CHAPTER V

DISCUSSION

Toxicity of *D. trifoliata* leaves

From this study, the 96-h LC_{50} of dichloromethane crude extract from *D. trifoliata* leaves was 1.99 mg/L that are lower than the LC_{50} value of the crude extract from other parts of this plant. Wattanasirmkit *et. al.*(1997) were reported the LC_{50} value of the ethanol crude extract from *D. trifoliata* leaves as 4.41 mg/L and Wattanasirmkit and Patamastan (1997) were also reported the LC_{50} value of the ethanol crude extract from *D. trifoliata* roots as 6.5 mg/L. A direct comparison of acute toxicity values in fish between a test to other tests is rather difficult. Since activities that affect the quality of the final toxicity data, such as (1) the source and condition of test organisms (2) condition and operation of equipment (3) solvent and extraction agent (4) test condition; temperature, DO, pH, food, dilution water quality.

However, the LC_{50} values of the bioassay tests could vary from test to test and depended on the differences in range of concentrations and sensitivity response of test organisms. The toxicity value of crude extract in this study was more effective, that may the effects of the extraction procedure and solvent agents. The dichloromethane crude extract is the secound fraction, it may contained more concentrate of the bioactive compound than the first fraction (ethanol crude extract). Since the crude is composed of many chemical compounds. The bioactivity to test fish of the crude extract may be the results of synergistic reaction.

However, all death fish in the experiments showed the same series of signs and symptoms; swam at the surface; pectoral fins anteriorly extended; partial loss of equilibrium; light pigmentation; excessive mucus; gill hemorrhage; and reduce respiration. The toxic compound may be acutely effected to the gill.

Symptoms and Gross anatomy of Nile tilapia

In 1st and 2nd month of experiment, all groups did not show any stress symptoms. But in the 4th month, some test fish showed the stress symptoms; swam at the surface; pectoral fins anteriorly extended; dark pigmentation; and reduce respiration. Especially in the last month, most of treated fish showed the same series of signs and symptoms. In other way, control fish did not show the stress symptom but they were much more intra aquarium competition from their highly growth rate.

Only treated liver displayed the distinctive lesion which indicate of liver injury : swelling, subcapsular hemorrhage and cyst forming. The long-term exposure to *D. trifoliata* leaves extract induce hepatic damage and disturb the life activity.

Light Microscopic and Ultrastructural Changes of Nile tilapia liver

The early stage of cellular injury; hydropic swelling; blood congestion and mild focal necrosis are seen in 1st month. From histochmistry study, the lipid deposition in the treated liver was very dense, the glycogen accumulation was similar in all groups. The ultrastructure change was observed easier than light microscope study. Mitochondria is the most effected organelle, which showed the condensation of metrix.

But in the secound month, the hydropic swelling, fatty degenerate and blood congestion were similar to sublethal toxicity of $CdCl_2$ (Rani and Ramamurthi, 1989). The focal necrosis of hepatocytes was noticed. Roberts (1978) reported that focal necrosis is commonly found in fish liver. Moreover, 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; Gingerich and Dalich, 1978) The diffuse focal necrosis is commonly observed in CCl_4 acute toxicity studies and subcapsular necrosis is also found (Gingerich *et al.*, 1978). The additional lesions which were found in this month was the hemorhagic inflamation, there were many erythrocytes inthe area of tissue damaged and the invasion of macrophage. Lipid deposition was very dense in treated liver. For the ultrastructure changes, proliferation of RER, swelling and rupture of mitochondria were the additional lesion which were found in this month.

In 3rd month treated fish, there were large area of necrosis tissue and infiltration of macrophages more than the previous month. Glycogen depostion in treated liver was less than control group, but lipid accumulation in treated liver was very dense. The depletion of glycogen were corresponding to significant reduction in the growth. Moreover, feeding rate in treatment group were decreased. For the ultrastructure changes, the parallel array of RER from 2nd month was changed to be fragmented RER scatter throughout cytoplasm.

The additional lesion in 4th month was the fibroplasia. It is indicated of the regeneration of the liver tissue. The chronic hepatic inflammation was characterized by the replacement of parenchyma with new fibrous connective tissue (Rand and Petrocelli, 1985). It may be seen occasionally in older wild fish but the most dramatic cirrhosis found in fish was the peribiliary cirrhosis of the hepatorenal syndrome of farmed marine flat fish. This condition was associated with dictary toxicity (Roberts, 1978). There were the depletion of glycogen in damage area. For the ultrastructure observation, the fragmented RER shifted to be SER were found. Braunbeck and Völkl (1991) suggested that the proliferation of SER by a relative shift from RER to SER, degranulation, reorganization and disintegration of the RER, and induction of peculiar ER changes were a set of sensitive cytological biomakers of organic contaminates.

The liver tissue showed the regeneration in response of chronic hepatic inflammation. The fibroplasia was found in many specimens of 5th month treated fish more than the previous month. The depleation of glycogen on damage area and accumulation of lipid were found. The ultrastructure changes were same as the previous month but more lipid droplets accumulation were seen.

Effects on Growth and Relative Liver Weight Index

Length and weight of treated fish in the first and the second month were not significant difference because the effect of the extract might not be express to reduce the growth. After that, in the third months the growth were significantly decreased. The growth of fish were related to feed quality, feed rate, feed conversion ratio and water quality. Since the factors that affected to the growth were the feed stuff, the

physical factors and water quality in all aquariums whuch were in the same condition of all experimental units and the competitive relation of intra aquarium were equalized by the loading factor. Therefore, the difference in growth among the experimental groups should be only the effect of toxicity potential of the dichloromethane crude extract from *D. trifoliata* leaves.

At 4th month, the relative liver weight index (%R) of treatment group was higher than both of control groups ($p \leq 0.05$). It indicated that the treated liver was inflamed. In the case of 5th month of experiment, the treated relative liver weight index stilled higher than the control groups but were not significant difference. There was not significant difference between 4th and 5th month of the treatment group which indicated that the treated livers are stilled inflammation. Normally, the relative liver weight ratio (%R) of common fish should be 1-2 % (Roberts, 1978). In this study, all the ratio are over than the standard ratio. It seem to be the species difference, the fed condition and stress from intensive stocking in laboratory condition.

Physical factors and water quality

In the experiment, physical factors (temperature and light period) and water quality parameters (DO, pH, hardness) were the same value in all experiment units. DO is the most important water quality parameter. Aeration during the experimental might alter the results and could reduce the apparent toxicity of the test solutions by stripping them of highly volatile toxic substances, or increased its toxicity by altering the pH. However, the DO in test solution must not be permitted to fall below 4.0 mg/L. Increasing in pH might occur in test solutions during the experiment from ammonia waste. And these problems can be avoided by replace the test dilution every 72 hours all over the experimental period.

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