CHAPTER II

LITERATURE REVIEW

Derris *Derris trifoliata* Lour.

Derris trifoliata Lour. is a member of family Leguminosae-Papilionatae, and its Thai common name is "*Thop thaep nam*". It is woody climber, usually not climbing up on tree but it is rather creeping on ground, 5-10 m long. *Derris trifoliata* is commonly found along the river in tidal forest and swampy ground which is not far from the sea coast. Its characteristics are dark brown to black stem and slender branches. *Leaves* imparipinnate, alternate; rachis including petiole, 10-15(-20) cm long, with 1-2 pairs of leaflets and a terminal ones; leaflets opposite; petiolules yellowish-brown, 3-10 by 1.5-5 cm, glabrous on both surfaces; apex acute to acuminate; base rounded to obtuse; secondary leaves, 8-10 pairs, faint but distinct on both surfaces. *Flowers* long. *Fruit* a flat pod, broadly oblong, oblique, with narrow winged-like ridges along both sutures, ca 3.5 by 3 cm; 1-seeded. *Seeds* kidney-shaped, 10-12 mm both ways. Flowering from May to August (Pengchray, 1991).

Chemical Composition of *Derris spp.* Extract

There are many groups of the chemical compound which were found in the *Derris spp.* extract such as 3-aryl-4-hydroxycoumarin, stilbene, isoflavone, flavonoid, aurones and auronol, steriod, triterpenoid, and other organic chemical compounds. The chemical composition of *Derris spp.* extract from the former studies are shown in Table 2.1

Table 2.1 The chemical composition of *Derris spp.* extract

Scientific Name	Parts of Plant	Chemical Compounds	Reference
D. amazonica	stem	1. (3S)-2′-O-methylvestitol	(Filho et. al., 1975a)
		2. β-sitosterol	
		3. lupeol	
		4. lupenone	
D. araripensis	root peak	1. methylenedioxy-(3′,4′)-5-hydroxy-6-methoxyfurano-(7,8,2″,3″)-flavanone	(Narcimento and Mors, 1981)
		2. methylenedioxy-(3′,4′)-5,6-dimethoxyfurano-(7,8,2″,3″)-flavanone	
		3. 3,4,5,6-tetramethoxyfurano-(7,8,2″,3″) -flavan	
		4. methylenedioxy-(3,4)-5′-hydroxy-2′,3′-methoxy-furano(3′,4′,2″,3″)-dihydrochalcone	
		5. 3,5,6-trimethoxyfurano-(7,8,2″,3″)-flavone	
		6. methylenedioxy-(3″,4″)-3,5,6-trimetoxyfurano-(7,8,2″,2″)-flavone	
		7. 3,5,6-trimethoxyfurano-(7,8,2″,3″)-flavanonol	
		8. methylenedioxy-(3,4)-3,6-dimethoxy-6″,6″-dimethylchromeno-(7,8,2″,3″)-flavone	
		9. 3,6-dimethoxy-6″,6″-dimethylchromeno-(7,8,2″,2″)-flavone	
D. elliptica	leaf	1. 2R,5R-dihydroxymethyl-3R,4R-dihydroxypyrrolidine	(Evan *et. al.*, 1985)

Table 2.1 (Continue)

Scientific Name	Parts of Plant	Chemical Compounds	Reference
D. floribunda	root	1. 3,5-dimethoxy-4-prenyl-stilbene 2. 3,5,4′-trimethoxy-4-prenylstibene 3. lonchocarpin 4. derricidin 5. 5,7-dihydroxy-6-prenyl-flavanone 6. 4-hydroxylonchocarpin	(Filho et. al., 1975b)
D. malaccensis	root	1. toxicarol 2. rotenone 3. elliptone 4. deguelin 5. malaccol 6. sumatrol	(Harper, 1940)
D. obtusa	root peak	1. methyldioxy-(3,4)-5′-hydroxy-2′-methoxy-furano-4′,3′,2″,3″)-chalcone 2. 5-hydroxy-6″,6″-dimethylchromeno-(7,8,2″,3″)-flavone 3. 3,6-dimethoxy-6″,6″-dimethylchromeno-(7,8,2″,3″)-flavone 4. furano-(6,7,2″,3″)-aurone 5. 4-hydroxyfurano-(6,7,2″,3″)-aurone	(Narcimento *et. al.*, 1976)

Table 2.1 (Continue)

Scientific Name	Parts of Plant	Chemical Compounds	Reference
		6. methylenedioxy-(3′,4′)-furano-(6,7,2″,3″)-aurone	(Narcimento *et. al.*, 1976)
		7. 4-methoxy-furano-(6,7,2″,3″)-aurone	
		8. derriobtusone A	
		9. derriobtusone B	
		10. β-sitosterol	
		11. heptocasanol	
D. rariflora	stem	1. 3,5-dimethxy-4-prenyl-stibebene	(Filho et. al., 1975a)
		2. 5,7-dihydoxy-6-prenyl-flavanone	(Filho et. al., 1975b)
		3. 5-hydroxy-7methoxy-6-prenylflavanone	
		4. β-sitosterol	
D. robusta	seed	1. derusnin	(Chibber and Sharma, 1979)
		2. robustone	
		3. robustone methyl ether	
		4. alpinumisolavone dimethyl ether	
		5. β-sitosterol	
		6. sitosterol- β-D-glucopy-ranoside	

Table 2.1 (Continue)

Scientific Name	Parts of Plant	Chemical Compounds	Reference
	inner seed	7. robustigenin	(Chibber and Sharma, 1979)
		8. robustigenin-5-O-methyl ether	
		9. derrugenin	
	root	10. derusnin	(Johnson and Peter, 1966)
		11. robustic acid	(East et. al., 1969)
		12. robustic acid methyl ether	
		13. robustin	
		14. robustin methyl ether	
		15. robustone	
		16. robustone methyl ether	
		17. derrubone	
		18. derrustone	
D. scandens	root	1. lonchocarpic acid	(Falshaw *et. al.*, 1969)
		2. scandenin	
		3. lonchocarpenin	
		4. warangalone	

Table 2.1 (Continue)

Scientific Name	Parts of Plant	Chemical Compounds	Reference
		5. chandalone	(Falshaw *et. al.*, 1969)
		6. scandinone	
		7. osajin	
D. sericea	root peak	1. lonchocarpin	(Nascimento and Mors, 1940)
		2. derricin	
		3. isolonchocarpin	
		4. derricidin	
		6. cerylic alcohol	
		7. carnaubylic alcohol	
D. spruceana	root and root peak	1. 3-methylenedioxy(3′,4′)-phenyl-4-hydroxy-5-methoxy-2″,2″-dimethyl-chromeno (5″,6″,7,8)-coumarin	(Garcia *et. al.*, 1986)
		2. 3-methylenedioxy(3′,4′)-phenyl-4,5,dimethoxy-2″,2″-dimethylchromeno(5″,6″,7,8)-coumarin	
		3. scandenin	
		4. 2,4-dimethoxy-2″,2″-dimethylchromene(5″,6″,3′,4′)-stilbene	
		5. 3′,4′-methylenedioxy-2″,2″ -dimethylchromeno(5″,6″,7,8)-isoflavone	
		6. β-sitosterol	

Table 2.1 (Continue)

Scientific Name	Parts of Plant	Chemical Compounds	Reference
D. trifoliata	leaf	1. rhamnetin3-O- β-neohesperidoside 2. quercetin3-O- β- neohesperidoside 3. campesterol 4. cholesterol 5. β-sitosterol 7. stigmasterol 8. stigmast-7-en-3- β-ol 9. β-amyrin 10. α-amyrin 11. lupeol 12. ceryl alcohol	(Rhamachandran and Seetharaman, 1986) (Sodachan, 1967) (Ghosh, 1985)
	root	13. dehydrorotenone 14. stigmasterol 15. lupeol 16. straight chain acid 17. hexacosanol	(Bose, 1976) (Sae Lim and Sutrummanukun, 1988)

Table 2.1 (Continue)

Scientific Name	Parts of Plant	Chemical Compounds	Reference
D. urucu	stem	1. rotenone 2. tephrosin 3. 12A-hydroxyrotenone 4. straight chain acid	(Filho et. al., 1975a)

Chemical Composition of *Derris trifoliata* Lour.

The chemical composition of the crude extract from *D. trifoliata* stem was studied by Sodachan (1967), the petroleum ether extract was composed of ceryl alcohol, lupeol, β-sitosterol, and stigmasterol. *D. trifoliata* root was extracted by petroleum ether, benzene, and ethanol, there were found dehydrorotenone, straight chain ketone, and lupeol respectively in the crude extract (Bose, Kirtaniya and Adityachaudhury, 1976).

Untawale *et. al.* (1978) measured the quantity of carbohydrate, protein, lipid, and organic matter in the leaves of esturine plant, they proposed that protein is the major composition of *D. trifoliata* leaf and the chemical compositions are vary along the season. Recently, the varying of the quantity of heavy metal in *D. trifoliata* leaves along the season was studied by Untawale, Wafar and Bhosle (1980), the heavy metal in which found were Fe, Mn, Cu, Ni, Co, and Pb. The quantity of heavy metal are varying along the season. In the rainy season Fe and Mn are the major composition of the heavy metal.

Ghosh *et. al.* (1985) extracted *D. trifoliata* leaves with the combination of the solvent (chloroform-methanol), the extract was composed of five types of steroid, campesterol, cholesterol, β-sitosterol, stigmasterol, stigmast-7-en-3β-ol and three types of triterpenopid, β-amyrin, α-amyrin, and lupeol. In the early year, Ramachandran and Seetharaman (1986) extracted *D. trifoliata* leaves by methanol and then the methanol extract were continued extracted by petroleum ether, ether, and ethyl acetate respectively. In the final fraction of ethyl acetate, found new compound "5,3',4'-trihydroxy-7-methoxy-3-O-β-D-(2-O-α-L-rhamnopyranosyl)-glucopyranosyl flavone" (rhamnetin 3-O-β-neohesperidoside) and 5,7,3',4'-tetrahydroxy-3-O-D-(2-O-α-L-rhamnopyranosyl) glucopyranosyl flavone (quercetin 3-O-β-neohesperidoside).

Moreover, the chemical composition of *D. trifoliata* root was studied by Sae Lim and Sutrummanukun (1988) and reported that the chloroform extract composed of hexacosanol, lupeol, stigmasterol, mixed matter of carboxylic acid and a compound which was decayed at 222 °C expected as the flavonoid compound, and the water

soluble fraction was found K^+, Na^+, Mg^+, Zn^{2+}, Fe^{2+}, Cl^-, amino acid, glucose, fructose, sucrose. Noppakundilograt (1991) also reported the chemical constituents of *D. trifoliata* root, fractionation of crude chloroform extract led to the isolation of nine compounds; sulfur; a mixture of unidentified esters; alcohol (C_{21}-C_{26}); carboxylic acids (C_{20}-C_{28}); lupeol; β-sitosterol; campesterol; stigmasterol; rotenone; 6a,12a-dehydro-α-toxicarol; and an unidentified compound obtain as yellow needles crystal m.p. 222 °C. And the chemical constituents of the essential oil from *D. trifoliata* root were identified. There were copaene, α-caryophyllene, phenyl acetonitrile, and dodecane which were identified.

Bioactivity of The *Derris trifoliata* Extraction

D. trifoliata is the mangrove plant and it can growing up among the abundant of insects and microorganisms which are cause of plant disease. Because some of the chemical constituents of *D. trifoliata* are the biotoxin which can defense the pest. The bioactivity of *D. trifoliata* extract had been widely studied.

De La Cruz *et. al* (1984) extracted roots, stems, leaves, and fruits with petroleum ether, water, and chloroform. The bioassay of the crude extract were tested against juvenile *Tilapia nilotica* (*Oreochromis niloticus*). The crude extract from roots, stem, and fruits were poisoned to all fish but the crude extract from leaves was not effected to the fish. For the fruit of *D. trifoliata* were continued study, the crude extract from fruit cover was non-toxic to the fish (milky sap) but the seed extract was poisoned to all of test fish. The anti-fungal property was studied by Udomsilp *et. al.* (1986), the ethanol crude extract from *D. trifoliata* root has an anti-fungal property for *Aerocylindrium oryzae, Fusarium moniliforme, Helminthosporium oryzae,* and *Pyricularia oryzae.*

After that, Noppakundilograt (1991) extracted roots, leaves, and stems of *D. trifoliata* with methanol, chloroform and water respectively, and also tested of the bioactivity of the extract. The extractions from chloroform fraction and water fraction were brought to bioassay testing. The chloroform and water extract from dry root can inhibit the growth of growing rice, and methanol and chloroform extract from fresh

leaves can inhibit the growth of growing rice too. The chloroform extract from fresh root and leaves can inhibit the growth of fungi, *Pythium ultimum* and *Rhizoctonia solani*. The leaf dipping method was tested of the toxicity on the third instar diamondback moth larvae. There were four parts of the extract, the chloroform and water extract from dry roots and methanol and chloroform from fresh leaves. The mortality percentage of the chloroform extract from dry root was 100% and for other parts the mortality percentage were only 0.8 %.

Moreover, Noppakundilograt (1991) performed the fish bioassay on *Gambrusia spp.* The bioassay experiments were conducted in 24 hours. The mortality percentage of chloroform extract from root was 100% of the concentration 50, 500, and 1500 mg/L. The water extract from root were 100% of the concentration 500, 2500, and 5000 mg/L. The chloroform extract from dry leaves was not effected to test fish in any concentration but in the water fraction was 5% mortality of the concentration 500, 2500, and 5000 mg/L. The chloroform extract from stem were 5, 50, 77% mortality of the concentration 50, 500, and 1500 mg/L. The water extract from stem, the concentration of 500, 2500 mg/L were not effected to test fish, the concentration of 5000 mg/L was 4% mortality.

Aquatic Ecosystem

The aquatic environment is complex and diverse. It includes several distinct ecosystem types (freshwater streams, lakes, ponds, rivers; estuaries, marine coastal, and deep ocean water) within many different biotic and abiotic components. The biotic or living components consist of many combinations of plants, animals, and microorganisms that inhabit specific ecological niches in each ecosystem. The abiotic or nonliving components include the physical environment within the boundaries of the ecosystem. Each aquatic ecosystem is thus a product of complex interactions of living and nonliving components. Since ecosystem involve complex interactions of physical, chemical, and biological factors. It is difficult to understand the response of the system to a chemical unless the relationship among components are well defined. Moreover, similar ecosystem are not necessarily affected in the same response by contamination of the same chemical. Minor difference in the physical, chemical

properties and biological composition can result difference in fate of a chemical and to different effects on the ecosystem (Rand and Petrocelli, 1985).

Aquatic Toxicology

Aquatic Toxicology is the qualitative and quantitative study of the adverse or toxic effects of chemicals and other anthropogenic materials or xenobiotics on aquatic organisms. Toxic effects may include both lethality and sub-lethal effects such as changes in growth, development, reproduction, phamacokinetic responses, pathology, biochemistry, physiology, and behavior. The toxicants enter aquatic ecosystem from (1) non-point sources such as agricultural runoff from land, contaminated ground water and bottom sediments, urban runoff, and atmospheric fallout, and (2) point sources such as discharges (effluents) from manufacturing plants, hazardous waste disposal sites, and municipal waste water treatment plants. The most innocuous chemical substances can have undesirable or distinctly harmful effects when taken up by an organism in sufficient amounts. In contrast, if minute quantities of toxic chemicals are uptake, it can result in no apparent adverse effect. Therefore, it is an important concept in toxicology that in general "no chemical is completely safe and no chemical is completely harmful" (Rand and Petrocelli, 1985). The factor that determines whether a chemical agent is potentially harmful or safe is the relationship between the concentration and the duration of exposure. In the aquatic environment the concentration, transport, transformation, and disposition of a chemical are primarily controlled by (1) the physical and chemical properties of the chemical compound, (2) the physical, chemical and biological properties of the ecosystem, (3) the sources and input rate of the chemical into the environment.

The major reason for carrying out toxicity tests with fish and other aquatic organisms is to determine which concentrations of a substance are harmful to the organisms and which have no apparent effect. The endpoints that have been considered in tests to determine the adverse effects of toxicants, including death and survival rate, decrease in reproduction and growth, locomotor activity, blood chemistry, and histopathology. From the results, a toxicologist can recommend maximum concentration for the well-being of aquatic organisms, engineers can design

treatment systems to achieve desired levels, and fisheries manager can evaluate chemical measurements in local bodies of water (Sprague, 1990).

Acute lethality is an obvious and easily observed effect, which is widely used for evaluate of the chemical toxicity in the early period. The results of these tests are usually expressed as the concentration, which is 50 % lethality (LC_{50}) of test organism during the particular time. The sub-lethal toxicity test is longer exposure period covering partial or complete life cycle of the test organism. It will be provided more observing subtle effects of the toxicants, such as reduction in growth and reproduction, and given more accurate to estimate the threshold and safe concentration.

All the data provided by toxicity potential is used for determine compliance with permit toxicity limits, to aid in the development and implementation of toxicity reduction plans, and for risk assessment. Furthermore, the data of toxicity test may be assembled to derive water quality criteria, to monitor the toxicity of chemicals and or effluents, and to evaluate the quality of surface waters. Using fish to assess the quality of an effluent is an economical and meaningful procedure, especially if many waste substances are present of if it is not known exactly what is present. Such tests could involve monitoring a cage of fish placed in a river below an industrial outfall or periodic standardized toxicity tests of an chemical or effluent (USEPA, 1994).

Nile tilapia *Oreochromis niloticus*

Nile tilapia were introduced into Thailand by Prince Arkihito of Japan at March 25, 1965. Nile tilapia were given for His Majesty King Bhumibol Adulyadej, to culture in Thailand. His Majesty, The King, called Nile tilapia as "*Pla nil*" in thai common name and gave 10,000 Nile tilapia to Department of Fisheries for fish culture, at 17 March, 1966 (Tungtrongpiroj *et al.*, 1993).

Taxonomy

Phylum	Vertebrata
Subphylum	Craniata
Superclass	Gnathostomata
Series	Pisces
Class	Teleostomi
Subclass	Actinopterygii
Order	Perciformes
Suborder	Labroidei
Family	Cichlidae
Genus	Oreochromis
Species	niloticus

Scientific name : *Oreochromis niloticus*

Synonyms / misidentifications : *Tilapia nilotica*

Common name : Nile tilapia

Thai common name : Pla Nil

Habitat and Biogeography : Nile tilapia is the native fish of Syria and Palestine, widely distribute on Africa. The fish occurs in freshwater, it is increasingly found in brackish water elsewhere by stocking pools and ponds. The fishes are found in many rivers and wetland areas in Sudan, Uganda. Nile tilapia were introduced into America and Asia for fish culture because it very well adapt to various environment and good reproductive performance. Nile tilapia can live in any freshwater resource or along the beach which the salinity are 20 ppt, it can tolerance the changes of temperature between 12 - 41 °C (Duangswasdi and Pupipat, 1982).

Biology and Characteristic : Nile tilapia have one nostril on each side of the snout. Body fairly elongate, moderately deep, greatly compressed. Dorsal and ventral profiles about equally convex. Caudal peduncle broadly short. Mouth slightly oblique, protractile broad and with swollen lips. Dorsal fin with long base, spinous dorsal fin with 16-17 spinous finrays, followed by 11-15 soft finrays of posterior soft dorsal fin. Anal fin relative short, consisting of 3 spinous finrays and 8-11 soft finrays. Posterior

part of both fins, especially in adult male fishes, usually and considerably extended. Pectoral fins moderately large and pointed, with 15 soft finrays. pelvic fins thoracic. Caudal fin broadly rounded in adult, but almost truncate in young. Scale fairly large, cycloid. Upper leteral line extending from upper corner of operculum to vertically below soft dorsal fin, with 19-25 scales while the lower portion which begins perpendicularly after and transposed to lower level at middle of caudal peduncle with 11-18 scales, totally consisting of 31-35 scales (Wongratana, 1996).

The reasons of selecting Nile tilapia *Oreochromis niloticus* as test organism in this study as follows ;

1. Widely available and abundant in Thailand
2. Good represent of the aquatic ecosystem that may receive the impact
3. Ecological and commercial importance
4. Amenable to routine maintenance and available techniques for culturing and rearing in the laboratory
5. Adequate background information

Fish Liver

The sensitivity of a species in responding to a range of contaminants is an important determinants for its usefulness as a bioindicator. Within individuals of a bioindicator species, careful analysis will reveal biomarkers as alterations in structure and function of specific organs, tissues and cells as a consequence of prior exposure to contaminants.

The liver of vertebrates not only represents an organ central to numerous vital functions in basic metabolism, but it is also a major site of accumulation, biotransformation and excretion of xenobiotic compounds. Thus, hepatocytes may be expected to be primary targets of toxic lesions. Selection of liver cells as appropriate targets should therefore provide an opportunity for detection of suitable biomarkers of environmental pollution. It is essential to realize that any physiological and biochemical alteration, if severe enough and protracted, will eventually result in

morphological effects, or, *vice versa*, structural modifications will ultimately be followed by functional consequences. Ultrastructural alterations of fish hepatocytes have repeatedly been used as monitor systems or sublethal effects of organic contaminants. However, the biomarker concept has specially been applied to particular hepatocellular changes (Braunbeck and Völkl, 1991).

Histological Alteration as a result of toxic injury : The most frequently encountered types of degenerative changes are those of ;

- hydropic degeneration
- cloudy swelling
- vacuolation
- focal necrosis
- pyknosis, karyorrhexis and karyolysis
- fatty degenerative changes
- zonal, massive and pericentral necrosis
- cirrhosis
- malignant hepatoma
- laminar or subcapsular necrosis

In general hepatocyte swelling, pyknosis of nuclei, and cytoplasmic vacuolation are also commonly found in toxic conditions. Acute and extensive necrosis of hepatocytes may occur in toxic conditions but focal necrosis is more common. Zonal necrosis of fish liver is difficult to ascertain because the fish liver is very much more diffuse (Roberts, 1978). Zonal and massive necrosis are rarely observed in the fish liver. However, focal hepatic necrosis is a regular lesion in which cause by the virus disease of salmonids and channel catfish. Pericentral necrosis has been reported in trout and catfish which received relatively high does of CCl_4 or MCB (Gingerich *et al.*, 1977) The diffuse focal necrosis is commonly observed in CCl_4 acute toxicity studies and subcapsular necrosis is also found (Gingerich *et al.*, 1977).

Histopathological of fatty degenerative, extremes cases of the hepatocytes are shown distention of every liver cell by a single large globule of fat and, where there is breakdown of liver cell membrane, macrophage invasion with ceroid or lipofuscin deposition also occurs (Roberts, 1978). The accumulation of lipophilic vacuoles had

been observed in a number of fish species following experimental intoxication with polychlorinated biphenyls (Hacking *et al.*, 1978). And the lipophilic vacuoles were also found in mammals of the PCB toxicational experiment (Fishbein, 1974).

Cirrhosis is the proliferative of chronic hepatic inflammation characterized by replacement of parenchyma by new fibrous connective tissue generally beginning at the portal triads (Rand and Petrocelli, 1985). It may be seen occasionally in older wild fish but the most dramatic cirrhosis found in fish is the peribiliary cirrhosis of the hepatorenal syndrome of farmed marine flat fish. This condition is associated with dictary toxicity (Roberts, 1978). A very distinctive hepatotoxin, alfatoxin, derived from *Aspergillus flavus* growing on oil seeds of the diet, produces a malignant hepatoma.

Histopathological alteration in the liver of freshwater teleost *Tilapia mossambica* in response to cadmium toxicity was studied by Rani and Ramamurthi (1989), they found the LC_{50} at 48-hour was 50 ppm, and 5 ppm as sublethal concentration of $CdCl_2$. The observations of *Tilapia mossambica* liver were engorged blood vessels, vacuolar degeneration of the hepatocytes, and fatty change in the peripancreatic hepatocytes.

Ultrastructure Alterations of Fish Liver : Klaunig et. al. (1979) studied the response of the chanel catfish liver to subacute 21 days exposure of PCB. The hepatocytes cytoplasm revealed, glycogen rich areas of variable size often associated with a single lipid droplet, RER in parallel arrays filling a major portion of cytoplasm, and numerous mitochondria. SER was restricted to glycogen rich areas. Three patterns of alteration were seen (1) increase in tubular SER within glycogen rich areas, (2) parallel stacks of SER which were continuous with RER on one or both ends, (3) membranous whorls contained multiple layers of smooth membrane separated by cytoplasm which often contained glycogen rosettes.

Braunbeck and Völkl (1991) studied the structural and functional alterarions in hepatocytes of golden ide *Leuciscus idus melanotus*, following a 4 weeks exposure to 5, 50, and 250 µg/L DNOC. The ultrastructural reaction was charecterized by deformation and dilatation of nuclear envelope, augmentation of nucleoli, deformation and proliferation of mitochondria, peroxisomes and lysosomes, lipid accumulation,

reduction and fenestration of RER, proliferation of SER, degeneration of golgi bodies, collagen accumulation in the space of Disse.