6.67	26.67	20.00	26.67	100	0

4.1.2 Definitive test

The concentration of liquors at 40,000 ppm were killed all fish within 24 hours, the percentages of mortality at 35,000, 30,000, 25,000, 20,000 and 15,000 ppm were 26.67, 20.00, 13.33, 6.67 and 0, respectively. The concentration of liquors at 35,000 ppm were killed all fish within 72 hours whereas the concentration at 30,000 ppm were killed all fish within 96 hours. Within 96 hours, the percentages of mortality at 40,000, 35,000, 30,000, 25,000, 20,000 and 15,000 ppm were 100, 100, 100, 86.67, 80.00 and 46.67, respectively (Table 4-2, Figure 4-1).

Table 4-2 Percentage of mean mortality of *O. niloticus* at various distilled liquors concentrations in definitive test (n=30).

Exposure time	Mean mortality (%) at distilled liquors concentrations (ppm)						
	15,000	20,000	25,000	30,000	35,000	40,000	control
24-hours	0	6.67	13.33	20.00	26.67	100	0
48-hours	26.67	40.00	46.67	53.33	86.67	100	0
72-hours	40.00	66.67	73.33	80.00	100	100	0
96-hours	46.67	80.00	86.67	100	100	100	0

4.1.3 Analysis for LC_{50} value

From Probit analysis, the median lethal concentration (LC_{50}) of distilled liquors for *O. niloticus* at 24, 48, 72 and 96 hours were 34,310.71, 24,284.81, 18,302.14 and 15,751.21 ppm, respectively (Table 4-3).

Table 4-3 Median lethal concentration (LC_{50}) of distilled liquors for Nile tilapia.

Exposure time	LC_{50} (ppm)
24-hours	34,310.71
48-hours	24,284.81
72-hours	18,302.14
96-hours	15,751.21

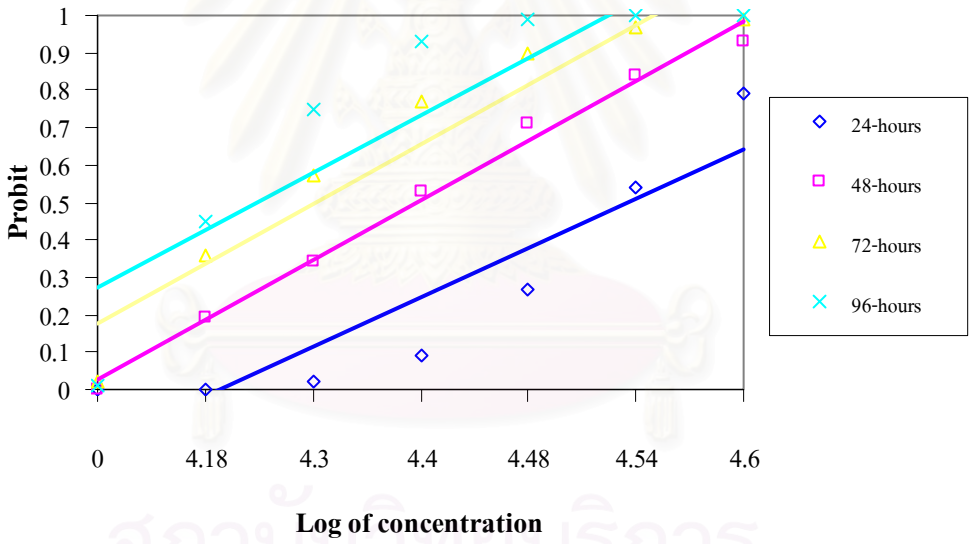


Figure 4-1 Median lethal concentration (LC_{50}) at 24, 48, 72 and 96 hours after exposure to various concentration of distilled liquors

The LC_{50} values of ethanol for many fish species have been reported. For example, the LC_{50} values for *Leuciscus idus*, blue gill *Lepomis macrochircus*, salmon *Salmo gairdneri*, fathead minnow *Pimelphales promelas* and rainbow trout *Oncorhynchus mykiss*, were determined at 8,000 mg/L (48 h), 93 ug/L (96 h), 13,000 mg/L (96 h), 14,740 mg/L (96 h) and 12,900-15,000 mg/L (96 h), respectively (American Methanol Institute, 1998). In the present study, the LC_{50} (96 h) value of disttilled liquors 30 degree for Nile tilapia was 15,751.21 ppm. Therefore, the toxicity of this liquors was determined as slightly toxic to tilapia. The values of acute toxicity may depend on the difference of experimental animals used (age and species), degree of alcohol and the duration of exposure.

4.2 Subchronic toxicity of distilled liquors on Nile tilapia *O. niloticus*

4.2.1 Liver somatic index (LSI)

The LSI values of control fish ranged from 1.65±0.13 at the beginning of sampling period to 1.50±0.11 at the end of the experiment. On the other hand, the LSI values of liquors treated group ranged from 3.17±0.18 at the beginning of sampling period to 3.16±0.18 at the end of the experiment. The LSI values of treated fish at all treated periods were significantly higher than control group ($p<0.05$). The body weights of liquors treated fish were significantly lower than the control group at all experimental periods ($p<0.05$) (Table 4-4).

Table 4-4 Mean body weight and LSI for Nile tilapia of control and liquors treated group at 4 months - 6 months (n=20).

Parameter	Experimental groups	Experimental periods (month)		
		4 th	5 th	6 th
		Mean±SE	Mean±SE	Mean±SE
Body weight (g)	Control	10.21 ± 0.39	14.46 ± 0.10	23.00 ± 1.15
	Treated	7.09 ± 0.45*	11.16 ± 0.71*	14.39 ± 0.74*
LSI	Control	1.65 ± 0.13	1.67 ± 0.07	1.50 ± 0.11
	Treated	3.17 ± 0.18*	2.90 ± 0.17*	3.16 ± 0.18*

* Significant difference observed between control and treatment groups ($p<0.05$).

In general, the LSI value of normal fish should be 1-2 % (Roberts, 1978). The increase of LSI value indicated the lipid accumulation or inflammation of liver. Hepatic inflammation of fish from other compounds was also studied. Schwaiger *et al*. (1996) reported inflammation of liver was also detected in rainbow trouts exposed to triphenyltin acetate and also detected in *Gambusia affinis* exposed to sumithion (Patwardhan and Gaikwad, 1991), rainbow trouts exposed to arsenic (Kotsanis and Greorgudak, 1999) and lake whitefish exposed to arsenic and nickel (Pedlar *et al*., 2002; Ptashynski *et al*., 2002). Lipid accumulation was found in *Boleophthalmus dussumieri* exposed to monocrotophos (Patil *et al*., 1992), *Tilapia mossambica* exposed to fenvalerate (Radhaiah and Jayan-Rao, 1992), rainbow trouts exposed to triphenyltin acetate (Schwaiger *et al*., 1996) and *Heteropneustes fosslis* exposed to cadmium (Ghosh and Chakrabarti, 1993).

4.2.2 Histopathology of liver

In normal liver, hepatocytes were arranged in branching cords or lamina, typically two cells thick which were separated by a maze of sinusoids. The nucleus of hepatocyte was round and contains a single prominent nucleolus. The cytoplasm of hepatocyte often contains vacuoles. Sinusoids, bile ductules and other blood vessels were randomly distributed within the liver parenchyma. The pancreatic tissue was surrounding the portal vessels entering the liver to form the hepatopancreas (Figure 4-2).

Semi-quantitative analyses of liver from treated fish demonstrated the severity and extent of alterations in liver. Within each sampling month, duration dependency was apparented especially in the occurrence and severity of inflammation and necrosis (Table 4-5).

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 4-5 Incidence of histopathological finding in Nile tilapia liver after exposure to distilled liquors 30 degree at 964.30 ppm from first through sixth month of experiment (n=5).

Lesion	Experimental periods (month)					
	1	2	3	4	5	6
[a] Lipid accumulation						
mild	0	0	0	1	0	1
moderate	1	3	1	0	1	0
marked	4	0	4	4	4	4
[b] Inflammation						
mild	0	4	4	1	0	1
moderate	0	1	1	0	1	0
marked	0	0	0	4	4	4
[b] Necrosis						
mild	4	0	2	1	0	0
moderate	1	4	3	0	0	1
marked	0	1	0	3	5	4
[b] Endothelial sloughing						
mild	0	2	2	3	2	2
moderate	0	3	3	0	1	1
marked	0	0	0	1	0	0
[b] Hyaline deposition						
mild	0	2	0	0	0	0
moderate	1	0	0	0	0	0
marked	4	0	0	0	0	0
[b] Regeneration nodule						
mild	0	0	0	1	0	2
moderate	0	0	0	0	0	0
marked	0	0	0	0	0	0

[a] Mild : ≤ 35 % of total area of one liver section;
Moderate : ≤ 70 % of total area of one liver section;
Marked : > 75 % of total area of one liver section

[b] Mild : ≤ 2 foci of lesions finding of one liver section;
Moderate : ≤ 4 foci of lesions finding of one liver section;
Marked : > 4 foci of lesions finding of one liver section

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Histopathological changes of the liquors treated group

After 1 month of exposure, the histopathological observed liver showed the presence of cytoplasmic vacuole which was positive stained with oil red O as in the control group. Hyaline granule accumulation was seen as highly eosinophilic deposition in the cytoplasm. Necrotic lesions were frequently near central vein of treated liver. In addition, the swelling of hepatocytes, dilatation of sinusoid and abnormal architecture of hepatic cord were also noticed (Figure 4-3, 4-4, Table 4-5).

After 2 months of exposure, the treated liver showed sloughing endothelium of central vein and infiltration of granulocytes in the liver parenchyma at the area adjacent to blood vessel. The dilated sinusoid and cellular debris which resulted from necrotic degeneration of hepatocyte were evidenced. In addition, hyaline granule accumulation in cytoplasm and hydropic swelling of hepatocytes were also found. From histochemistry studies, hepatocytes of exposed fish were positive stained with oil red O (Figure 4-5, 4-6, Table 4-5).

After 3 months of exposure, liver still exhibited endothelial sloughing of central vein. Swollen hepatocytes and the lost of liver parenchyma architecture were observed. Abnormal architectures had obscured liver sinusoids. The necrotic areas were seen near central vein and liver capsule. Inflammation was characterized by the presence of granulocytes infiltration. Hepatocytes of exposed fish were positive stained with oil red O as in the control fish (Figure 4-7, 4-8, Table 4-5).

After 4 months of exposure, treated livers of Nile tilapia were noticeably altered. The alterations consisted of swollen and necrotic hepatocytes as well as inflammation in liver parenchyma. The infiltration of lymphocytes at the area adjacent to hepatopancreas was markedly observed. In addition, sloughing of central vein endothelium with

granulocytes infiltrated and thickening liver capsule were also detected. Furthermore, the islands of regenerating hepatocytes with basophilic cytoplasm were also observed in some livers. Hepatocytes of exposed fish were more positive stained with oil red O than the control fish (Figure 4-9, 4-10, Table 4-5).

After 5 months of exposure, liver of Nile tilapia showed similar alterations as those observed in previous month. The sloughing of central vein endothelium was still observed. Necrotic degeneration of hepatocytes and inflammation were still observed. While hepatocytes of exposed fish were positive stained with oil red O, hepatocytes of the control group were slightly stained with oil red O (Figure 4-11, 4-12, Table 4-5).

After 6 months of exposure, the most consistently observed histopathologies were sloughing of endothelium in central vein as well as necrotic degeneration of hepatocytes and inflammation at the area adjacent to central vein. The inflammation indicated by presence of granulocytes and lymphocytes infiltration. Moreover, the islands of regenerating hepatocytes were also observed in some fish. Hepatocytes of exposed fish were positive stained with oil red O and hepatocytes of the control group were slightly stained with oil red O (Figure 4-13, 4-14, Table 4-5).

The majority of the liver after being exposed to ethanol showed some types of cytologic alteration. These were fat accumulation, hydropic swelling of hepatocyte, necrotic degeneration, inflammatory infiltration and sloughing of blood vessel endothelium. As were observed for hyaline granule accumulation, sinusoid dilatation, hepatocyte regeneration and thickening of liver capsule in small numbers.

Histological alterations in liver of Nile tilapia in this study were similar to the studies of ethanol on mammals. The alterations consisted of lipid accumulation, hepatocyte necrosis and inflammation. Iimuro *et al*. (1997a, 1997b) reported lipid

accumulation in liver was observed in ethanol fed rats and also observed in human who consumed ethanol (Ishak *et al*., 1991; Woods *et al*., 1993; Levinthal, 1996). Hepatic inflammation had been observed in ethanol fed rats (Takahashi, 1987; Iimuro *et al*., 1997a, 1997b) and human consumed ethanol (Ishak *et al*., 1991; Woods *et al*., 1993). Hepatocyte necrosis was observed in ethanol fed rats (Adachi *et al*., 1994). The similarities of liver pathologies observed in this study and those seen in ethanol-treated mammals, indicated that liver may also be the target organ of ethanol toxicity. In addition, sloughing of central vein endothelium, sinusoid dilatation, hepatocyte swelling and thickening hepatic capsule were reported in liver of treated fish. The hepatocyte necrosis that was observed in this study resembles to the result from the experiment on ethanol fed rats in hypoxia condition (French *et al*., 1984). The acute and chronic ethanol intake increased hepatic oxygen consumption in ethanol fed rats for the ethanol oxidation by alcohol dehydrogenase (ADH) and microsomal ethanol oxidixing system (MEOS) (Videla *et al*., 1973 cited in Thurman *et al*., 1990). The ethanol increase venous blood flow, but this increase was not adequate for the compensation of the increased oxygen consumption and would lead to the hypoxia-induced necrosis (Tsukamoto and Xi, 1989 cited in Thurman *et al*., 1990). Additionally, the increasing hepatocyte size after chronic ethanol consumption which resulted in obscured sinusoid could lead to the reduced flow or oxygenation through the sinusoid (Israel *et al*., 1982 cited in Tsukamoto *et al*., 1986; Vidins *et al*., 1985). Moreover, these lesions may be caused by acetaldehyde and free radicals which derrived from MEOS. Hasumura (1997) reported the acetaldehyde, a product of the metabolism of ethanol, was chemically highly reactive and also easily bound to plasma membrane, oganelle membrane, structural protein and enzyme protein. For this reason, the mechanism which results in hepatocyte alterations may involve lipid peroxidation which was a process in which the polyunsaturated fatty acid of cellular and subcellular phospholipid membranes were damaged by free radicals (Di Giulio, 1991 cited in Cooley *et al*., 2000). Thus, acetaldehyde may bind to plasma membrane and endoplasmic reticulum and causes membrane damage. The swelling of SER and dissociation of ribosomes from RER have

produced and interfered with ability to synthesize protein to complicate with lipid. Consequently, the result was lipid accumulated in hepatocytes (Kumar *et al*., 2002). Similarly, the swelling of hepatocytes may also involve with lipid peroxidation resulting in modification of membrane permeability. Hyaline granules present within hepatocytes are indicators of liver parenchyma damage. These inclusions were eosinophilic aggregated of intermediate filaments which presumably resist degradation (Kumar *et al*., 2002). The appearance of leukocytes infiltration in the liver supports the inflammatory reaction indicated by necrosis. Following necrosis, liver parenchyma must undergo adaptation. Regenerating hepatocytes, then, occur to replace necrotic hepatocyte.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Figure 4-2 Photomicrograph of *O. niloticus* liver of control group (H&E stain).

A. The liver of control group shows hepatocytes (H) with moderate degree of vacuolation, central vein (CV) and sinusoids (S) between hepatocytes. Bar scale = 25 μm.

B. The liver of control group shows pancreas surround the thin wall veins (V) and acinar cell of exocrine pancreas with zymogen granules (P). Bar scale = 100 μm.

C. The liver of control group shows bile ductules (BD). The bile ductules wall consists of the mucosa layer composed of simple columnar epithelium and submucosa or muscularis layer. Bar scale = 25 μm.

D. The liver of control group shows hepatocytes capsule simple squamous epithelium (→). Bar scale = 25 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

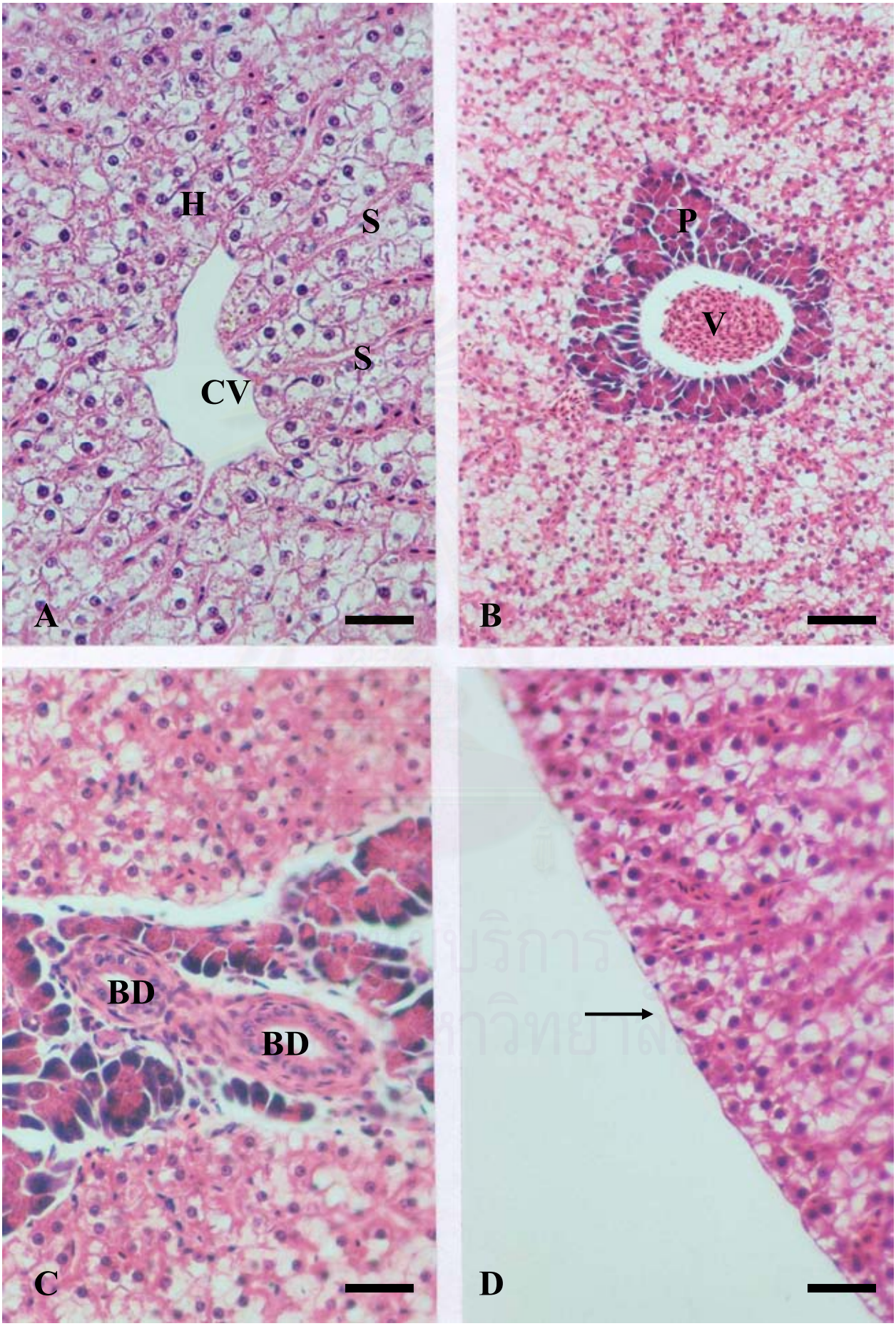

H
S
S
CV
A
P
V
B
BD
BD
C
D

Figure 4-3 Photomicrograph of *O. niloticus* liver at 1 month experimental period (H&E stain).

A. The liver of control group. Most hepatocytes show clear cytoplasm and still normal appearance. Central vein (CV) and sinusoid (S) show normal profile. Bar scale = 25 μm.

B. The liver of treated fish shows accumulation of hyaline granule (→) in cytoplasmic hepatocytes**.** Necrotic hepatocytes are found (*). Bar scale = 25 μm.

C. High magnification of micrograph shows hyaline accumulation (→) in cytoplasmic hepatocytes of treated fish liver. Bar scale = 10 μm.

D. The liver of treated fish shows dilated sinusoid (S) and the area of necrotic hepatocytes. The swelling of some hepatocytes (*) and cellular debris (→) are seen. Bar scale = 25 μm.

E. The liver of treated fish shows debris resulting from necrotic degeneration (→) near central vein (CV) and dilatation of sinusoid (S). Bar scale = 25 μm.

F. The liver of treated fish shows the area of necrosis (*) near central vein (CV) and abnormal architecture of liver tissue. The swelling (→) and hyaline accumulation (➤) are also found in hepatocytes. Bar scale = 25 μm.

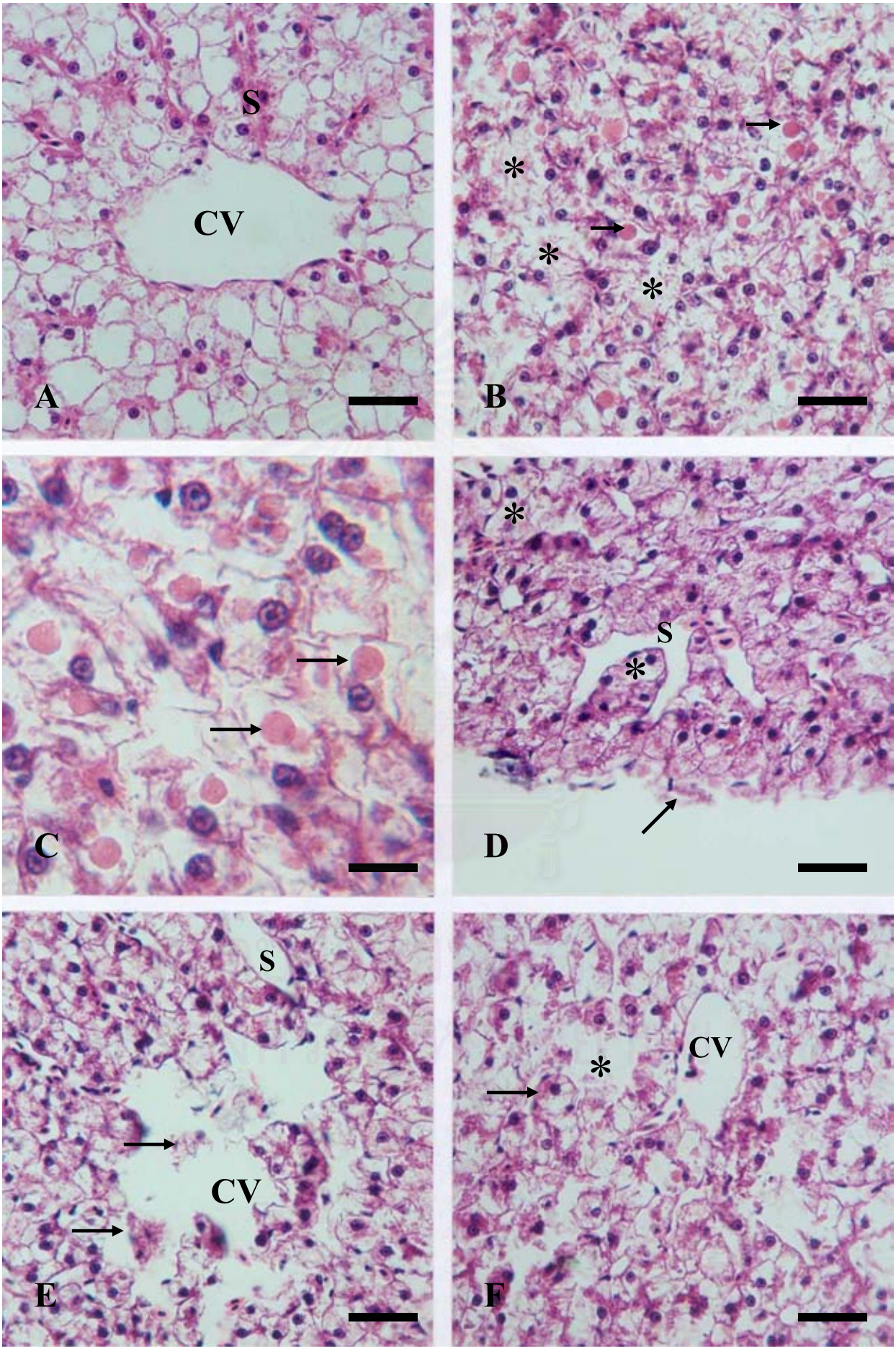

S
CV
A
B
C
S
D
S
CV
E
CV
F

Figure 4-4 Photomicrograph of *O. niloticus* liver at 1 month experimental period. (H&E, oil red O stain).

A. The liver of control group shows normal appearance with clear cytoplasm. Bar scale = 25 μm. (H&E stain).

B. The liver of treated group shows normal appearance with clear cytoplasm. Bar scale = 25 μm. (H&E stain).

C. The liver of control fish shows the small size of lipid droplets deposition in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

D. The liver of treated fish shows the large size of lipid droplets deposition in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

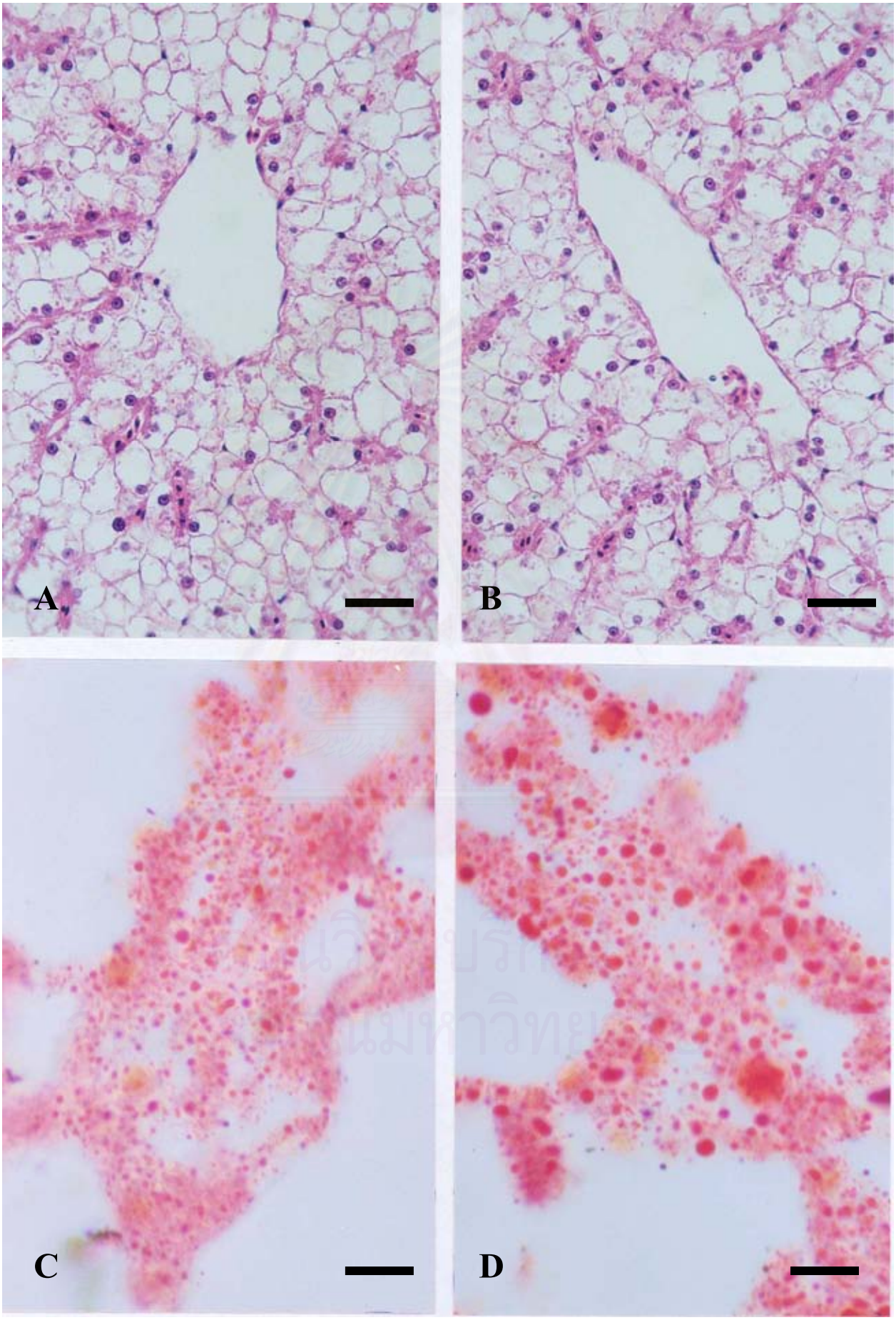

A
B
C
D

Figure 4-5 Photomicrograph of *O. niloticus* liver at 2 months experimental period (H&E stain).

A. The liver of control group with normal hepatic cord and moderate clear cytoplasm. CV: Central vein. Bar scale = 25 μm.

B. The liver of treated fish shows dilatation of sinusoid (S). CV: Central vein. Bar scale = 25 μm.

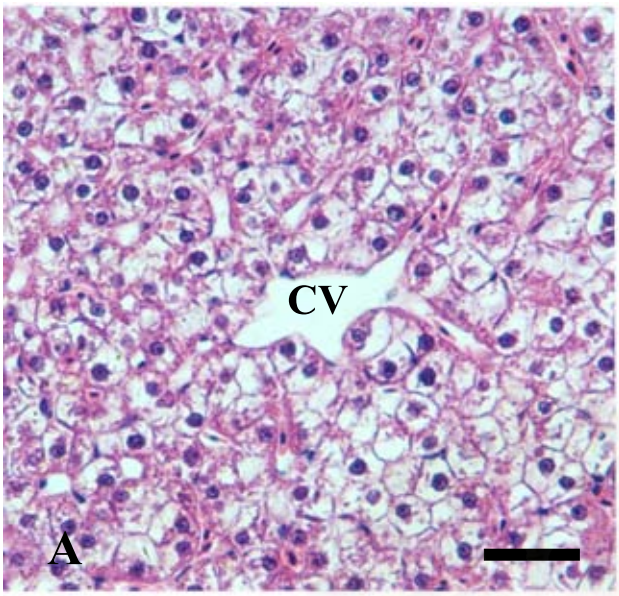
C. The liver of treated fish shows the accumulation of hyaline granule (→) in cytoplasm of some hepatocytes. Bar scale = 10 μm.

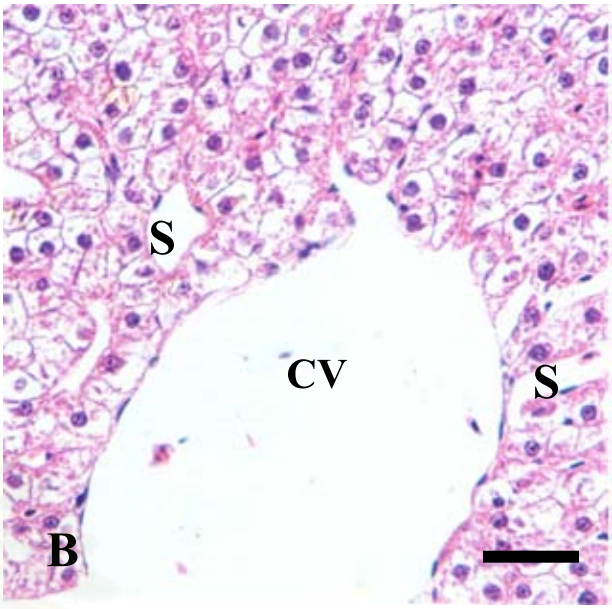
D. The liver of treated fish shows cellular debris (CD) resulting from necrotic degeneration of hepatocytes. Bar scale = 25 μm.

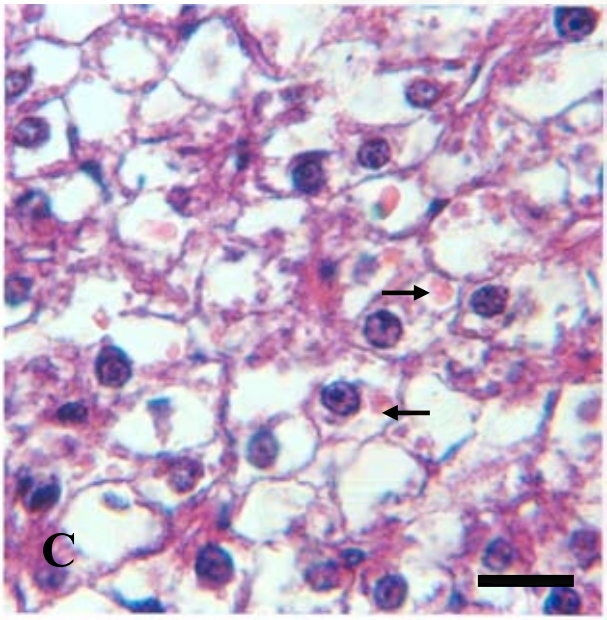
E. The liver of treated fish shows inflammation near the central vein (CV) and swelling of hepatocytes (*). The inflammation characterized by the presence of granulocytes (→). Bar scale = 25 μm.

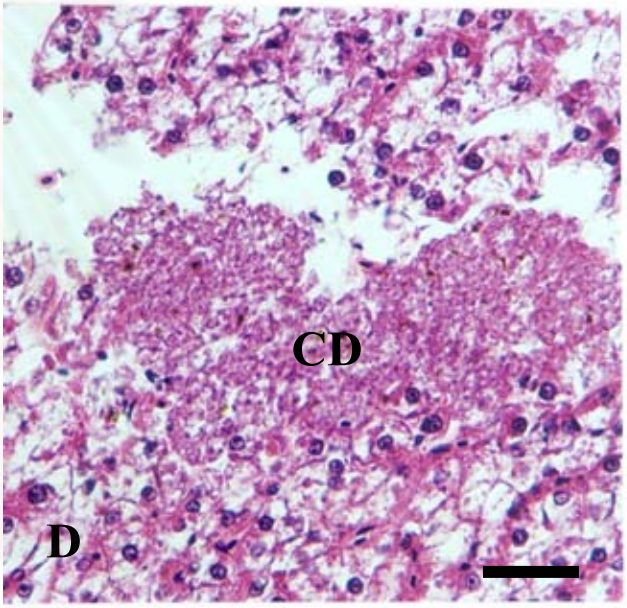
F. The liver of treated fish shows sloughing of central vein endothelium with inflammation, indicated by granulocyte infiltration (→). The swelling of hepatocytes (*) is also found. Bar scale = 25 μm.

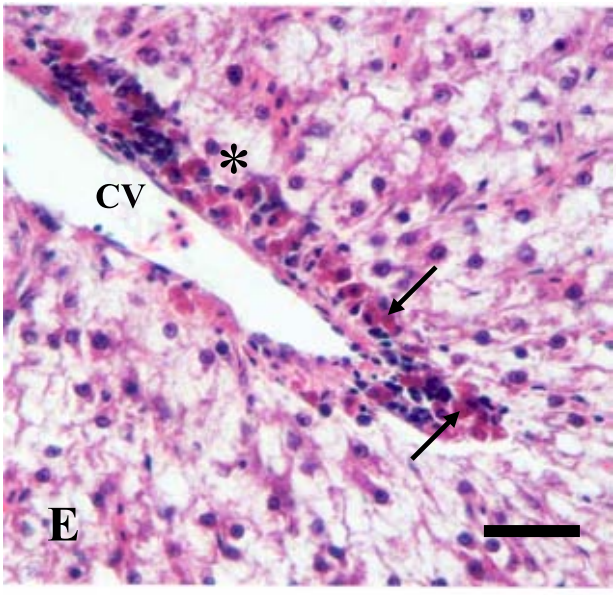
สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย


CV
A


S
CV
S
B


C


CD
D


CV
*
E

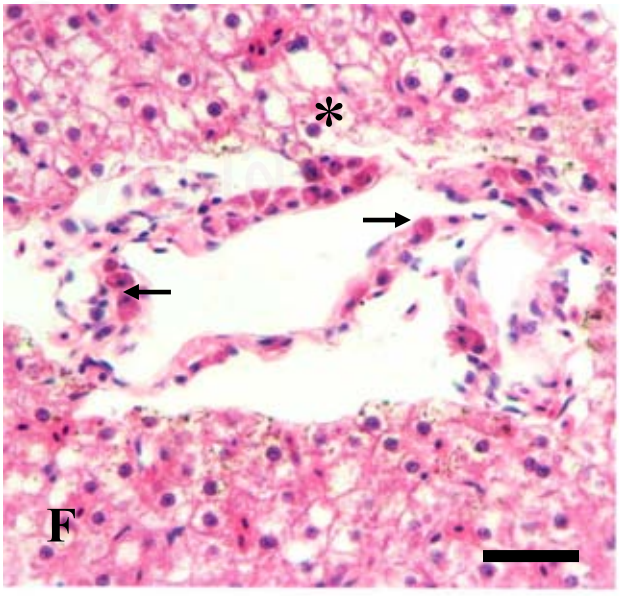

*
F

Figure 4-6 Photomicrograph of *O. niloticus* liver at 2 months experimental period. (H&E, oil red O stain).

A. The liver of control group shows normal appearance. Bar scale = 25 μm. (H&E stain).

B. The liver of treated group shows normal appearance with rather prominent vacuole. Bar scale = 25 μm. (H&E stain).

C. The liver of control fish shows the slight deposition of lipid droplets in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

D. The liver of treated fish shows the small size of lipid droplets in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

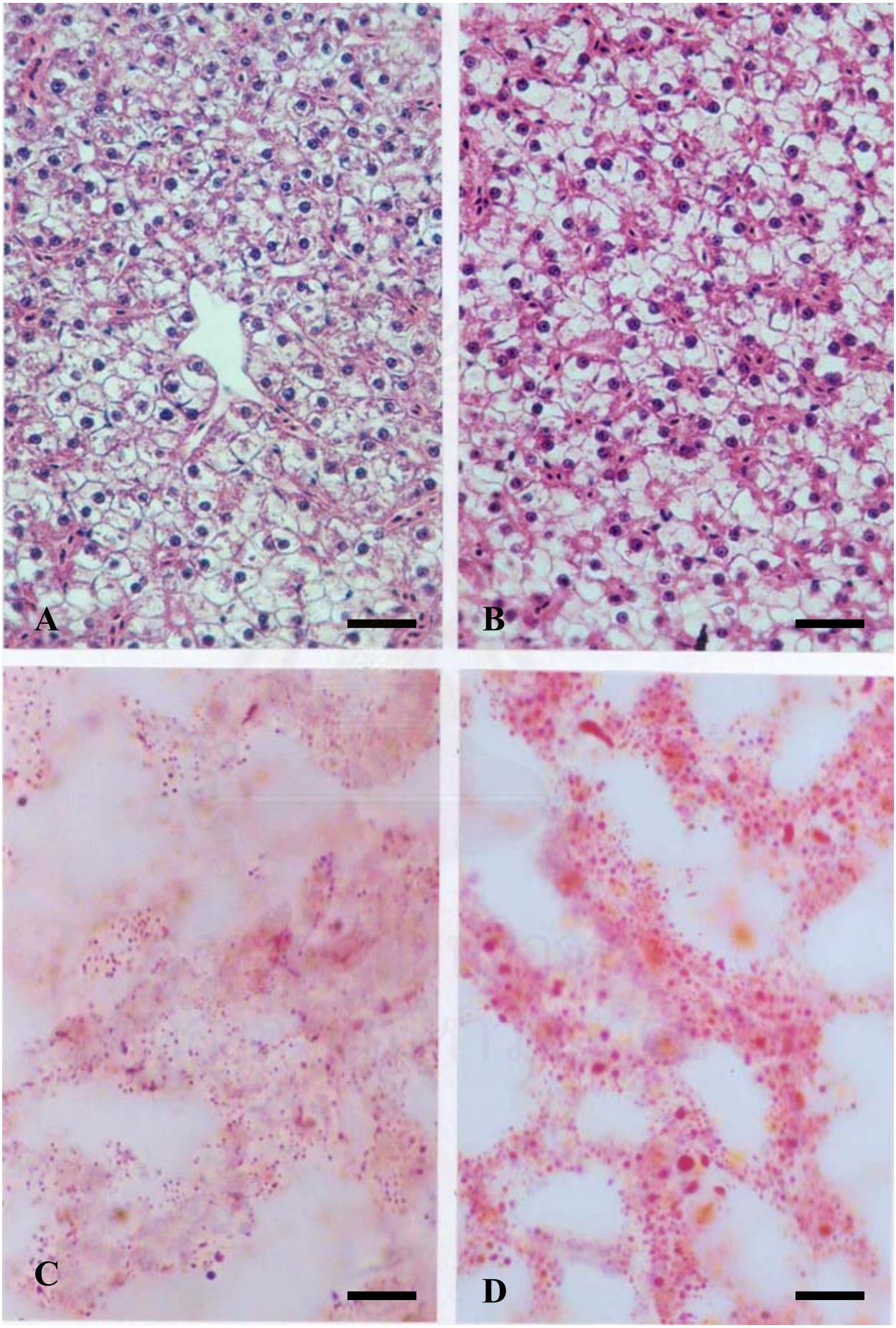

A
B
C
D

Figure 4-7 Photomicrograph of *O. niloticus* liver at 3 months experimental period (H&E stain).

A. The liver of control group shows normal appearance of hepatic plate and sinusoid. Most of hepatocytes show clear cytoplasm. Bar scale = 25 μm.

B. The liver of treated fish shows sloughing of central vein endothelium (→) and the fatty degeneration in cytoplasmic hepatocytes throughout the section. Bar scale = 50 μm.

C. The liver of treated fish shows inflammation near central vein (CV) characterized by granulocytes (→) infiltration. Fatty degeneration is found throughout the liver parenchyma. The swelling of hepatocytes (*) is also noticed. Bar scale = 25 μm.

D. The liver of treated fish shows granulocytes infiltration (→) near the bile ductules. Fatty degeneration and the loss of parenchymal architecture are found throughout the section. The swelling of hepatocytes (*) is also observed. Bar scale = 25 m.

E. The liver of treated fish in low magnification shows of necrotic degeneration (*) near liver capsule. Bar scale = 50 μm.

F. The liver of treated fish shows area of necrotic degeneration (*) and disruption of cord architecture near blood vessels. Bar scale = 25 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

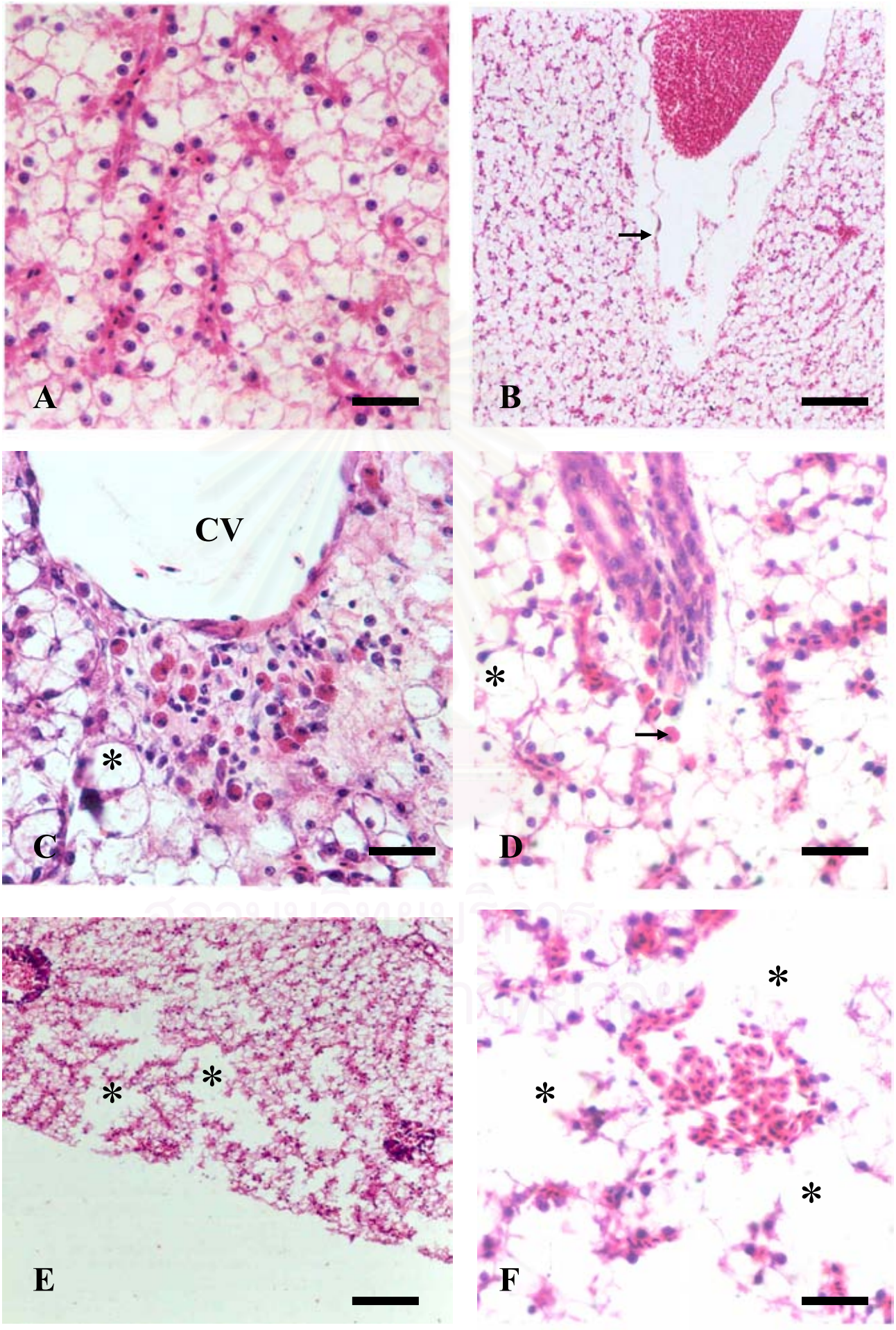

A
B
CV
C
D
E
F

Figure 4-8 Photomicrograph of *O. niloticus* liver at 3 months experimental period. (H&E, oil red O stain).

A. The liver of control group shows normal hepatocyte with clear cytoplasm. Bar scale = 25 μm. (H&E stain).

B. The liver of treated group. There is no significant change from control group. Bar scale = 25 μm. (H&E stain).

C. The liver of control fish shows the small and large size of lipid droplets deposition in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

D. The liver of treated fish shows the small and large size of lipid droplets deposition in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

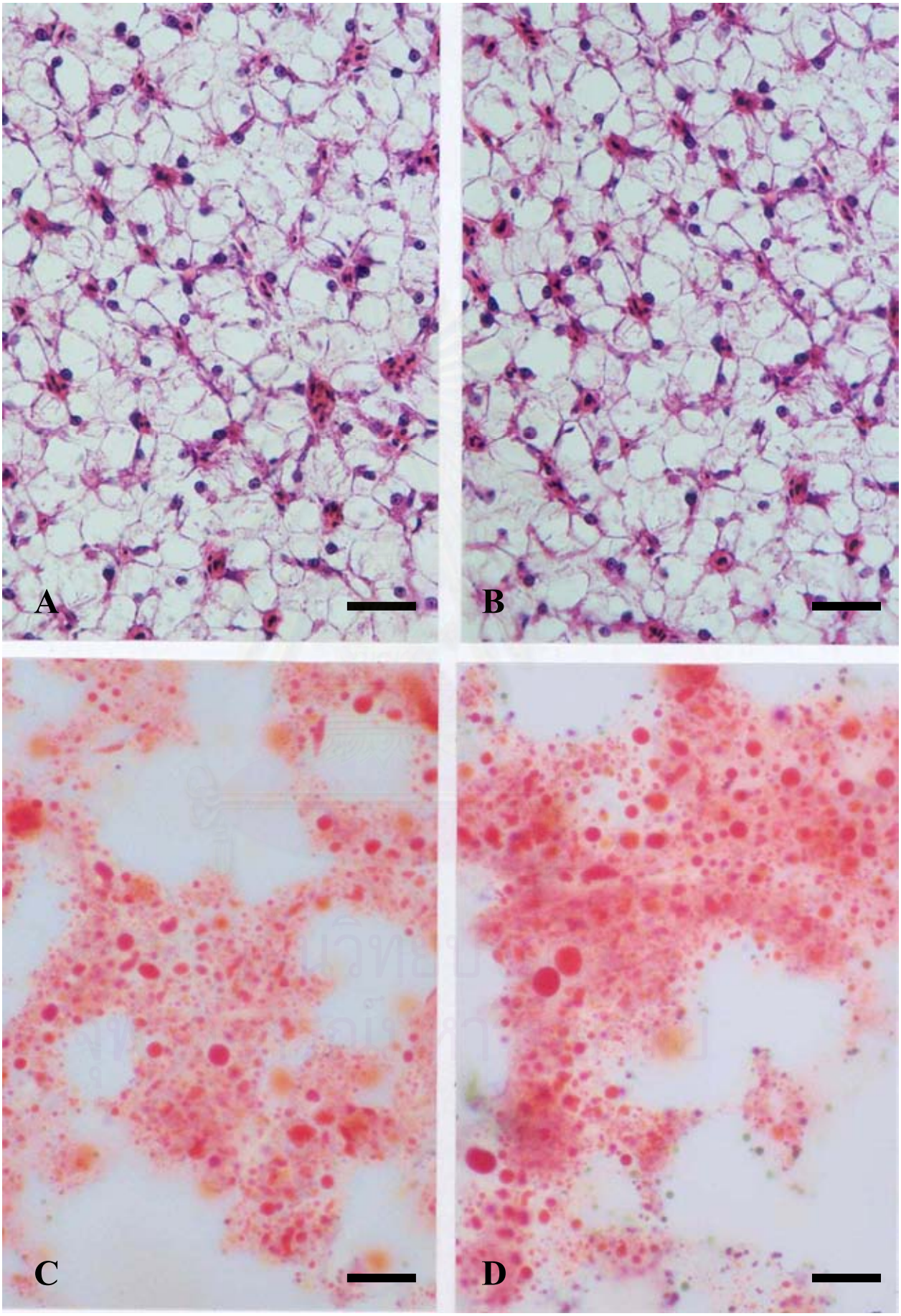

A
B
C
D

Figure 4-9 Photomicrograph of *O. niloticus* liver at 4 months experimental period (H&E stain).

A. The liver of control group shows the normal appearance of hepatic capsule and hepatic plate. Hepatocytes show less vacuolation. Bar scale = 25 μm.

B. The liver of treated fish shows the large area of necrotic degeneration (*) near liver capsule. Bar scale = 25 μm.

C. The liver of treated fish shows the area of necrotic degeneration (*) and thickening of liver capsule (→). Bar scale = 25 μm.

D. The liver of treated fish shows intensive infiltration of lymphocytes (between arrows) adjacent to pancreas (P). Bar scale = 25 μm.

E. The Liver of treated fish shows sloughing of central vein endothelium with granulocytes infiltration (→). Bar scale = 25 μm.

F. Liver of treated fish shows regenerating hepatocytes (RH) in the area of swelling of hepatocyte (*). Bar scale = 25 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

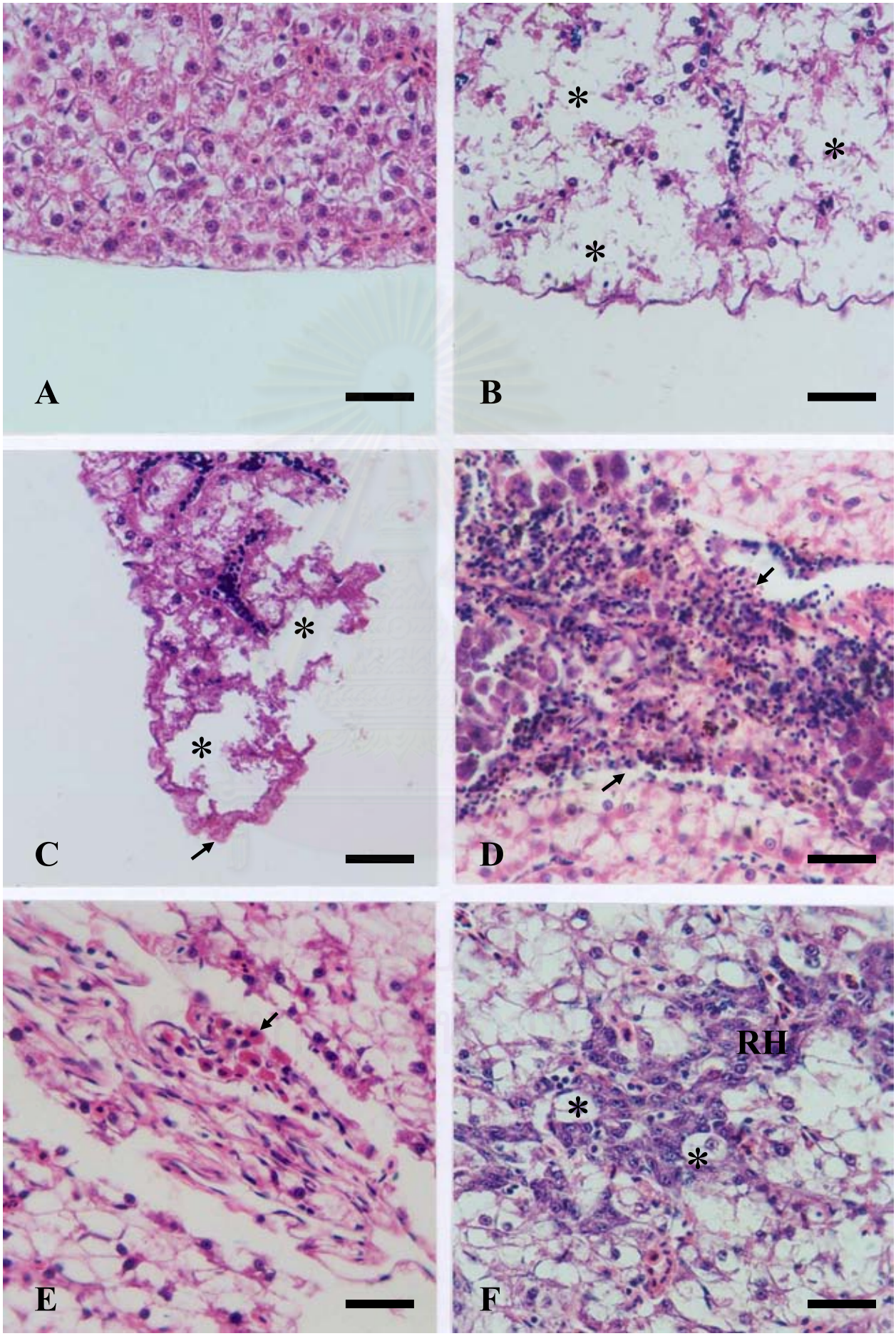

A
B
C
D
E
F
RH

Figure 4-10 Photomicrograph of *O. niloticus* liver at 4 months experimental period. (H&E, oil red O stain).

A. The liver of control group shows normal appearance of hepatocytes with less vacuolation. Bar scale = 25 μm. (H&E stain).

B. The liver of treated group shows normal appearance of hepatocytes with clear cytoplasm. Bar scale = 25 μm. (H&E stain).

C. The liver of control fish shows the slight accumulation of lipid droplets in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

D. The liver of treated fish shows the small size of lipid droplets in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

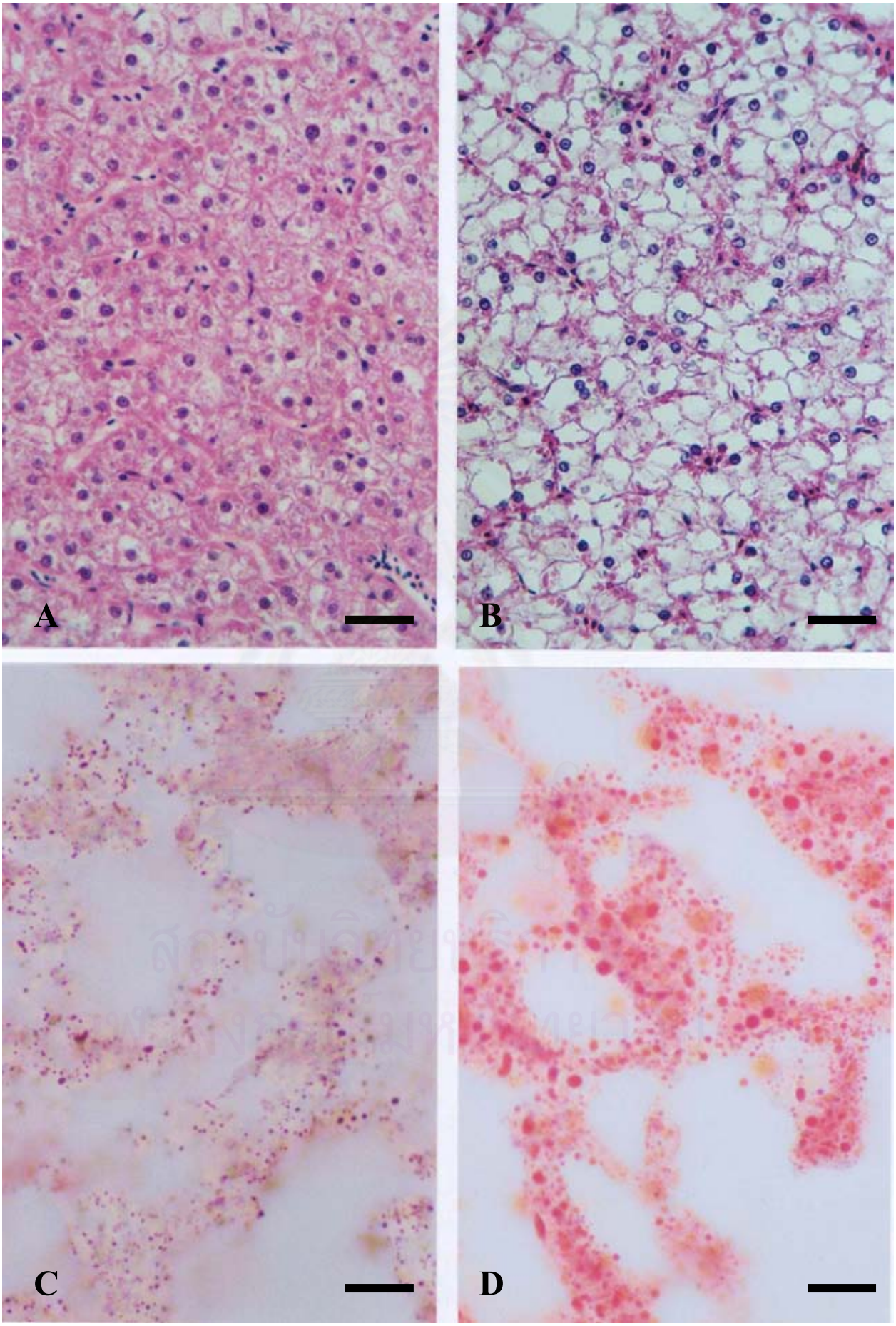

A
B
C
D

Figure 4-11 Photomicrograph of *O. niloticus* liver at 5 months experimental period (H&E stain).

A. The liver of control group shows normal profile of hepatic cord. CV: Central vein. Bar scale = 25 μm.

B. The liver of treated fish shows sloughing of central vein endothelium (→). Bar scale = 25 μm.

C. The liver of treated fish shows the large area of necrotic degeneration (*) near central vein (CV). Bar scale = 25 μm.

D. The liver of treated fish shows granulocytes (→) and lymphocytes (➤) infiltration near bile ductules (BD). Bar scale = 25 μm.

E. The liver of treated fish shows lymphocytes infiltration (➤) into the area of necrotic hepatocytes (*). Bar scale = 25 μm.

F. The liver of treated fish shows infiltration of granulocytes (→) and lymphocytes (➤) into the area of necrotic degeneration (*). Bar scale = 25 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

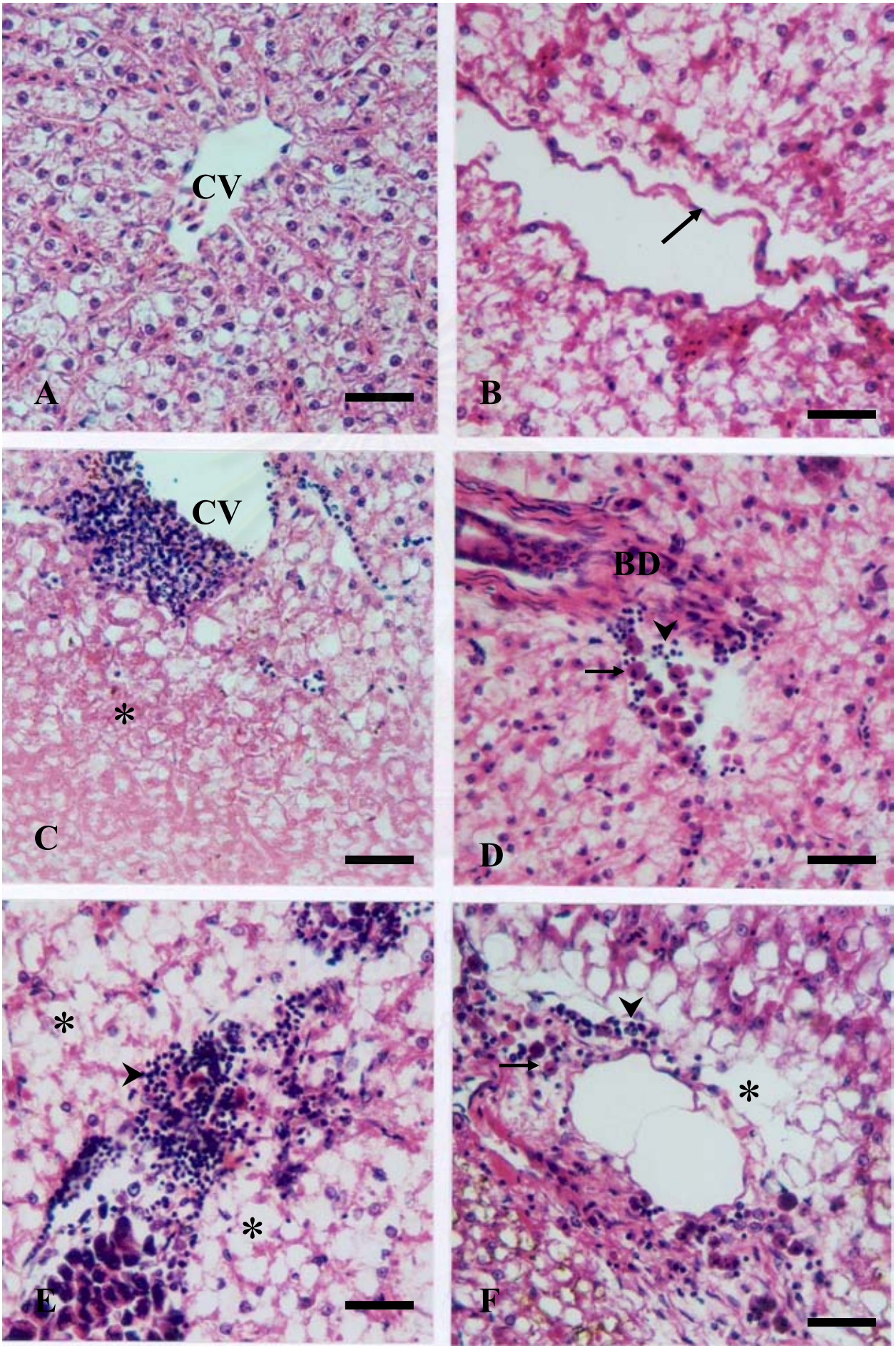

CV
A
B
CV
C
BD
D
E
F

Figure 4-12 Photomicrograph of *O. niloticus* liver at 5 months experimental period. (H&E, oil red O stain).

A. The liver of control group shows normal hepatocyte with less vacuolation. Bar scale = 25 μm. (H&E stain).

B. The liver of treated group shows clear cytoplasm of hepatocyte. Bar scale = 25 μm. (H&E stain).

C. The liver of control fish shows the slight deposition of lipid droplets in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

D. The liver of treated fish shows the small and large size of lipid droplets in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

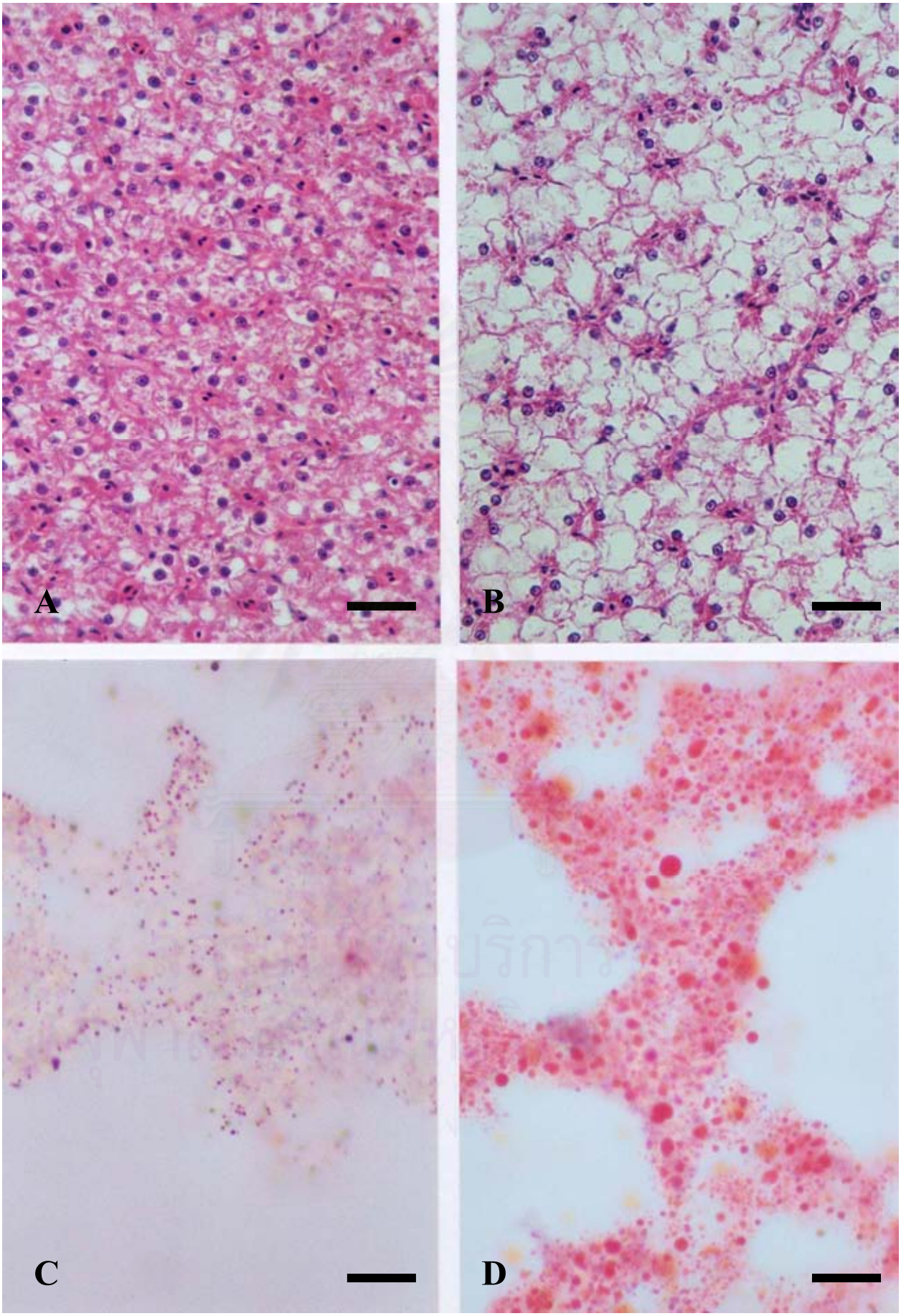

A
B
C
D

Figure 4-13 Photomicrograph of *O. niloticus* liver at 6 months experimental period (H&E stain).

A. The liver of control group shows normal hepatic cord. CV: Central vein. Bar scale = 25 μm.

B. Liver of treated fish shows sloughing of central vein endothelium (→). Bar scale = 25 μm.

C. The liver of treated fish shows the large area of necrotic degeneration (*) near central vein (CV). Bar scale = 25 μm.

D. The liver of treated fish shows the area of inflammation indicated by the infiltration of granulocytes (→) and lymphocytes (➤). Bar scale = 25 μm.

E. The liver of treated fish shows inflammation at subcapsular space characterized by the infiltration of lymphocytes (➤). Bar scale = 25 μm.

F. The liver of treated fish shows the cluster of regenerating hepatocytes (between arrows). Bar scale = 25 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

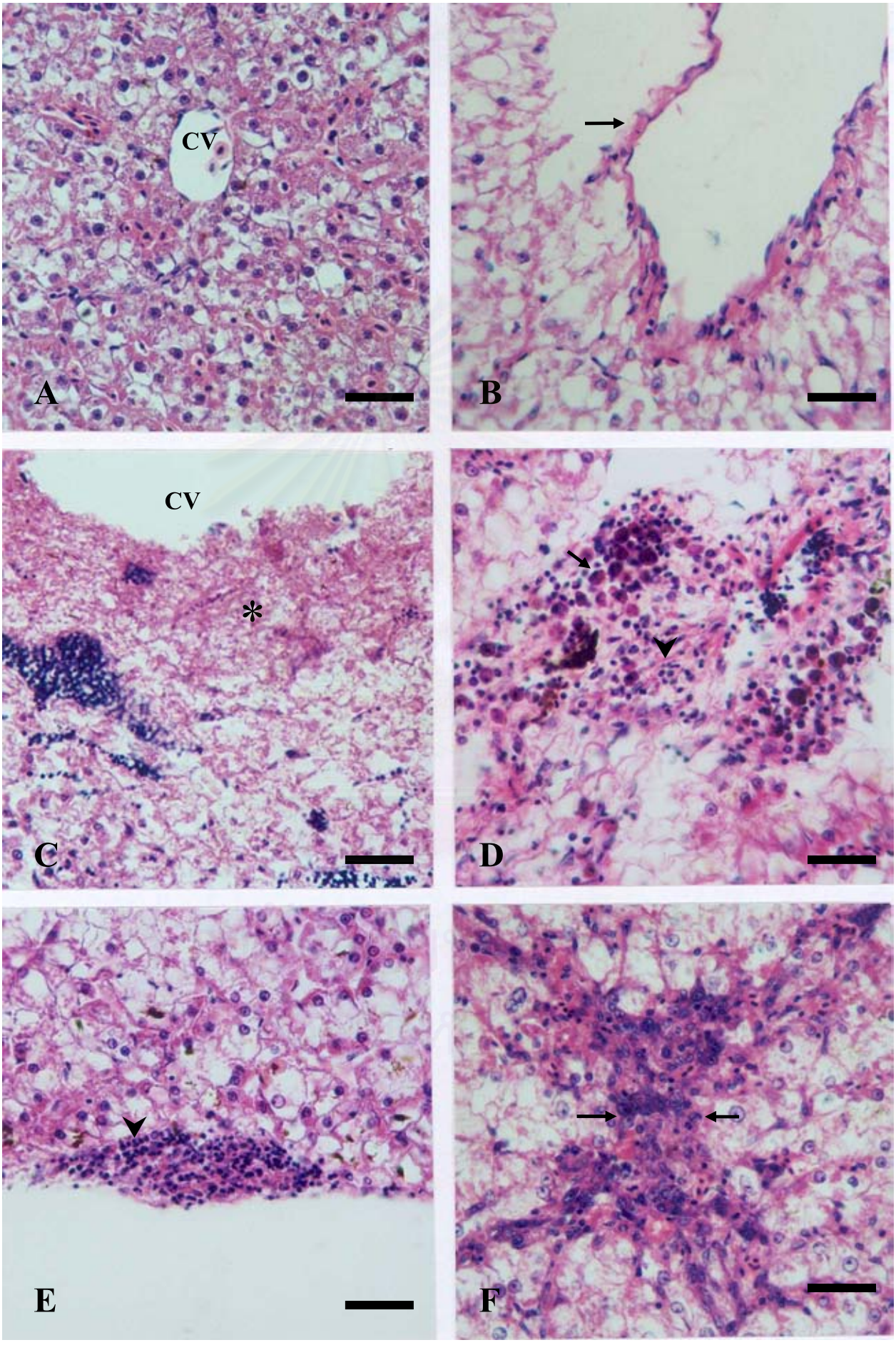

CV
A
B
CV
*
C
D
E
F

Figure 4-14 Photomicrograph of *O. niloticus* liver at 6 months experimental period. (H&E, oil red O stain).

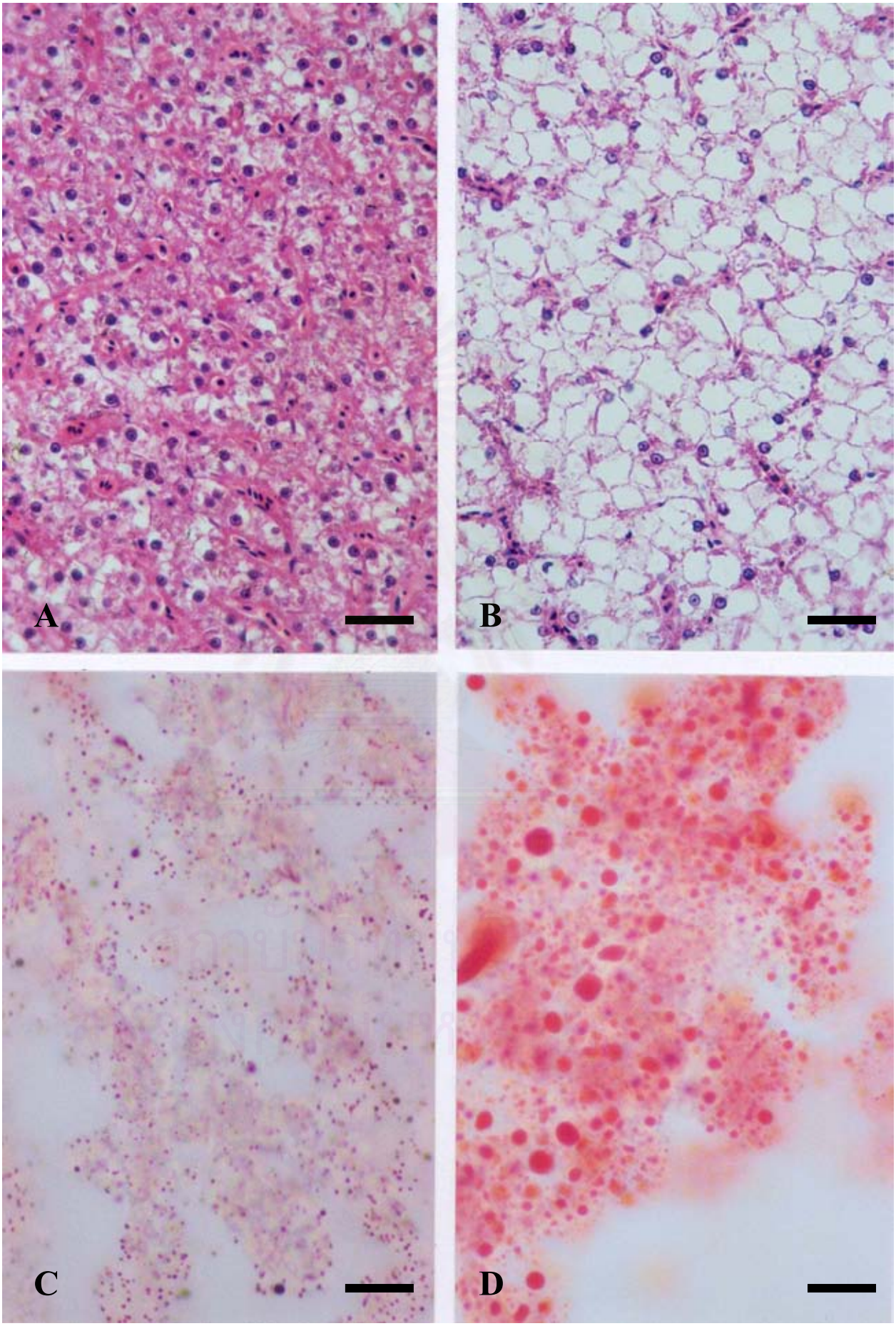
A. The liver of control group shows less vacuolation in hepatocytes. Bar scale = 25 μm. (H&E stain).

B. The liver of treated group shows prominent vacuolation in hepatocytes. Bar scale = 25 μm. (H&E stain).

C. The liver of fish control shows the slight accumulation of lipid droplets in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

D. The liver of treated fish shows the small and large size of lipid droplets in hepatocytes. Bar scale = 10 μm. (Oil red O stain).

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย


A
B
C
D

4.2.3 Histopathology of kidney

Kidney of Nile tilapia was composed of nephron unit and glomerulus. The renal corpuscle consisted of Bowman's capsule, a double-layered epithelial structure. The visceral layer covers the exposed surface of the glomerulus. The parietal layer forms the boundary of the capsule. The glomerulus composed of capillaries. The renal tubule was composed of proximal tubule, distal tubule and collecting duct. The proximal tubule was characterized by a dense luminal brush border of microvilli, increasing the surface of the apical membrane and was lined by columnar cells. The distal tubule is lined by low columnar or cuboidal cells with inconspicuous brush border (Figure 4-15).

Kidney of Nile tilapia exposed to liquors, the alterations were most observed in glomerulus and proximal tubule. The most consistently presented histopathologies were shrinkage of glomerulus, hyaline deposition in epithelial cells, hydropic swelling, necrotic epithelial cell and accumulation of debris in tubular lumen. However, these changes were not time-related and they occurred with same degree of severity among all exposure periods. The degree of renal alteration was rather slight (Table 4-6).

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Table 4-6 Incidence of histopathological finding in Nile tilapia kidney after exposure to distilled liquors 30 degree at 964.30 ppm from first through sixth month of experiment (n=5).

Lesion	Experimental periods (month)					
	1	2	3	4	5	6
Glomerulus						
Glomerular shrinkage						
mild	3	3	4	4	4	4
moderate	1	1	1	1	0	0
marked	1	1	0	0	1	1
Proximal tubules						
Necrosis						
mild	5	5	4	5	5	5
moderate	0	0	1	0	0	0
marked	0	0	0	0	0	0
Hyaline deposition						
mild	4	0	0	5	5	5
moderate	1	0	0	0	0	0
marked	0	0	0	0	0	0
Hydropic swelling						
mild	0	1	0	0	0	1
moderate	0	0	0	0	0	0
marked	0	0	0	0	0	0

Mild : $\leq$ 2 foci of lesion finding of one liver section;

Moderate : $\leq$ 4 foci of lesion finding one liver section ;

Marked : $>$ 4 foci of lesion finding one liver section

After 1 month of exposure, the histopathological changes in kidney showed shrinkage of glomerulus, hyaline deposition in epithelial cells as well as the appearance of karyolysis and pycnotic nuclei of proximal tubules. Ultimately, the debris which resulting from necrotic degeneration of tubular epithelium was presented (Figure 4-16, Table 4-6).

After 2 months of exposure, epithelial cells of affected proximal tubules were frequently necrosis. As were noticed for the shrinkage of glomerulus and intensive swollen epithelial cells of proximal tubules. In addition, cellular debris in proximal tubular lumens which resulting from necrotic epithelium was also found (Figure 4-17, Table 4-6).

After 3 months of exposure, kidneys of *O. niloticus* showed intensively shrinkage of glomerulus which leading to the enlargement of Bowman's space. Renal tubular necrosis was indicated by presence of condense and hyperbasophilic nuclei and karyolysis as well as the disintegration of tubular epithelium (Figure 4-18, Table 4-6).

After 4 months of exposure, the treated fish showed histopathological changes of the kidney. Renal tissues showed the empty Bowman's space which resulting from shrinkage of glomerulus. Necrosis with pyknosis and karyolysis and hyaline accumulation in epithelium were noticed in proximal tubule. In addition, cellular debris in tubular lumen was also noticed (Figure 4-19, Table 4-6).

After 5 months of exposure, the alterations were generally characterized by the large empty Bowman's space which resulting from the shrinkage of glomerulus. Necrosis and hyaline deposition in epithelial cells of proximal tubules. Tubular necrosis was characterized by the presence of pyknosis or karyolysis. Cellular debris was also seen in proximal tubular lumen (Figure 4-20, Table 4-6).

After 6 months of exposure, the histopathological alterations showed the same evidence as previous months. These alterations were characterized by the presence of intensive shrinkage of glomerulus, swollen and necrotic epithelial cells in proximal tubules. Tubular necrosis was indicated by the appearance of pyknosis or karyolysis. In addition, hyaline deposition in epithelial cell of proximal tubules and cellular debris accumulation in lumen were also observed (Figure 4-21, Table 4-6).

Renal pathology of fish from other compounds was also studied. Sastry and Sharma (1981) reported the shrinkage of glomerulus was detected in *Ophiocephalus punctatus* after exposure to diazinon and also detected in gold fish exposed to haxachlorobutadiene (Reimschuessel *et al*., 1989), coho salmon exposed to tobromycin (Schneider *et al*., 1989) and in mullet exposed to mercury (Pandey, 1994). Other effects such as hyaline accumulation in proximal tubule had been observed in rainbow trout and common carp exposed to cadmium (Forlin *et al*., 1986; Singhal and Jain, 1997) and tench exposed to 2,4-D (Gomez *et al*., 1999). The swelling of epithelial cell was found in common carp after exposure to uranium (Singhal and Jain, 1997) and also lake whitefish exposed to uranium and nickel (Cooley *et al*., 2000; Ptashynski *et al*., 2002).

Glomerular lesions observed in this study was the shrinkage of glomerulus. Hyaline accumulation in proximal tubules was an indicator of glomerular damage. The accumulation of these inclusions was possibly due to extensive damaged to the filtration barrier (Gomez *et al*., 1999). In general, the small amount of protein filtered through the glomerulus was reabsorbed by pinocytosis in the proximal tubules. In glomerular damage, the heavy protein was leaked across the glomerular filter and pinocytotic reabsorbtion of the protein was increased. Subsequently, the fusion of these vesicles with lysosomes resulted in the appearance of hyaline droplets (Kumar *et al*., 2002). In addition, the intensive accumulation of hyaline droplets in the epithelial cells of proximal tubules could often appear to displace the nucleus leading to tubular necrosis (Singhal and Jain, 1997).

Tubular necrosis was observed in many kinds of fish such as coho salmon after exposure to tobromycin (Schneider et al., 1989), toadfish exposed to gentamycin (Jones *et al*., 1987), goldfish exposed to HCBD (Reimschuessel *et al*., 1989), mullet exposed to dichlorvos and mercury (Mophatra and Noble, 1992; Pandey, 1994), *Puntius conchonius* exposed to cadmium (Gill *et al*., 1989), common carp exposed to cadmium and gallium (Singhal and Jain, 1997; Yang and Chen, 2003), lake whitefish exposed to uranium and nickel (Cooley *et al*., 2000; Ptashynski *et al*., 2002) and lusitanian toadfish exposed to vanadium (Borges *et al*., 2003). Additionally, necrotic debris in tubular lumen had also been found in coho salmon after exposure to tobromycin (Schneider *et al*., 1989), *Ophiocephalus punctatus* exposed to diazinon (Sastry and Sharma, 1981) and lake whitefish exposed to nickel (Ptashynski *et al*., 2002).

Tubular necrosis has been reported in almost all histologic examminations of xenobiotic-induced kidney damage in fish. The propensity of proximal tubule effect was probably related to the high capacity for membrane transport in the tubular epithelial cells (Larsen and Perkins, 2001). In addition, the lesions which had occurred in glomerulus and proximal tubules perhaps because they are the first to contact with toxic compounds. The swelling of proximal tubular epithelium may result from the impairment of Na^+/K^+-ATPase. In *in vitro* studies by Parenti *et al*. (1991) and Rothman *et al*. (1992), they showed that ethanol interfered with Na^+/K^+-ATPase of rat membrane which involved in the active regulation of cells volume. This alteration also observed in experiment of van Thiel *et al*. (1979), renal hypertrophy in rat occured after chronic ethanol ingestion. Intratubular casts have also been reported as the markers of damage to the tubule cell themselves.

Figure 4-15 Photomicrograph of *O. niloticus* kidney of control group (H&E stain).

A. The kidney of control fish shows the glomerulus (GL) as a cluster of capillaries surrounded by the Bowman' s capsule (→). Bar scale = 10 μm.

B. The kidney of control fish shows the proximal tubule (PT) with columnar epithelium and prominent brush border. Bar scale = 10 μm.

C. The kidney of control fish shows the distal tubule (DT) lined by cuboidal cell with inconspicuous brush border. Bar scale =10 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

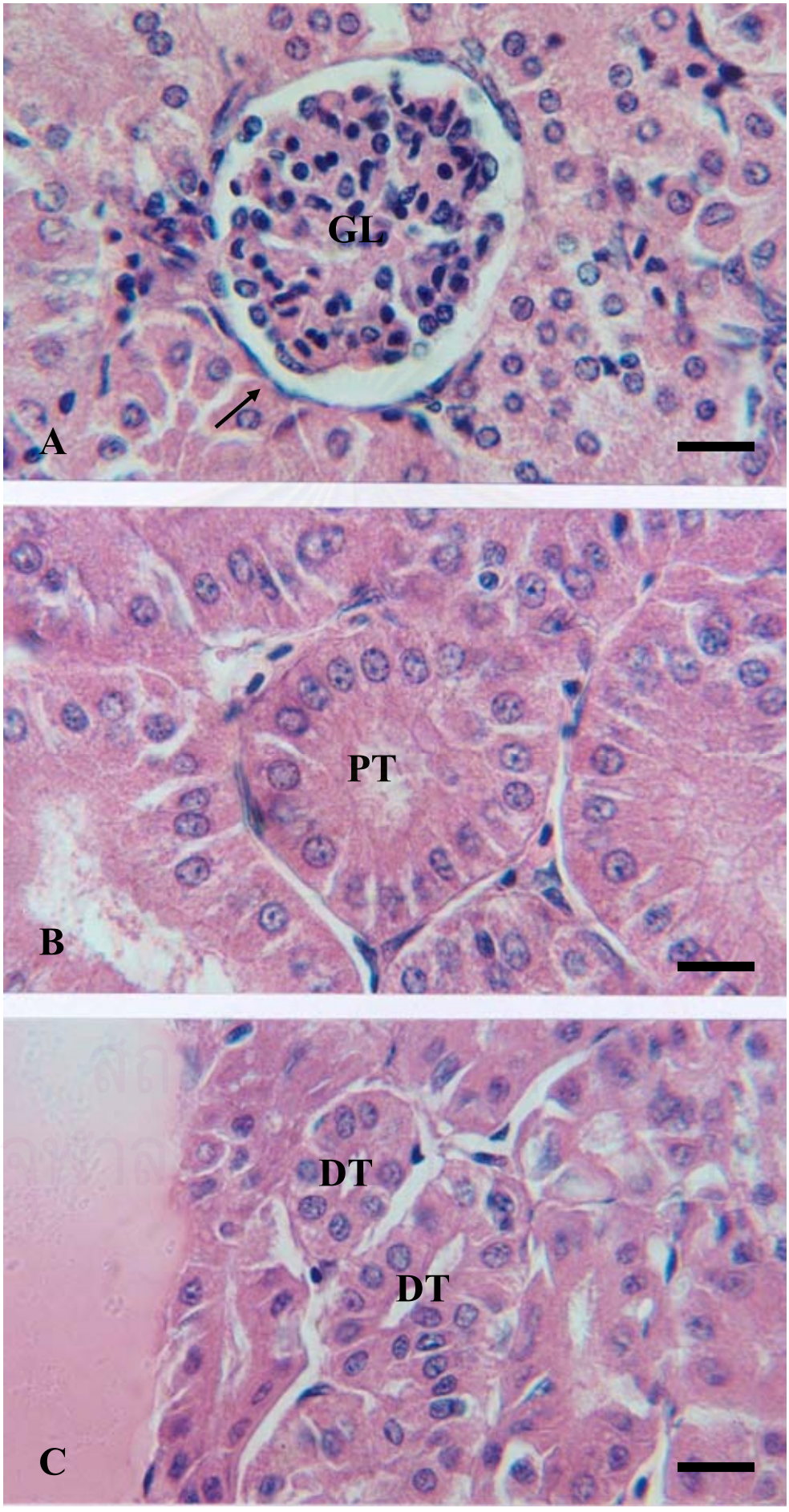

GL
A
PT
B
DT
DT
C

Figure 4-16 Photomicrograph of *O. niloticus* kidney at 1 month experimental period (H&E stain).

A. The kidney of control group shows normal appearance of glomerulus (GL) and all of renal tubule (*). Bar scale = 25 μm.

B. The kidney of treated fish shows empty of Bowman's space (*) resulting from the shrinkage of glomerulus (GL). Bar scale = 10 μm.

C. The kidney of treated fish shows necrosis in epithelium of proximal tubules (→). Bar scale = 10 μm.

D. The kidney of treated fish shows hyaline accumulation (→) in cytoplasm of proximal tubules epithelium. Necrosis of epithelial cells (➤) is also noticed. Bar scale = 10 μm.

E. The kidney of treated fish in low magnification shows cellular debris (→) in proximal tubule. Bar scale = 10 μm.

F. The kidney of treated fish in higher magnification shows cellular debris resulting from necrotic degeneration in proximal tubular epithelium (→). Bar scale = 10 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

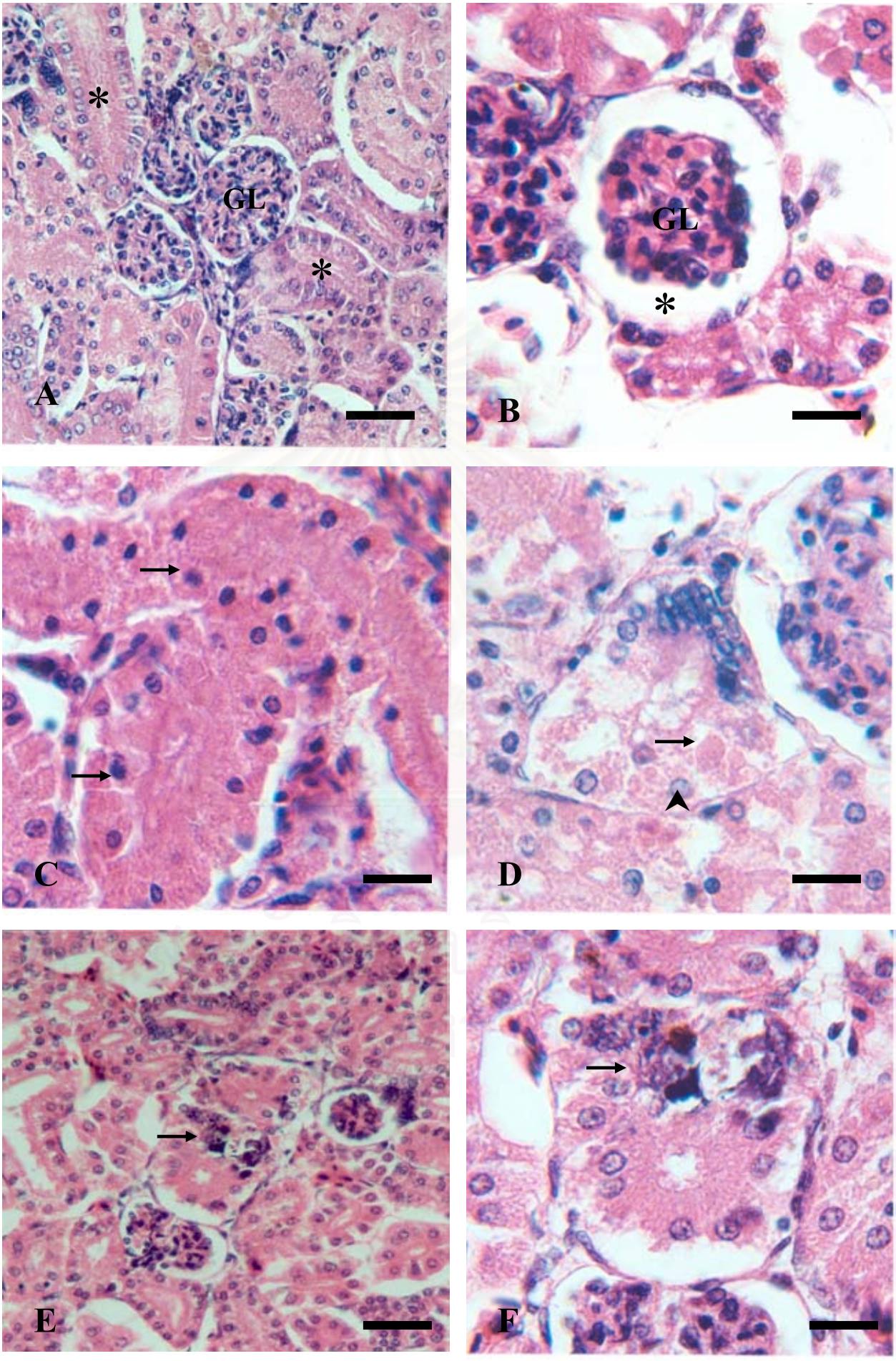

GL
A
GL
B
C
D
E
F

Figure 4-17 Photomicrograph of *O. niloticus* kidney at 2 months experimental period (H&E stain).

A. The kidney of control group shows normal appearance of glomerulus (GL) and epithelial cells of all renal tubule (*). Bar scale = 25 μm.

B. The kidney of treated fish shows empty of Bowman's space (*). Bar scale = 10 μm.

C. The kidney of treated fish shows the intensive swelling (*) of proximal tubules. Bar scale = 10 μm.

D. The kidney of treated fish shows pyknotic nuclei of epithelial cells (→) in proximal tubules. Bar scale = 10 μm.

E. The kidney of treated fish in low magnification shows cellular debris accumulation in proximal tubular lumen. Bar scale = 25 μm.

F. The kidney of treated fish in higher magnification shows debris in proximal tubular lumen (→) resulting from necrotic degeneration of epithelial cells. Bar scale = 10 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

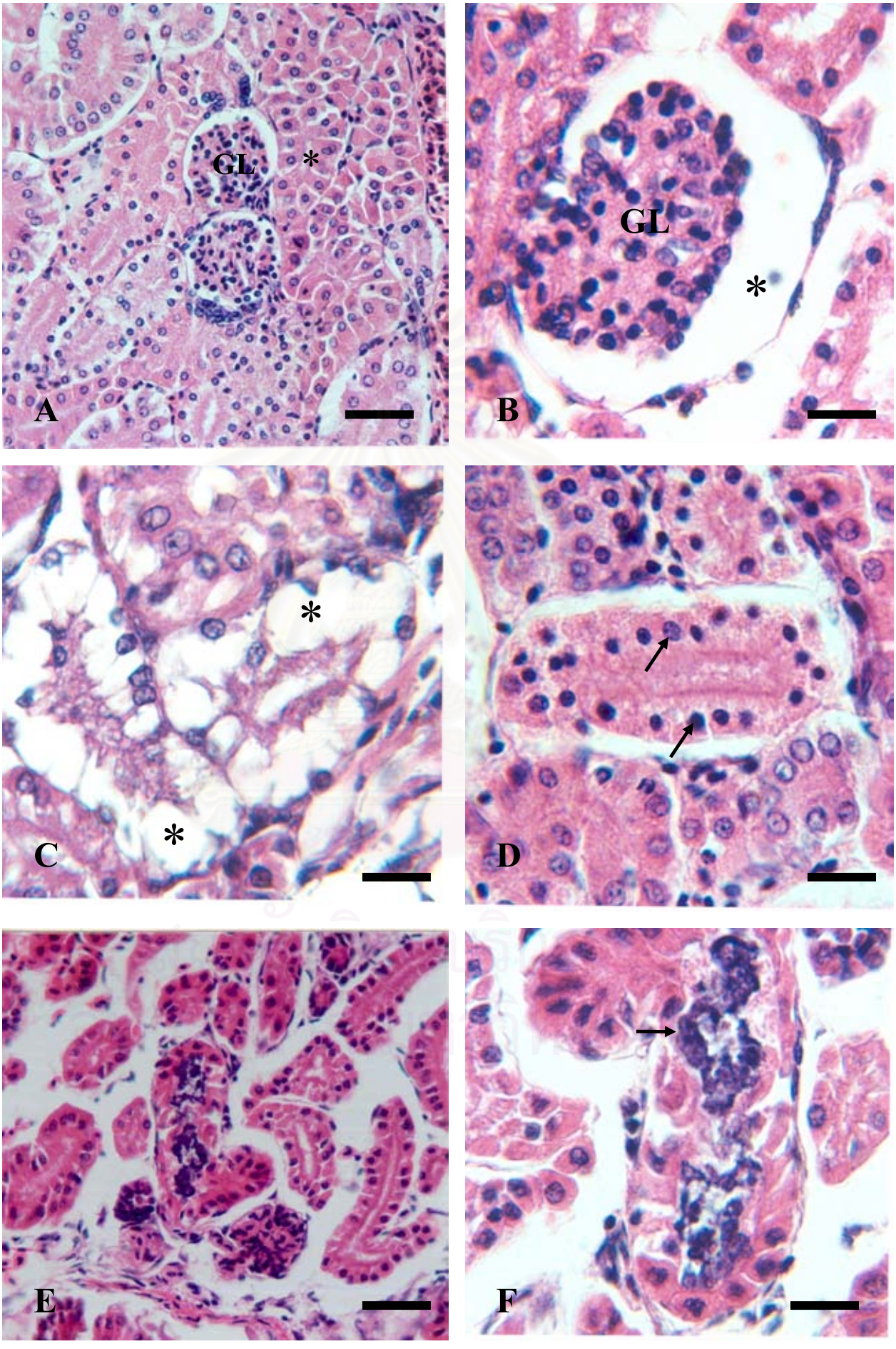

GL
*
A
GL
*
B
*
*
C
D
E
F

Figure 4-18 Photomicrograph of *O. niloticus* kidney at 3 months experimental period (H&E stain).

A. The kidney of control group shows well develop glomerulus (GL) and renal tubule (*). Bar scale = 25 μm.

B. The kidney of treated fish shows large empty Bowman's space (*) resulting from the shrinkage of glomerulus (GL). Bar scale = 10 μm.

C. The kidney of treated fish shows hyperbasophilic nuclei of epithelial cells in proximal tubule (→). Bar scale = 10 μm.

D. The kidney of treated fish shows necrosis of epithelial cells in proximal tubule (→) with disintegrating of epithelium (*). Bar scale = 10 μm.

E. The kidney of treated fish in low magnification shows disintegrating of proximal tubule epithelium (*). Bar scale = 25 μm.

F. The kidney of treated fish in higher magnification shows disintegrating of proximal tubule epithelium (*). Bar scale = 10 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

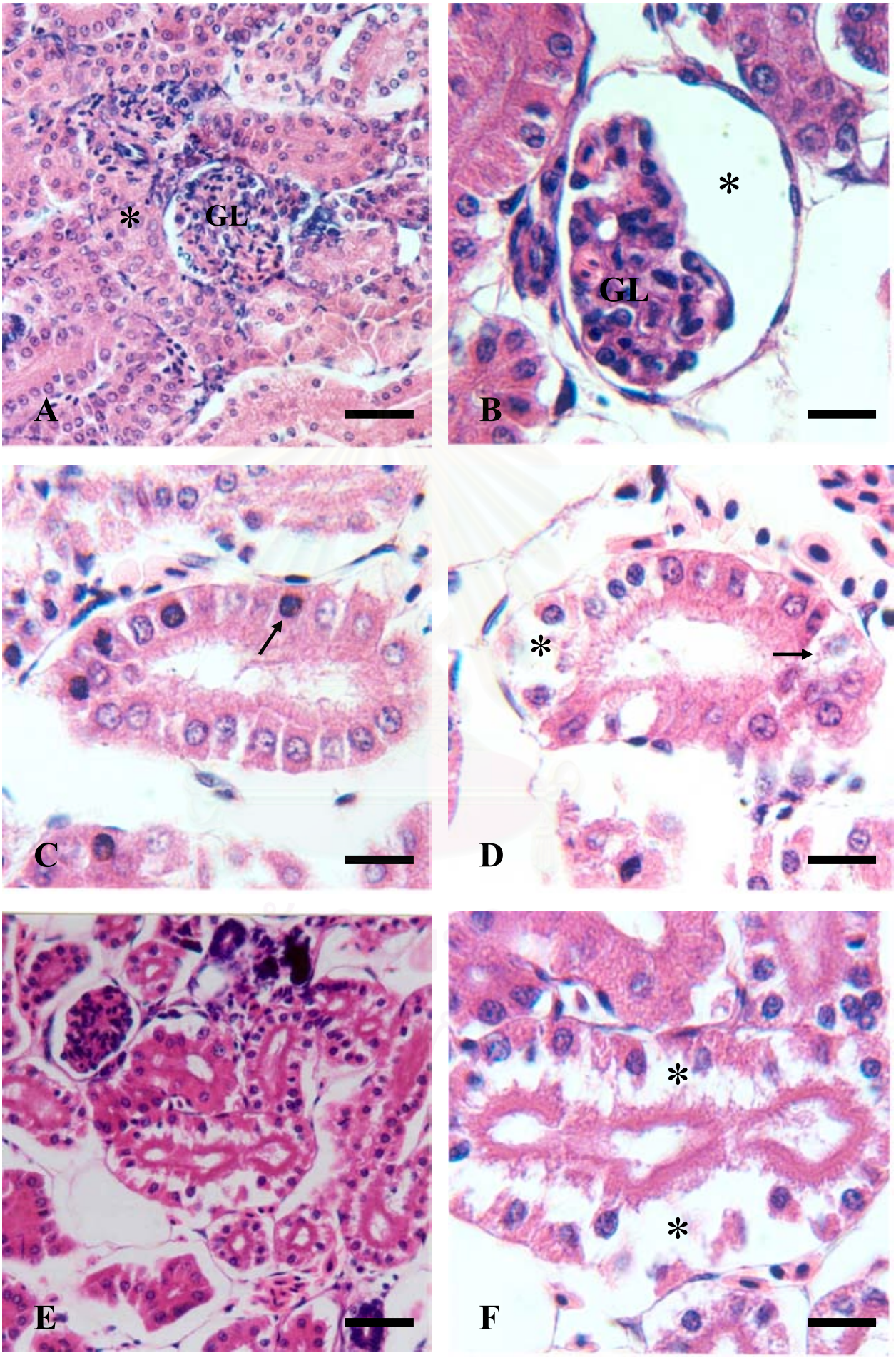

*
GL
A
*
GL
B
C
*
D
E
*
*
F

Figure 4-19 Photomicrograph of *O. niloticus* kidney at 4 months experimental period (H&E stain).

A. The kidney of control group. The architecture is not altered. Two glomerulus (GL) show normal appearance. Renal tubule (*) reveals normal profile. Bar scale = 25 μm.

B. The kidney of treated fish shows shrinkage of glomerulus (GL) resulting in empty Bowman's space (*). Bar scale = 10 μm.

C. The kidney of treated fish shows hyperbasophilic nuclei of epithelial cell (→) of proximal tubules. Bar scale = 10 μm.

D. The kidney of treated fish shows necrotic epithelial cells (→) with hyaline granule accumulation in renal tubule epithelium. Bar scale = 10 μm.

E. The kidney of treated fish shows necrosis of epithelial cells of proximal tubules indicated by hyperbasophilic nuclei (→) and karyolysis (➤). Bar scale = 10 μm.

F. The kidney of treated fish in low magnification shows cellular debris accumulation in tubular lumen (→).Bar scale = 25 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

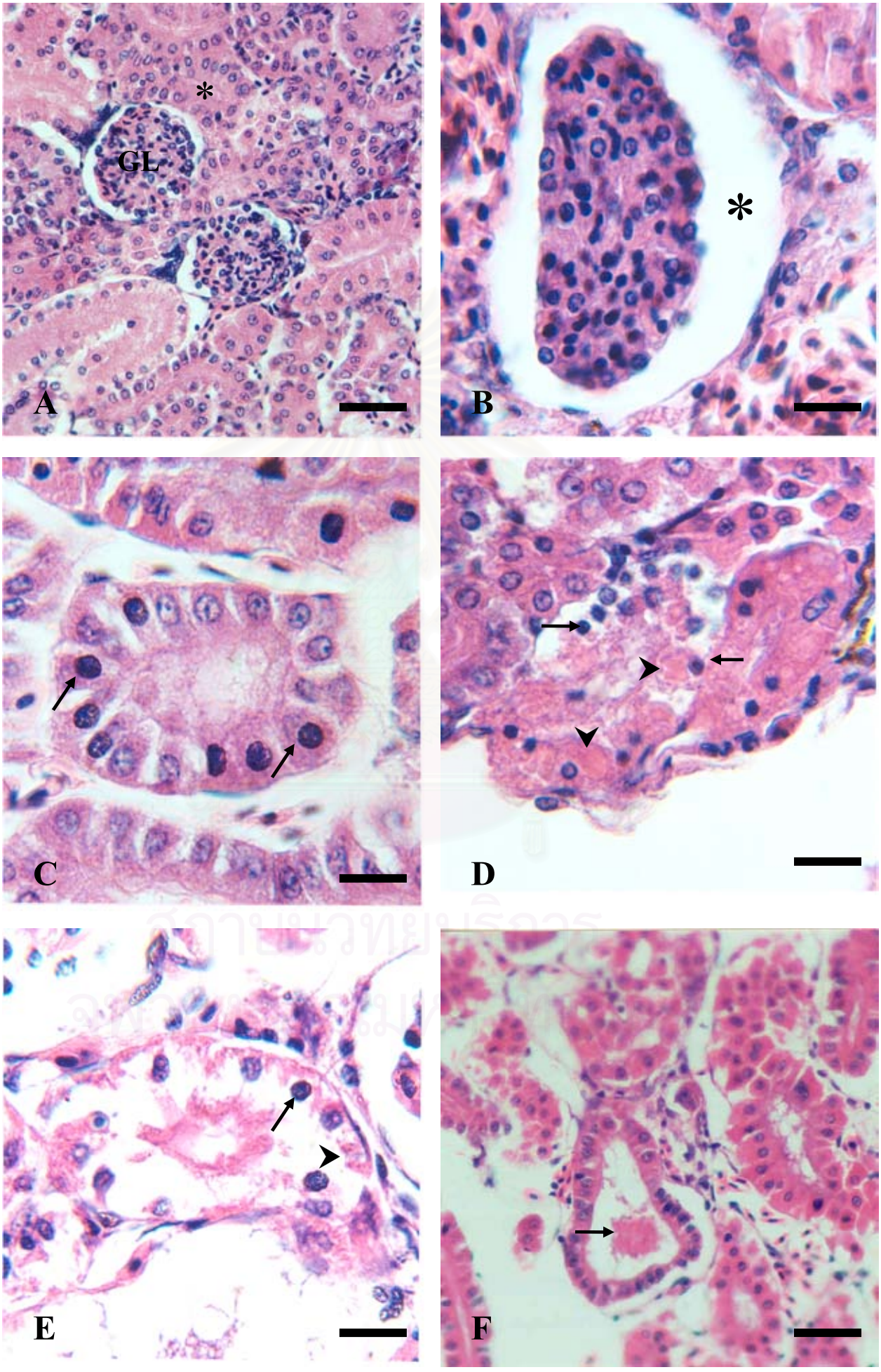

GL
A
B
C
D
E
F

Figure 4-20 Photomicrograph of *O. niloticus* kidney at 5 months experimental period (H&E stain).

A. The kidney of control group. Glomerulus (GL) shows normal appearance. Renal tubule (*) reveals normal profile. Bar scale = 25 μm.

B. The kidney of treated fish shows large empty of Bowman's space (*). Bar scale = 10 μm.

C. The kidney of treated fish shows necrotic epithelial cells (→) of proximal tubules with hyaline accumulation (➤) in cytoplasm. Bar scale = 10 μm.

D. The kidney of treated fish shows necrotic degeneration of renal tubule characterized by pyknosis (→) and karyolysis (➤). Bar scale = 10 μm.

E. The kidney of treated fish in low magnification shows disintegrating of tubular epithelium and appearance of debris in lumen. Bar scale = 25 μm.

F. The kidney of treated fish shows necrotic epithelial cells of proximal tubules (→) with cellular debris appearance in lumen (*). Bar scale = 10 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

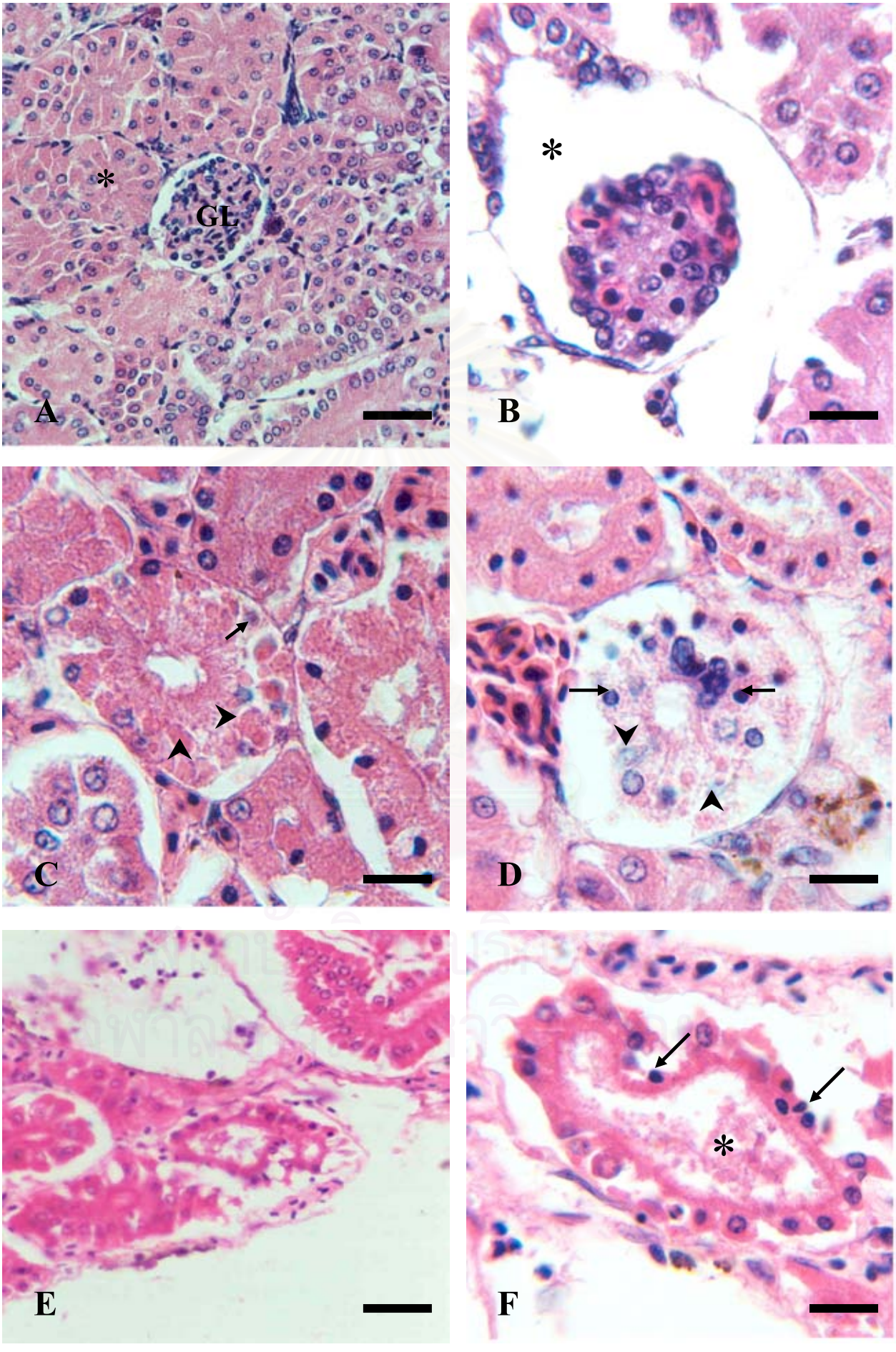

A
GL
B
C
D
E
F

Figure 4-21 Photomicrograph of *O. niloticus* kidney at 6 months experimental period (H&E stain).

A. The kidney of control group. There are normal appearance of glomerulus (GL) and all of renal tubule (*). Bar scale = 25 μm.

B. The kidney of treated fish shows enlargement of Bowman's space (*) resulting from the shrinkage of glomerulus. Bar scale = 10 μm.

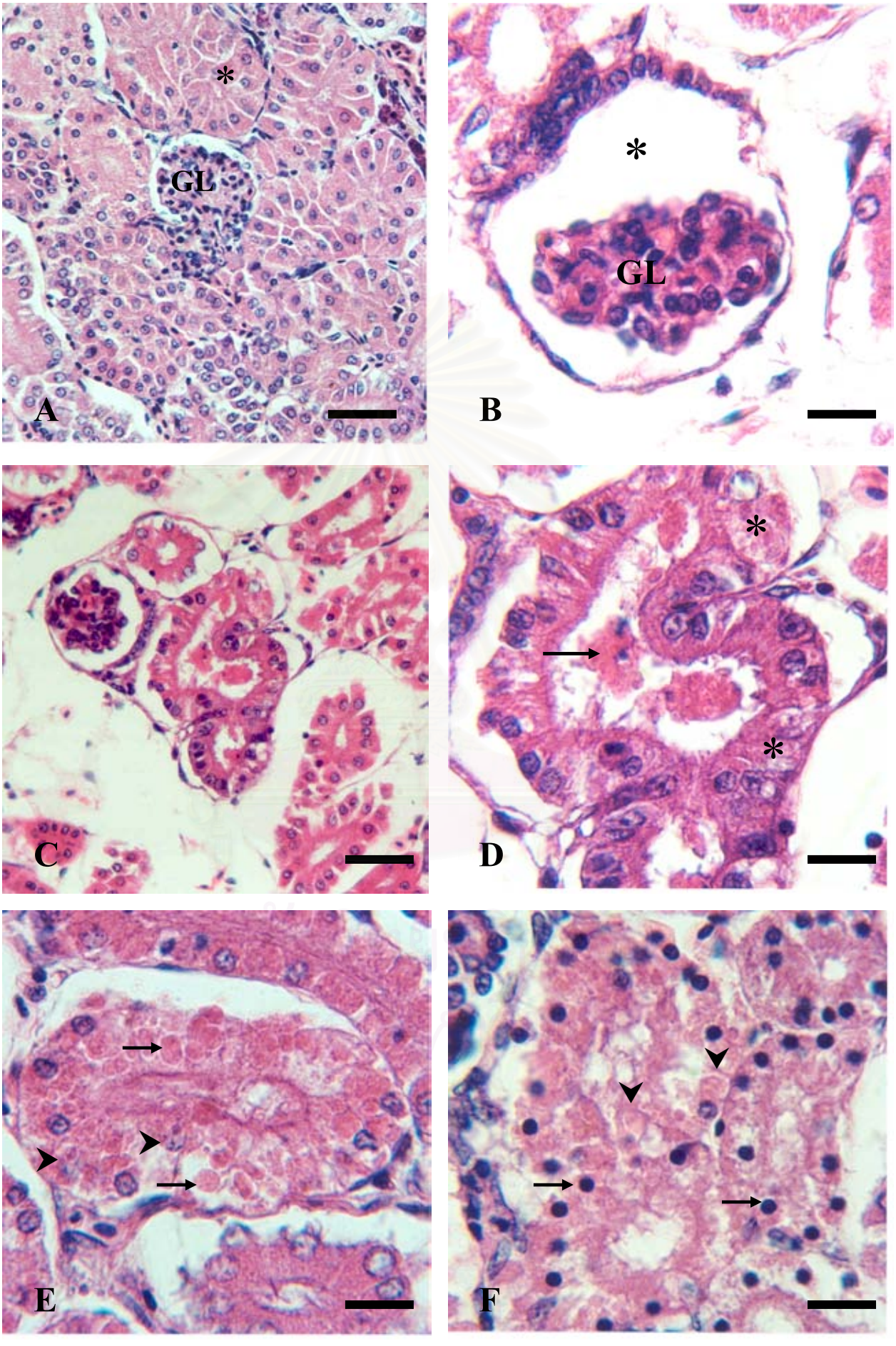
C. The kidney of treated fish in low magnification shows cellular debris accumulation in tubular lumen and swelling of some epithelial cells. Bar scale = 25 μm.

D. The kidney of treated fish in higher magnification shows accumulation of cellular debris (→) in proximal tubular lumen. Swelling of some epithelial cells is also observed (*). Bar scale = 10 μm.

E. The kidney of treated fish shows necrotic degeneration of proximal tubules (➤). Hyaline accumulation (→) is also found throughout the epithelium. Bar scale = 10 μm.

F. The kidney of treated fish shows necrotic epithelial cells (→) and hyaline deposit (➤) in proximal tubules. Bar scale = 10 μm.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย


GL
A
GL
B
C
D
E
F

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. Distilled liquors 30 degree caused histopathological alterations of Nile tilapia liver and kidney at sublethal concentration. Histopathological changes of liver consisted of fat accumulation, necrosis and inflammation. Histopathological alterations of kidney consisted of glomerulus shrinkage and tubular damage.
2. The severity of histopathological alterations of liver was higher than kidney and depended on exposure time.

5.2 Recommendations

1. To provide more information about toxicity of distilled liquors, the ultrastructural alterations of liver tissue or renal tissue should be performed.
2. In this study the direct effects of acetaldehyde, the first metabolite of ethanol oxidation, on liver and kidney tissue have not been studied. So, further studies about hepatotoxicity and nephrotoxicity of this compound are recommended to compare its effects with ethanol.
3. Toxicity testing of various chemicals should be conducted on various organisms.

REFERENCES

Adachi, Y., Bradford, B. U., Gao, W., Bojes, H. K. and Thurman, R. G. 1994. Inactivation of Kupffer cells prevents early alcohol-induced liver injury. Hepatology 20: 453-460.

American Methanol Institute. 1998. Evaluation of the fate and transport of ethanol in the environment. Washington: Malcolm Pirnie.

American Society for Testing and Materials. 1980. Standard practice for conducting toxicity tests with fishes, macroinvertebrates and amphibians. ASTM E 729-80, Philadelphia: ASTM.

Anderson, W. A. D. and Kissane, J. M. 1977. Pathology. Missouri: Mosby Company.

Arteel, G. E., Imuro, Y., Yin, M., Raleigh, J. A. and Thurman., R. G. 1997. Chronic enteral ethanol treatement causes hypoxia in rat liver tissue *in vivo*. Hepatology 25: 920-926.

Augusto, J., Smith, B., Smith, S., Robertson, J. and Reimschuessel, R. 1996. Gentamicin-induced nephrotxicity and nephrogenesis in *Oreochromis niloticus*, a tilapian fish. Diseases of Aquatic Organisms 26: 49-58.

Balasubramanian, P., Saravanan, T. S. and Palaniappan, M. K. 1998. Biochemical and histopathological changes in certain tissue of *Oreochromis mossambicus* (Trewaves) under ambient urea stress. Bull. Environ. Contam. Toxicol. 63: 117–124.

Banerjee, S. and Bhattacharya, S. 1994. Histopathology of kidney of *Channa punctatus* exposed to chronic nonlethal level of elsan, mercury and ammonia. Ecotoxicology and Environmental Safety 29: 265-275.

Bautrup, E., Gissel, M. and Danschner, G. 1986. Histochemical demonstration of two mercury pools in trout tissue : Mercury in kidney and liver after mercuric chloride exposure. Ecotoxicology and Environmental Safety 12: 267-282.

Borges, G., Mendonca, P., Joaquim, N., Coucelo, J. and Aureliano, M. 2003. Acute effects of vanadate oligomers on heart, kidney and liver histology in the Lusitanian toadfish *Halobactrachus didatylus*. Arch. Environ. Contam. Toxicol. 45: 415-422.

Bradford, B. U., Misra, U. K. and Thurman, R. G. 1993. Kupffer cells are requiured for the swift increase in alcoholic metabolism. Res. Common. Subst. Abuse. 14: 1-6.

Cogwill, U. M. and Milazzo, D. P. 1991. The sensitivity of *Ceriodaphnia dubia* and *Daphnia magna* to seven chemicals utilizing the tree-brood test. Arch. Environ. Comtam. Toxicol. 20(2): 211-217.

Cooley, H. M., Evans, R. E. and Klaverkamp, J. F. 2000. Toxicological effects of dietary uranium in lake whitefish *Coregonu clupeaformis*. Aquatic Toxicology 48: 495 -515.

Culling, C. F. A. 1963. Handbook of histopathological technique. 2nd ed. London: Butter worth.

Davidson, S. V. 1980. Alcoholism and health. Maryland: Aspen System.

Decker, T., Lohman-Matthes, M. L., Karch, U., Petrs, T. and Decker, K. 1989. Comparative study of cytotoxicity, tumor necrosis factor and prostaglandin release after stimulation of rats Kupffer cells, murine Kupffer cells and murine inflammatory liver macropharges. J. Leukoc. Biol. 45: 139-146.

Finney, D. J. 1971. Probit analysis. London: Cambridge University Press.

Folmar, L. C., Gardner, G. R., Hickey, J., Bonomelli, S. and Moody, T. 1993. Serum chemistry and histopathological evaluations of brown bullheads *Ameiurus nebulosus* from the Buffalo and Niagara rivers, New York. Arch. Environ. Contam. Toxicol. 25: 298-303.

Folmar, L. C., Harsbarger, J., Baumann, P. C., Gardner, G. and Bonomelli, S. 1995. Pathological and serum chemistry profiles of brown bullheads *Ameiurus nebulosus* from the Black river and Old Woman Creek, Ohio. Bull. Environ. Contam. Toxicol. 54: 50-59.

Forlin, L., Haox, C., Karlsson-Norrgren, L., Runn, P. and Larson, A. 1986. Biotransformation enzyme activities and histopathology in salmon *Salmo gairdneri* treated with cadmium. Aquat. Toxicol. 8: 51-64.

Foss, S. S. and Ray, B. 1997. Effect of culture duration on toxicity of ethanol to developing embryos of the grass shrimp, *Palaemontes pugio*. Bull. Environ. Contam. Toxicol. 59: 467-471.

French, S. W., Benson, N. C. and Sun, P. S. 1984. Centrilobular liver necrosis induced by hypoxia in chronic ethanol-fed rats. Hepatology 4: 912-917.

Georgudaki, L. J. and Kotsanis, N. 2001. Toxic effects of cadmium and mercury in rainbow trout *Oncorhynchus mykiss* : A short-term bioassay. Bull. Environ. Contam. Toxicol. 66: 77-85.

Gerundo, N., Alderman, D. J., Clifton-Hadley, R. S. and Feish, S.W. 1991. Pathological effects of repeated doses of malachite green. A preliminary study. J. Fish Dis. 14(5): 521-532.

Ghosh, A. R. and Chakrabarti, P. 1993. Histopathlogical and histochemical change in liver, pancreas and kidney of the freshwater fish *Heteropnuestes fossilis* Bloch exposed to cadmium. Environment and Ecology 11(1): 185-188.

Gill, T. S., Pant, J. C. and Tewari, H. 1987. Cadmium nephrophathy in a freshwater fish *Puntius conchonius* Hamilton. Ecotoxicology and Environmental Safety 18(2): 165-172.

Gomez, L., Soler, F., Martinez, S., Gazquez, A., Duran, E. and Roncero, V. 1999. 2, 4-D treatment in tench *Tinca tinca* L.: Pathological processes on the excretory kidney. Bull. Environ. Contam. Toxicol. 62: 600-607.

Hasumura, Y. 1997. Metabolism of alcohol and its effects on the body system. Asian Med. J. 40(8): 375-380.

Hijioka, T., Sato, N., Matsumura, T., Yoshihara, H., Takei, Y., Fukui, H., Oshita, M., Kawano, S. and Kamada, T. 1991. Ethanol-induced disturbance of hepatic microcirculation and hepatic hypoxia. Biochem. Pharmacol. 11: 1551-1557.

Hill, D. B. 1998. Alcoholic liver disease. Postgraduate medicine 103(4): 205-213.

Humason, G. L. 1979. Animal tissue technique. 4th ed. San Francisco: Freeman.

Iimuro, Y., Frankenberg, M. V., Arteel, G. E., Bradford, B. U., Wall, C. A. and Thurman, R. G. 1997a. Female rats exhibit greater susceptibility to early alcohol-induced injury than males. Am. J. Physiol. Gastrointes. Liver Physiol. 272(35): G1186-1194.

Iimuro, Y., Gullucci, R. M., Luster, M. I. and Thurman, R. G. 1997b. Antibodies to tumor necrosis factor-α attenuate hepatic necrosis and inflammation due to chronic exposure to ethanol in the rat. Hepatology 26: 1530-1537.

Iimuro, Y., Kono, H., Connor, H. D., Bradford, B. U., Mason, R. P., Wall, C. A. and Thurman, R. G. 1996. Increased susceptibility to alcoholic hepatitis in female rats is associated with elevated free radical formation. Hepatology 24: 1243 (Abstract).

Ishak, K. G., Zimmerman, H. J. and Ray, M. B. 1991. Alcoholic liver disease: Pathological and clinical aspects. Alcohol Clin. Exp. Res. 15(1): 45-66.

Jones, J., Kinnel, M., Christenson, R. and Reimschuessel, R. 1997 Gentamicin concentration in toadfish and goldfish serum. Journal of Aquatic Animal Health 9: 211-215.

Jonsson, C. M. and Toledo, M. C. F. 1993. Acute toxicity of endosulfan to the fish *Hyphessobrycon bifasciatus* and *Brachydanio rerio*. Arch. Environ. Contam.Toxicol. 24: 151-155.

Kalbfleisch, J. M., Linderman, R. D. and Ginn, H. E. 1963. The effects of ethanol administration on urinary excretion of magnesium and other electrolytes in alcoholic and normal subjects. J. Clin. Invest. 42: 1471-1475.

Khan, R. A. 1997. Effect of sediment from two sulphite-bleaching paper mills on winter flounder *Pleuronectes amricanus* following chronic exposure. Bull. Environ. Contm. Toxicol. 59: 139-145.

Khan, R. A. 1998. Influence of petroleum at a refinery terminal on feral winter flounder, *Pleuronectes americanus*. Bull. Environ. Contam. Toxicol. 61: 770-777.

Khan, R. A. 2000. Comparison of tissue lesions in four species of benthic fish sampled in 1972–1973 and 1997–1998 on the Grand Bank off Newfoundland. Bull. Environ. Contam. Toxicol. 65: 78-83.

Khan, R. A. 2003. Stress-related bioindicator anomalies in feral male winter flounder *Pleuroectes americanus* exposed to effluent from two pulp and paper mills in Newfoundland. Bull. Environ. Contam. Toxicol. 70: 401-407.

Khan, R. A., Barker, D. E., Hooper, R., Lee, E. M., Ryan, K. and Nag, K. 1994. Histopathology in winter flounder *Pleuronectes americanus* living adjacent to a pulp and paper mill. Arch. Environ. Contam. Toxicol. 26: 95-102.

Kiatisevi, F. 2001. Alcoholic liver diseases. Bangkok: Bangkok Medical Journal .

Kotsanis, N. and Georgudaki, L. J. 1999. Arsenic induced liver hyperplasia and kidney fibrosis in rainbow trout *Oncorhynchus mykiss* by microinjection technique: A sensitive animal bioassay for environmental metal-toxicity. Bull. Environ. Contam.Toxicol. 62: 169-178.

Kumar, V., Cotran, R. S. and Robbins, S. L. 2002. Robbins basic pathology. 7th ed. Philadelphia: W. B. Saunders.

Kumar, S. and Pant, S. C. 1984. Organal damage caused by aldicarb to a freshwater teleost *Barbus conchonius* Hamilton. Bull. Environ. Contam. Toxicol. 33: 50-55.

Kumuri, S. A. and Kumuri, N. S. 1995. Effect of water pollution on histology of fish *Channa Striatus* in Hussainsagar, Huderrabad, India. Environmental and Ecology 13(4): 932-934.

Lagderspetz, K. Y., Tiiska, A. and Senius, K. E. 1993. Low sensitivity of cilliary in the gills of *Anodonta cygnea* to some ecotoxicals. Comp. Biochem. Physiol. 10(3): 393-395.

Larsen, B. K. and Perkins, E. J. 2001. Target organ toxicity in the kidney. In Schlenk, D. and Benson, W. H. (eds.), Target organ toxicity in marine and freshwater teleosts, pp. 91-150. London: Tayler& Francis.

Lauren, D. J., Wishkovsky, A, Groff, J. M., Hedrick, R. P. and Hinton, D. E. 1989. Toxicity and pharmacokinetics of the antibiotic, fumagillin, in yearling salmon *Salmo gaindneri*. Toxicology and Applied Pharmacology 98: 444-453.

Levinthal, C. F. 1996. Drugs, behaviors and modern society. Boston: Allyn & Bacon.

Lorimier, A. A. 2000. Alcohol, wine and health. Am. J. Surgery 180: 357-361.

Martinez, F. E., Abril, E. R., Earnest, D. L. and Watson, R. R. 1992. Ethanol and cytokine secretion. Alcohol 9: 455-458.

Moden, K., Arii, S., Itai, S., Sasaoki, T., Adachi, Y., Funali, N., Higashitsuji, H. and Tobe, T. 1991. Enhancement and hepatocyte modulating effect of chemical mediators and monokines produced by hepatic macrophage in rats with induced sepsis. Res. Exp. Med. (Berl.) 191: 177-187.

Mophatra, B. C. and Noble, A. 1992. Liver and kidney damage in grey mullet *Liza parsia* (Hamilton and Buchanan) on exposure to an organophosphate 'Nuran'. Journal of the Marine Biology Association India 34 : 218-221.

Myers, M. S., Stehr, C. M., Olson, O. P., Johnson, E., McCain, B. B., Chan, S. L. and Varanasi, U. 1994. Relationship between toxicipathic hepatic lesions and exposure to chemical contaminant in Enlish sole *Parophry vetulus*, starry flounder *Platichthys stellatus*, and white croaker *Genyonemus lineatus* from selected marine sites on the Pacific Coast, USA. Environmental Health Perspectives 102: 200-215.

Nakano, M., Worner, T. M. and Lieber, C. S. 1982. Perivenular fibrosis in alcoholic liver injury: ultrastructure and histologic progression. Gastroenterology 83: 777-785.

Neskovic, N. K., Poleksic, V., Elezovic, I., Karan, V. and Budimir, M. 1996. Biochemical and histopathological effects of glyphosate on carp *Cyprinus carpio* L. Bull. Environ. Contam. Toxicol. 56: 295-302.

Nowak, B. 1996. Relationship between endosulfan residue level and ultrastructural changes in the liver of catfish *Tandanus tandanus*. Arch . Environ. Contam. Toxicol. 30: 195-202.

Ohnishi, K. 1997. Alcohol and liver disease. Asian Med. J. 40(8): 389-395.

Oronsaye, J. A. O. 1989. Histological changes in the kidney and gills of the stickle back *Gasterosteus aculeatus* L. exposed to dissolved cadmium in hard water. Ecotoxicology and Environmental Safety 17: 279-290.

Pandey, A. K. 1994. Branchial and renal lesions in the estuarine mullet *Liza parsia* exposed to sublethal concentration of mercury. Proceedings of the National Academy of science, India 648: 281-287.

Parenti, P., Giordana, B. and Hanozet, G. M. 1991. In vitro effect of ethanol on sodium and glucose transport in rabbit renal brush border membrane vesicles. Biophys. Acta. 1070: 92-98.

Patil, V. T., Shinde, S. V. and Kulkarni, A. B. 1992. Histopathological changes induced by monocrotophos in the liver of the fish *Boleophthalmus dussumieri*. Environ. Ecol. 10(1): 52-54.

Patwardhan, S. A. and Gaikwad, S. A. 1991. Effect of sumithion on liver, hepatopancreas and mesenteric pancreas of freshwater fish *Gambusia affinis*. Environment and Ecology 9(2): 352-355.

Pedlar, R. M. P., tashynski, M. D., Evans, R. and Klaverkamp, J. F. 2002. Toxicological effects of dietary arsenic exposure in lake whitefish *Coregonus clupeaformis*. Aquat Toxicol. 57: 167-189.

Philipart, J. C. and Ruwet, J. C. 1982. Ecology and distribution of tilapias. Proceedings of the International Conference on the Biology and Culture of Tilapias 1: 15-59.

Phillips, M. J., Poucell, J., Patterson, J. and Valenria, P. 1987. The liver- an atlas and text of ultrastructural pathology. New York: Raven Press.

Phumipat, T. 1981. Life History of Pla Nil, Tilapia nilotica (Linnaeus). Technical Paper No. 7, National Inland fisheries Institute, Department of Fisheries. 34 pp. (in Thai),

Ptashynski, M. D., Pedlar, R. M., Evans, R. E, Baron, C. L. and Klaverkamp, J. F. 2002. Toxicological effects of dietary nickel in lake whitefish *Coregonus clupeaformis*. Aquat. Toxicol. 58: 229-247.

Qu, W., Zhong, Z., Goto, M. and Thurman, R. G. 1996. Kupffer cell prostaglandin E_2 stimulates parenchyma cell O_2 consumption: Alcohol and cell-cell communication. Am. J. Physiol. Gastrointest. Liver Physiol. 270(33): G574-G580.

Radhaiah, V. and Jayantha-Rao, K. 1992. Fenvalerate toxicity to the liver in a freshwater teleost *Tilapia mossambica* Pefers. Comparative Phisiology and Ecology 17(2): 48-53.

Rand, G. M. and Petrocelli, S. R. 1985. Fundamental of aquatic toxicology. Washington: Hemisphere Publishing.

Reimschuessel, R., Bennett, R. O., May, E. B. and Lipsky, MM. 1989. Renal histophatological changes in the goldfish *Carassius auratus* after sublethal exposure to hexachlorobutadiene. Toxicology 15: 169-180.

Reimschuessel, R., Bennett, R. O., May, E. B. and Lipsky, M. M. 1993. Pathological alterations and new nephron development in rainbow trout *Oncorhynchus mykiss* following tetrachloroethylene contamination. Journal of Zoo and Wildlife Medicine 24: 503-507.

Reinschuessel, R. and Gonzalez, C. M. 1998. Renal alterations following sublethal mercury toxicity: a fish model for aquatic environmental contamination. In Salem, H. and Katz, S. A. (eds.), In advances in animal alternatives for safety and efficacy testing, pp 399-401. Washington: Tayler & Franscis.

Reimschuessel, R. and Williams, D. 1995. Development of new nephrons in adult kidney following gentamicin-induced nephrotoxicity. Renal Failure 17: 101-106.

Roberts, R. J. 1978. Fish Pathology. 1st ed. New York: MacMillan.

Rolf, L. L., Setser, M. D. and Walker, J. L. 1986. Pharmacokinetics and tissue residues in channel catfish *Ictalurus punctatus* given intracardiac and intramuscular injections of gentamicin sulfate. Veterinary and Human Toxicology 28(1): 25-31.

Rothman, A., Proverbio, T., Fernandez, E. and Proverbio, F. 1992. Effect of ethanol on the Na^+ and the Na^+/K^+- ATPase activity of basolateral plasma membranes of kidney proximal tubular cells. Biochem. Pharmacol. 43: 2034-2036.

Rudolph, A., Yanez, R. and Troncoso, L. 2001. Effects of exposure of *Oncorhynchus mykiss* to the water-accommodate fraction of petroleum hydrocarbons. Bull. Environ. Contam. Toxicol. 66: 400-406.

Sam-angsri, C. 2002. Brine shrimp cytotoxicity test. Department of Biology, Faculty of Science, Chulalongkorn University.

Sastry, K. V. and Sharma, K. 1981. Diazinon-induced histophatological and hematological alterations in a freshwater teleost *Ophiocephalus punctatus*. Ecotoxicology and Environmental Safety 5: 329-340.

Schneider, S. R., Hendricks, J. D., Constantine, G. H. and Larson, R. E. 1980. Tobromycin nephrotoxicity and lethality in coho salmon. Toxicology and Appplied Pharmacology 54: 399-404.

Schwaiger, J., Fent, K., Stecher, H., Ferling, H. and Negele, R. D. 1996. Effects of sublethal concentretions of triphenyltinacetate on rainbow trout *Oncorhynchus mykiss*. Arch. Environ. Contam. Toxicol. 30: 327-334.

Singhal, R. N. and Jain, M. 1997. Cadmium-induced change in the histology of kidneys in common carp *Cyprinus carpio* (Cyprinidae). Bull. Environ. Contam. Toxicol. 58: 456-462.

Sprague, J. B. 1990. Aquatic toxicology. In Schreck, C. B. and Moyle, P. B. (eds.), Methods for fish biology, pp. 491-528. Betheda: American Fisheries Society.

Srijunngam, J. 1998. Subchronic effects of neem Azadirachta indica A. Juss. Seed extract on female reproductive system of tilapia *Oreochromis niloticus* Linn. Master's Thesis, Department of Biology, Graduate School, Chulalongkorn University.

Takahashi, T., Kamimura, T. and Ichida, F. 1987. Ultrastructural findings on polymorphonuclear leukocyte infiltration and acute hepatocellular damage in alcoholic hepatitis. Liver 7: 347-358.

Theile, D. L. 1989. Tumor necrosis factor, the acute phase response and the pathogenesis of alcoholic liver disease. Hepatology 9: 497-499.

Thongra-ar, N. 1991. Study on blood alcohol concentration of the drivers and passengers involved in the fatal road traffic accidents in Bangkok and the near-by provinces. Master's Thesis, Department of Pharmacology, Graduate School, Chulalongkorn University.

Thurman, R. G. 1998. Machanisms of hepatic oxicity. II. Alcoholic liver injury involved activation of Kupffer cells by endotoxin. Am. J. Physiol. 275(38): G605-G611.

Trewavas, E. 1982. Tilapias: Taxonomy and Speciation. Proceeding of the International Conference on the Biology and Culture of Tilapias. 1: 3-13.

Tsukamoto, H., Gaal, K. and French, S. W. 1990. Insights into the pathogenesis of alcoholic liver necrosis and fibrosis: status report. Hepatology 12(3): 599-608.

Tsukamoto, H., Towner, S. J., Ciofalo, L. M., and French, S. W. 1986. Ethanol-induced liver fibrosis in rats fed high fat diet. Hepatology 6(5): 814-822.

Vamvakas, S. Teschner, M., Bahner, U. and Heidland, A. 1998 Alcohol abuse. Clinical Nephrology 49(4): 205-213

Van Thiel, D. H., Gavaler, J. S., Little, J. M. and Lester, R. 1979. Alcohol: Its effect on the kidney. Adv. Exp. Med. Biol. 85: A449-457.

Vindins, E. I., Britton, R. S. and Medline, A. 1985. Sinusoidal caliber in alcoholic and nonalcoholic liver disease: Diagnostic and pathogenic implications. Hepatology 5: 408-414.

Wattanasirmkit, K. and Patamastan, P. 1997. Effects of acute exposure to *Derris trifoliate* root extract on liver of tilapia *Oreochromis niloticus*. In Rodcharoen, J., Wongsiri, S. and Mulla, S. (eds.), Biopesticides: Toxicity, safety development and proper use. Proceedings First International Symposium on Biopesticides. pp. 75-81. Bangkok: Chulalongkorn University Press.

Woods, S. E., Hitchcock, M. and Meyer, A. 1993. Alcohol hepatitis. American Family Physician 47(5): 1171-1178.

Wu, W. Z., Zhou, B. S., Xu, Y., Schramm, K. W. and Kettrup, A. 1999. Cytological and Biochemical alterations in *Carassius auratus* hepatocytes from exposure to sediment containing dioxins and related compounds. Arch. Environ. Contum. Toxicol. 37 : 358-363.

Yamada, S., Moshida, S., Ohno, A., Hirata, K., Oggata, I., Ohta, Y. and Fujiwara, K. 1991. Evidence for enhanced secretory function of hepatic macrophages after long- term ethanol feeding in rats. Liver 11: 220-224.

Yang, J. L. and Chen, H. C. 2003. Growth retardation and histopatology of common carp *Cyprinus carpio* exposed to gallium. Bull. Environ. Contam. Toxicol. 71: 240-247.

Yin, M., Ikejima, K., Wheeler, M. D., Bradford, B. U., Seabra, V., Forman, D. T., Sato, N. and Thurman, R. G. 2000. Estrogen is involved in early alcohol-induced liver injury in a rat enteral feeding model. Hepatology 31: 117-123.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDICES

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX I

ACUTE TOXICITY TEST DATA

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LC_{50} at 24 hours

* PROBIT ANALYSIS *

DATA Information

7 unweighted cases accepted.

0 cases rejected because of missing data.

1 case is in the control group.

MODEL Information

ONLY Normal Sigmoid is requested.

---

* PROBIT ANALYSIS *

Parameter estimates converged after 18 iterations.

Optimal solution found.

Parameter Estimates (PROBIT model: (PROBIT(p)) = Intercept + BX):

	Regression Coeff.	Standard Error	Coeff./S.E.
CONC	.00014	00002	7.09129

Intercept	Standard Error	Intercept/S.E.
-4.91483	.65406	-7.51440

Pearson Goodness-of-Fit Chi Square = 21.557 DF = 5 P = .001

Since Goodness-of-Fit Chi square is significant, a heterogeneity factor is used in the calculation of confidence limits.

---

* PROBIT ANALYSIS *

Observed and Expected Frequencies

CONC	Number of Subjects	Observed Responses	Expected Responses	Residual	Prob
.00	30.0	.0	.000	.000	.00000
15000.00	30.0	.0	.085	-.085	.00284
20000.00	30.0	2.0	.606	1.394	.02019
25000.00	30.0	4.0	2.734	1.266	.09115
30000.00	30.0	6.0	8.054	-2.054	. 26846
35000.00	30.0	8.0	16.180	-8.180	.53933
40000.00	30.0	30.0	23.774	6.226	.79245

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

********** PROBIT ANALYSIS **********

Confidence Limits for Effective CONC

		95% Confidence Limits	
Prob	CONC	Lower	Upper
.01	18070.35053	-24689.04635	24986.85179
.02	19973.37809	-17151.51085	26229.79096
.03	21180.78807	-12395.86413	27045.07391
.04	22089.07556	-8836.94127	27676.94443
.05	22827.89737	-5956.95898	28205.84927
.06	23456.75076	-3518.56796	28668.95660
.07	24008.13217	-1392.29270	29086.72778
.08	24501.82846	500.57918	29471.74607
.09	24950.82548	2211.59840	29832.37681
.10	25364.12798	3776.41378	30174.51862
.15	27075.31073	10115.12952	31731.11514
.20	28435.30454	14907.18730	33214.00125
.25	29602.05813	18731.11435	34773.41733
.30	30649.83891	21829.71619	36509.22400
.35	31620.76396	24332.46340	38486.27675
.40	32542.07688	26341.53345	40728.09877
.45	33433.45780	27960.87781	43221.54169
.50	34310.70621	29291.77947	45938.22062
.55	35187.95461	30420.83196	48856.74872
.60	36079.33553	31415.50393	51974.86404
.65	37000.64845	32327.00102	55314.25903
.70	37971.57350	33195.92593	58925.13407
.75	39019.35428	34058.52713	62896.94138
.80	40186.10787	34954.11003	67384.70160
.85	41546.10168	35937.55052	72676.20502
.90	43257.28443	37111.89160	79397.17619
.91	43670.58693	37387.71271	81028.31226
.92	44119.58395	37684.55024	82803.12470
.93	44613.28024	38007.88957	84757.67553
.94	45164.66165	38365.61707	86943.99447
.95	45793.51504	38769.71716	89441.39274
.96	46532.33685	39239.82347	92380.17355
.97	47440.62434	39811.79517	95998.99524
.98	48648.03432	40563.56712	100818.15296
.99	50551.06188	41732.63875	108429.55600

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LC_{50} at 48 hours

* PROBIT ANALYSIS *

DATA Information

7 unweighted cases accepted.

3 cases rejected because of missing data.

1 case is in the control group.

MODEL Information

ONLY Normal Sigmoid is requested.

- -

* PROBIT ANALYSIS *

Parameter estimates converged after 16 iterations.

Optimal solution found.

Parameter Estimates (PROBIT model: (PROBIT(p)) = Intercept + BX):

	Regression Coeff.	Standard Error	Coeff./S.E.
CONC	.00009	.00001	7.63788

Intercept	Standard Error	Intercept/S.E.
-2.29701	33025	-6.95528

Pearson Goodness-of-Fit Chi Square = 8.959 DF = 5 P = .111

Since Goodness-of-Fit Chi square is significant, a heterogeneity factor is used in the calculation of confidence limits.

- -

* PROBIT ANALYSIS *

Observed and Expected Frequencies

CONC	Number of Subjects	Observed Responses	Expected Responses	Residual	Prob
.00	30.0	.0	324	-.324	.01081
15000.00	30.0	8.0	5.697	2.303	.18991
20000.00	30.0	12.0	10.279	1.721	.34263
25000.00	30.0	14.0	15.809	-1.809	.52697
30000.00	30.0	16.0	21.168	-5.168	.70560
35000.00	30.0	26.0	25.338	662	.84459
40000.00	30.0	30.0	27.943	2.057	.93142

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

********** PROBIT ANALYSIS **********

Confidence Limits for Effective CONC

		95% Confidence Limits	
Prob	CONC	Lower	Upper
.01	-310.14976	-21924.12963	8254.23046
.02	2571.86173	-16752.44802	10314.24268
.03	4400.40562	-13480.79991	11630.87245
.04	5775.94796	-11025.94396	12627.59921
.05	6894.84554	-9033.89459	13443.14611
.06	7847.20311	-7342.30175	14141.25867
.07	8682.23440	-5862.53769	14756.79876
.08	9429.90533	-4540.66624	15311.02226
.09	10109.88212	-3341.31098	15817.90030
.10	10735.80187	-2239.95811	16287.13797
.15	13327.27687	2286.21335	18263.63101
.20	15386.89905	5830.48421	19887.47176
.25	17153.87149	8815.76417	21335.97137
.30	18740.66735	11434.71021	22698.69642
.35	20211.07020	13789.61790	24033.40038
.40	21606.33870	15939.14575	25384.95318
.45	22956.27713	17918.44825	26792.98673
.50	24284.81280	19750.39492	28294.67118
.55	25613.34846	21453.70574	29924.99147
.60	26963.28689	23049.39548	31716.63778
.65	28358.55540	24565.00165	33702.11226
.70	29828.95825	26036.51564	35920.20992
.75	31415.75411	27510.00603	38428.39063
.80	33182.72654	29047.04690	41325.12933
.85	35242.34873	30741.87322	44798.41461
.90	37833.82373	32776.51357	49266.43875
.91	38459.74348	33256.27077	50357.27211
.92	39139.72027	33773.40505	51546.37112
.93	39887.39119	34337.68005	52858.19107
.94	40722.42248	34963.13941	54328.03586
.95	41674.78006	35671.13561	56009.74506
.96	42793.67764	36496.67160	57991.80534
.97	44169.21998	37503.72575	60436.33391
.98	45997.76387	38831.48126	63696.85627
.99	48879.77536	40904.67372	68855.35764

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LC_{50} at 72 hours

* PROBIT ANALYSIS *

DATA Information

7 unweighted cases accepted.

3 cases rejected because of missing data.

1 case is in the control group.

MODEL Information

ONLY Normal Sigmoid is requested.

- -

* PROBIT ANALYSIS *

Parameter estimates converged after 16 iterations.

Optimal solution found.

Parameter Estimates (PROBIT model: (PROBIT(p)) = Intercept + BX):

	Regression Coeff.	Standard Error	Coeff./S.E.
CONC	.00011	.00001	7.60100

Intercept	Standard Error	Intercept/S.E.
-2.02037	.33818	-5.97419

Pearson Goodness-of-Fit Chi Square = 6.938 DF = 5 P = .225

Since Goodness-of-Fit Chi square is NOT significant, no heterogeneity

factor is used in the calculation of confidence limits.

- -

* PROBIT ANALYSIS *

Observed and Expected Frequencies

CONC	Number of Subjects	Observed Responses	Expected Responses	Residual	Prob
.00	30.0	.0	.650	-.650	.02167
15000.00	30.0	12.0	10.732	1.268	.35773
20000.00	30.0	20.0	17.230	2.770	.57434
25000.00	30.0	22.0	23.105	-1.105	.77016
30000.00	30.0	24.0	27.051	-3.051	.90170
35000.00	30.0	30.0	29.021	.979	.96736
40000.00	30.0	30.0	29.751	.249	.99169

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

********** PROBIT ANALYSIS **********

Confidence Limits for Effective CONC

		95% Confidence Limits	
Prob	CONC	Lower	Upper
.01	-2771.81298	-11660.38457	2606.45511
.02	-302.39013	-8367.41132	4604.11286
.03	1264.37950	-6282.03594	5875.47324
.04	2442.99926	-4715.76037	6834.33921
.05	3401.71551	-3443.55122	7616.13791
.06	4217.73361	-2362.18265	8283.05263
.07	4933.22186	-1415.29261	8869.06503
.08	5573.85625	-568.57322	9394.87694
.09	6156.48759	200.48422	9874.08180
.10	6692.80070	907.48251	10316.11100
.15	8913.28025	3823.40693	12157.46740
.20	10678.04701	6124.23583	13637.57450
.25	12192.05975	8081.97311	14923.54364
.30	13551.68999	9823.16457	16095.30080
.35	14811.59003	11418.05465	17199.69173
.40	16007.11195	12910.32401	18268.77608
.45	17163.79326	14329.50779	19327.73166
.50	18302.13582	15697.08796	20399.00089
.55	19440.47837	17030.01986	21504.91839
.60	20597.15968	18343.31826	22669.75935
.65	21792.68161	19652.62742	23921.80391
.70	23052.58164	20977.49742	25296.21492
.75	24412.21188	22346.25048	26840.41047
.80	25926.22462	23804.42334	28625.94403
.85	27690.99138	25433.21172	30778.09164
.90	29911.47093	27403.57811	33565.00608
.91	30447.78404	27869.61586	34247.99582
.92	31030.41538	28372.41596	34993.45803
.93	31671.04977	28921.52646	35816.87883
.94	32386.53802	29530.69451	36740.61321
.95	33202.55612	30220.83696	37798.75405
.96	34161.27237	31026.26149	39047.33737
.97	35339.89213	32009.70374	40589.03668
.98	36906.66176	33307.70000	42647.77616
.99	39376.08461	35337.10837	45908.99880

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

LC_{50} at 96 hours

********** PROBIT ANALYSIS **********

DATA Information

7 unweighted cases accepted.

3 cases rejected because of missing data.

1 case is in the control group.

MODEL Information

ONLY Normal Sigmoid is requested.

********** PROBIT ANALYSIS **********

Parameter estimates converged after 18 iterations.

Optimal solution found.

Parameter Estimates (PROBIT model: (PROBIT(p)) = Intercept + BX):

	Regression Coeff.	Standard Error	Coeff./S.E.
CONC	.00016	.00003	6.17200

Intercept	Standard Error	Intercept/S.E.
-2.49073	.50843	-4.89887

Pearson Goodness-of-Fit Chi Square = 2.738 DF = 5 P = .740

Since Goodness-of-Fit Chi square is NOT significant, no heterogeneity

factor is used in the calculation of confidence limits.

********** PROBIT ANALYSIS **********

Observed and Expected Frequencies

CONC	Number of Subjects	Observed Responses	Expected Responses	Residual	Prob
.00	30.0	.0	.191	-.191	.00637
15000.00	30.0	14.0	13.582	.418	.45272
20000.00	30.0	24.0	22.475	1.525	.74916
25000.00	30.0	26.0	27.846	-1.846	.92820
30000.00	30.0	30.0	29.636	.364	.98788
35000.00	30.0	30.0	29.965	.035	.99883
40000.00	30.0	30.0	29.998	.002	.99994

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

********** PROBIT ANALYSIS **********

Confidence Limits for Effective CONC

		95% Confidence Limits	
Prob	CONC	Lower	Upper
.01	1039.52340	-7606.06616	5649.02964
.02	2763.42336	-5107.50250	6984.96060
.03	3857.18263	-3525.23565	7835.55764
.04	4679.97511	-2336.83390	8477.30464
.05	5349.25333	-1371.54567	9000.70123
.06	5918.91420	-551.04591	9447.30606
.07	6418.39535	167.43108	9839.83192
.08	6865.62115	809.91693	10192.11661
.09	7272.35512	1393.49048	10513.24707
.10	7646.75440	1929.99069	10809.52839
.15	9196.86748	4142.99816	12044.45893
.20	10428.84820	5889.72057	13038.04834
.25	11485.77797	7376.62502	13902.08744
.30	12434.93357	8699.83570	14690.09672
.35	13314.46768	9912.77343	15433.51891
.40	14149.05955	11048.69835	16153.98825
.45	14956.53683	12130.08470	16868.68484
.50	15751.21187	13173.14521	17593.23104
.55	16545.88691	14190.32580	18343.65716
.60	17353.36420	15191.97658	19138.08931
.65	18187.95606	16187.91685	19998.54331
.70	19067.49018	17189.55091	20953.26917
.75	20016.64577	18213.37330	22040.66675
.80	21073.57554	19287.33170	23317.65189
.85	22305.55627	20464.14286	24881.15256
.90	23855.66934	21858.32507	26934.90835
.91	24230.06862	22184.14835	27441.86660
.92	24636.80259	22534.25662	27996.46234
.93	25084.02839	22915.08935	28610.40015
.94	25583.50954	23335.91212	29300.58023
.95	26153.17042	23810.81405	30092.78290
.96	26822.44863	24362.88991	31029.39186
.97	27645.24112	25034.34600	32188.08450
.98	28739.00038	25916.98227	33738.31213
.99	30462.90035	27290.86204	36198.92698

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX II

LSI DATA

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Data sheet of control (M_4C_x) group (n=20)

Number	Body weight	Liver weight	LSI
1	12.3	0.22	1.7886
2	13.68	0.24	1.7544
3	10.02	0.26	2.5948
4	9.88	0.16	1.6194
5	9.50	0.17	1.7895
6	8.88	0.17	1.9144
7	7.98	0.15	1.8797
8	9.07	0.21	2.3153
9	13.05	0.14	1.0728
10	9.97	0.07	0.7021
11	9.75	0.09	0.9231
12	8.56	0.07	0.0818
13	13.08	0.22	1.6820
14	8.90	0.14	1.5730
15	9.15	0.21	2.2951
16	9.73	0.14	1.4389
17	12.66	0.25	1.9747
18	8.98	0.18	2.0045
19	10.02	0.19	1.8962
20	9.02	0.16	1.7738
Mean	10.27	0.17	1.6884
SE	1.7005	0.0553	0.5031

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Data sheet of control (M_5C_x) group (n=20)

Number	Body weight	Liver weight	LSI
1	12.93	0.21	1.6241
2	11.28	0.18	1.5957
3	10.94	0.22	2.0110
4	10.12	0.18	1.7787
5	12.29	0.20	1.6273
6	10.04	0.13	1.2948
7	11.09	0.22	1.9838
8	10.31	0.21	2.0369
9	11.71	0.24	2.0495
10	14.43	0.15	1.0395
11	12.94	0.24	1.8547
12	13.68	0.24	1.7544
13	13.05	0.14	1.0728
14	12.29	0.20	1.6273
15	18.72	0.38	2.0299
16	16.54	0.23	1.3906
17	21.83	0.40	1.8323
18	19.57	0.34	1.7374
19	26.19	0.41	1.5655
20	19.34	0.28	1.4478
Mean	14.47	0.24	1.6677
SE	4.3474	0.0803	0.2959

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Data sheet of control (M_6C_x) group (n=20)

Number	Body weight	Liver weight	LSI
1	24.49	0.46	1.8783
2	21.13	0.41	1.9404
3	18.41	0.33	1.7925
4	16.35	0.34	2.0795
5	23.46	0.27	1.1509
6	21.97	0.18	0.8193
7	23.82	0.24	1.0076
8	13.65	0.31	2.2711
9	20.52	0.46	2.2417
10	26.41	0.45	1.7039
11	20.38	0.29	1.4230
12	29.54	0.20	0.6771
13	24.34	0.30	1.2326
14	23.96	0.46	1.9199
15	15.96	0.30	1.8797
16	32.16	0.35	1.0873
17	33.12	0.35	1.0568
18	19.54	0.26	1.3306
19	22.62	0.21	0.9284
20	28.12	0.43	1.5292
Mean	23.00	0.33	1.4975
SE	5.0235	0.0885	0.4703

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Data sheet of treated (M_4T_x) group (n=20)

Number	Body weight	Liver weight	LSI
1	5.70	0.20	3.5263
2	5.31	0.18	3.3710
3	5.41	0.10	1.9224
4	5.42	0.14	2.6384
5	8.94	0.36	4.0268
6	9.78	0.22	2.2495
7	5.65	0.21	3.7876
8	11.50	0.31	2.7217
9	7.74	0.26	3.3075
10	6.15	0.22	3.6260
11	8.80	0.16	1.8523
12	7.76	0.27	3.5052
13	4.53	0.11	2.3179
14	6.84	0.13	1.9591
15	6.53	0.26	3.9510
16	6.04	0.25	4.1887
17	5.90	0.25	4.2203
18	11.17	0.41	3.6347
19	7.09	0.27	3.7377
20	5.58	0.16	2.8495
Mean	7.09	0.22	3.1697
SE	1.9674	0.0783	0.7855

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Data sheet of treated (M_5T_x) group (n=20)

Number	Body weight	Liver weight	LSI
1	16.00	0.50	3.1254
2	10.78	0.27	2.5046
3	8.52	0.23	2.6995
4	8.51	0.24	2.8202
5	14.54	0.42	2.8885
6	11.31	0.31	2.7409
7	8.07	0.28	3.4696
8	7.94	0.18	2.2670
9	7.12	0.19	2.6685
10	13.88	0.35	2.5216
11	11.06	0.32	2.8933
12	9.65	0.19	1.9689
13	7.16	0.20	2.7933
14	15.18	0.33	2.1739
15	8.41	0.19	2.2592
16	16.77	0.46	2.7430
17	15.74	0.70	4.4473
18	12.76	0.27	2.1160
19	10.25	0.45	4.3902
20	9.60	0.44	4.5833
Mean	11.25	0.32	2.8214
SE	3.1653	0.1313	0.6485

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Data sheet of treated (M_6T_x) group (n=20)

Number	Body weight	Liver weight	LSI
1	13.89	0.43	3.0958
2	12.66	0.43	3.3965
3	11.72	0.28	2.3870
4	10.26	0.40	3.8986
5	21.73	0.60	2.7612
6	17.29	0.57	3.2967
7	11.01	0.38	3.4514
8	10.99	0.36	3.2757
9	17.31	0.76	4.3905
10	15.48	0.46	2.9716
11	12.32	0.30	2.4351
12	14.33	0.36	2.5122
13	12.27	0.24	1.9560
14	21.92	0.42	1.9161
15	13.94	0.41	2.9412
16	15.32	0.71	4.6345
17	15.47	0.44	2.8442
18	16.12	0.47	2.9156
19	12.93	0.42	3.2483
20	10.83	0.52	4.8015
Mean	14.58	0.44	3.0630
SE	3.2010	0.1300	0.6922

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

T-Test of body weight compared between control and treated group at 4 months

Group Statistics

	1-control, 2-treated	N	Mean	Std. Deviation	Std. Error Mean
Body weight	1.00	20	10.209000	1.723390	.385362
	2.00	20	7.087500	2.002803	.447840

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Body weight	Equal variances assumed	.487	.489	5.283	38	.000	3.121500	.590817	1.925454	4.317546
	Equal variances not assumed			5.283	37.173	.000	3.121500	.590817	1.924580	4.318420

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

T-Test of body weight compared between control and treated group at 5 months

Group Statistics

	1-control, 2-treated	N	Mean	Std. Deviation	Std. Error Mean
Body weight	1.00	20	14.4645	4.4603	.9974
	2.00	20	11.1625	3.1866	.7125

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Body weight	Equal variances assumed	1.571	.218	2.694	38	.010	3.3020	1.2257	.8206	5.7834
	Equal variances not assumed			2.694	34.387	.011	3.3020	1.2257	.8120	5.7920

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

T-Test of body weight compared between control and treated group at 6 months

Group Statistics

	1-control, 2-treated	N	Mean	Std. Deviation	Std. Error Mean
Body weight	1.00	20	22.9975	5.1540	1.1525
	2.00	20	14.3895	3.3081	.7397

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Body weight	Equal variances assumed	2.716	.108	6.286	38	.000	8.6080	1.3694	5.8357	11.3803
	Equal variances not assumed			6.286	32.383	.000	8.6080	1.3694	5.8198	11.3962

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

T-Test of liver weight compared between control and treated group at 4 months

Group Statistics

	1-control, 2-treated	N	Mean	Std. Deviation	Std. Error Mean
Liver weight	1.00	20	.172000	5.51171E-02	1.23246E-02
	2.00	20	.223500	8.07384E-02	1.80536E-02

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Liver weight	Equal variances assumed	2.249	.142	-2.356	38	.024	-5.150000E-02	2.18593E-02	-9.575184E-02	-7.248163E-03
	Equal variances not assumed			-2.356	33.549	.024	-5.150000E-02	2.18593E-02	-9.594544E-02	-7.054565E-03

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

T-Test of liver weight compared between control and treated group at 5 months

Group Statistics

	1-control, 2-treated	N	Mean	Std. Deviation	Std. Error Mean
Liver weight	1.00	20	.2400	8.240E-02	1.842E-02
	2.00	20	.3260	.1345	3.008E-02

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Liver weight	Equal variances assumed	3.911	.055	-2.438	38	.020	-8.6000E-02	3.528E-02	-.1574	-1.4587E-02
	Equal variances not assumed			-2.438	31.496	.021	-8.6000E-02	3.528E-02	-.1579	-1.4099E-02

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

T-Test of liver weight compared between control and treated group at 6 months

Group Statistics

	1-control, 2-treated	N	Mean	Std. Deviation	Std. Error Mean
Liver weight	1.00	20	.330000	9.07860E-02	2.03004E-02
	2.00	20	.448000	.131653	2.94386E-02

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Liver weight	Equal variances assumed	.895	.350	-3.300	38	.002	-.118000	3.57594E-02	-.190391	-4.560882E-02
	Equal variances not assumed			-3.300	33.737	.002	-.118000	3.57594E-02	-.190693	-4.530726E-02

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

T-Test of LSI compared between control and treated group at 4 months

Group Statistics

	1-control, 2-treated	N	Mean	Std. Deviation	Std. Error Mean
Liver somatic index	1	20	1.653705	.584996	.130809
	2	20	3.169680	.789069	.176441

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Liver somatic index	Equal variances assumed	4.980	.032	-6.902	38	.000	-1.515975	.219642	-1.960617	-1.071333
	Equal variances not assumed			-6.902	35.040	.000	-1.515975	.219642	-1.961853	-1.070097

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

T-Test of LSI compared between control and treated group at 5 months

Group Statistics

	1-control, 2-treated	N	Mean	Std. Deviation	Std. Error Mean
Liver somatic index	1.00	20	1.667700	.303604	6.78879E-02
	2.00	20	2.903710	.764497	.170947

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Liver somatic index	Equal variances assumed	6.466	.015	-6.720	38	.000	-1.236010	.183934	-1.608364	-.863656
	Equal variances not assumed			-6.720	24.848	.000	-1.236010	.183934	-1.614946	-.857074

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

T-Test of LSI compared between control and treated group at 6 months

Group Statistics

	1-control, 2-treated	N	Mean	Std. Deviation	Std. Error Mean
Liver somatic index	1.00	20	1.497490	.490456	.109669
	2.00	20	3.156485	.796517	.178107

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Liver somatic index	Equal variances assumed	1.938	.172	-7.932	38	.000	-1.658995	.209163	-2.082424	-1.235566
	Equal variances not assumed			-7.932	31.597	.000	-1.658995	.209163	-2.085260	-1.232730

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

BIOGRAPHY

Miss Sirinnapa Pumchae was born on the 8th of December 1978 in Nongbualamphu, Thailand. She graduated her bachelor's degree of science in biology from the Faculty of Science, Prince of Songkhla University in 2000. She continued her graduated study for a master's degree of science in zoology at the Chulalongkorn University in 2001.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย