

## EFFECTS OF MENTHA PIPERITA (L.) EXTRACT MIXED DIET ON HEPATIC HISTOLOGICAL ALTERATION AND DISEASE RESISTANCE AGAINST AEROMONAS HYDROPHILA INFECTION IN ROHU (LABEO ROHITA HAM.)

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**Summary:** Rohu (*Labeo rohita* Ham.) fingerlings with average weight of  $35\pm 2$ g were randomly divided into four groups (13 fish/tank in duplicate) viz., the control (fed on normal diet) and three treated groups namely  $T_1$ ,  $T_2$  and  $T_3$  treatments fed on *Mentha piperita* (L.) extract mixed diet with varying doses i.e. 10, 20 and 30 ml/100 g feed, respectively. After three weeks of treatment, fish from all the groups were sampled (3 fish/tank) for hepatic histological examination. Then, all the fish groups were immunized intraperitoneally (i.p.) with a dose of  $10^6$  cells/fish of formalin-killed *Aeromonas hydrophila* suspension. After two weeks of immunization, all the treatments were challenged (i.p) with a dose of  $10^6$  cells/fish of live *A. hydrophila* suspension. After challenge, all the fish treatments were fed on the same normal diet and observed daily cumulative mortality. Moribund infected fish and survival fish after two weeks of challenge were sampled for histopathological examination. The results indicated that there was no histological alteration in liver tissues of *M. piperita* (L.) extract treated fish. However, *A. hydrophila* infected fish showed histopathological alteration such as degenerative hepatocytes and congestion of liver tissues. Disease resistance against *A. hydrophila* increased in the *M. piperita* (L.) extract treated groups with the highest in the  $T_3$  treated group.

### I. INTRODUCTION

Fish histopathology is used as a tool for aquatic toxicology (Simpson et al, 1989). Histopathological alterations in liver may be useful indicators of arsenic (As) toxicity in environmental monitoring programs that also measure As concentrations in those tissues. The nuclear, architectural and structural alterations were observed in liver of adult lake whitefish fed with As contaminated diets (Pedlar et al., 2002). Recently, the use of immunostimulants was introduced as a prophylactic measure (Mulero et al., 1998) in aquaculture. Since such uses have so far not shown any of the negative side effects that antibiotics and live vaccines may have on the fish and on the environment, they are an attractive alternative way of controlling bacterial infections (Siwicki et al., 1994; Mulero et al., 1998).

Present study was carried out to estimate possible hepatic histological alterations and disease resistance against *A. hydrophila* infection in Rohu fingerlings (*Labeo rohita* Ham.) fed on *Mentha piperita* (L.) extract mixed diet.

## II. MATERIALS AND METHODS

Rohu (*Labeo rohita* Ham.) with similar weight ( $35\pm 2$ g) were randomly divided into four groups (13 fish each in duplicate) viz., the control group (fed on normal diet) and three treated groups namely T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups fed on *Mentha piperita* (L.) extract mixed diet with varying doses i.e. 10, 20 and 30 ml/100 g feed, respectively. After three weeks of treatment, three fish from each group were sampled for hepatic histopathological examination. Then, all the fish groups were immunized intraperitoneally (*i.p.*) with a dose of  $10^6$  cells/fish of formalin-killed *Aeromonas hydrophila* suspension. After two weeks of immunization, all the groups were challenged (*i.p.*) with a dose of  $10^6$  cells/fish of live *A. hydrophila* suspension. After challenge, all the fish groups were fed on the same normal diet and observed daily mortality. Moribund fish after challenge and survival fish after two weeks of challenge were sampled for the hepatic histopathological examination.

Histological studies were carried out following the procedure of Roberts (2001). Prepared sections were stained in haematoxylin (H) and eosin (E), observed under high power (40 and 100 X) on the microscope.

Relative percent survival (RPS), considered as the degree of protection against experimental *A. hydrophila* infection of fish, was estimated by following formula: RPS =

$$\left(1 - \frac{T}{N}\right) \times 100 \text{ (Logambal et al, 2000)}$$

Where: T: Percent mortality of test groups

N: Percent mortality of normal saline injected control group

## III. RESULTS AND DISCUSSION

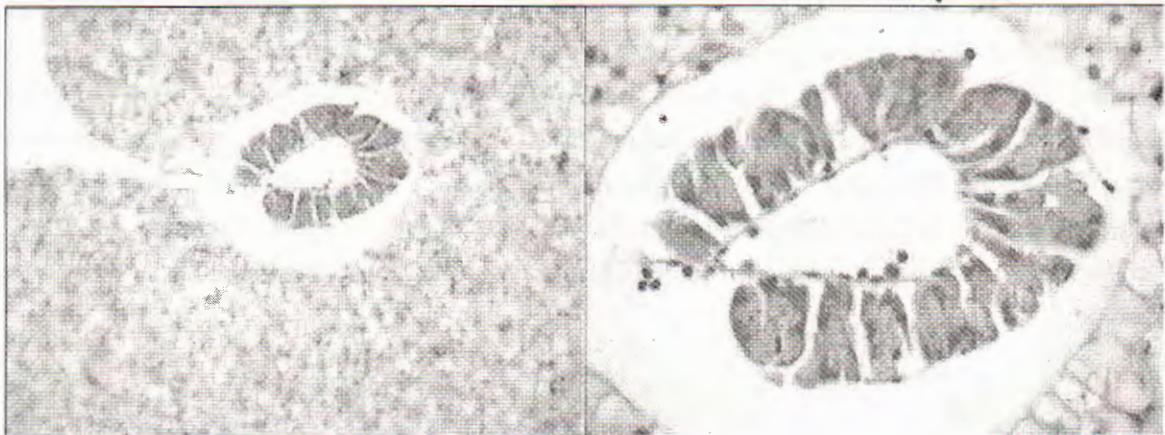
### 1. Histological study

#### a. After three weeks of treatment

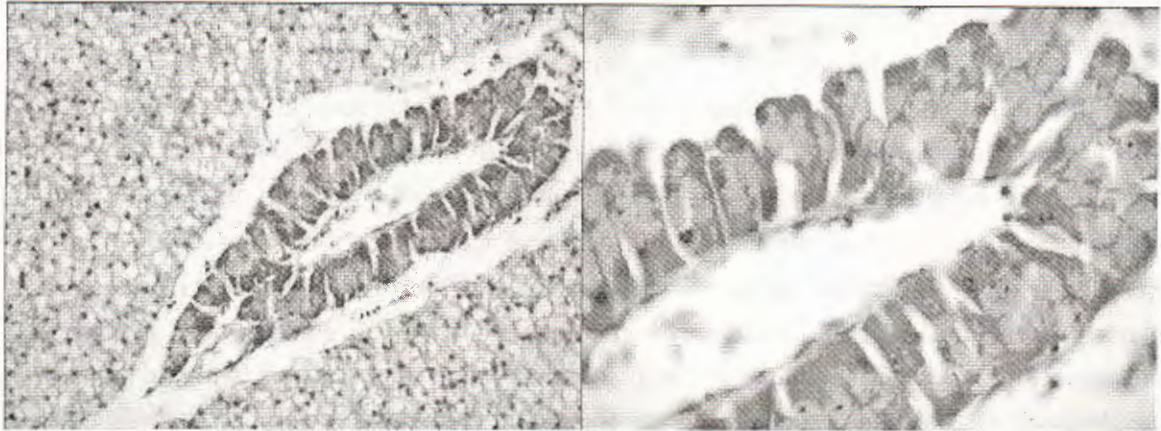
After three weeks of treatment with the *Mentha piperita* (L.) extract mixed diet, the histological appearance of liver tissues of the control and three treated groups has been depicted in the Figures 1 and 2. The results showed that there was no histological alteration in the liver tissues of the treated fish compared to those of the control.



However, hepatocellular vacuolation with varying sizes was observed in both the control and treated fish liver tissues (Figures 1 and 2). The hepatopancreas in the liver tissues of the *M. piperita* (L.) treated fish showed normal histological appearance with mild vacuolation of the hepatic cells (Figure 2). Fish liver is composed of parenchymal cells namely hepatocytes and lattice fibres. Hepatic cells play an important role in protein, lipid and carbohydrate metabolism and detoxification. Moreover, they are involved in hematopoiesis during larval life and antibody production (Hibiya, 1982). In the present study, after three weeks of treatment with the plant extract mixed diet, the structure of liver tissues of both control and treated fish showed identical histological appearance. Neither there was any hypertrophy nor any hyperplastic changes in the liver tissues of the three treated groups. The results indicated that the plant extract mixed diet did not affect the normal functionalities of the important organ of the treated fish. This confirmed that the extract was well accepted in the feed although it might not have helped in the growth rate. However, as per the histopathological results, vacuolation of the hepatocytes with varying sizes from mild to moderate in the liver tissues of both the control and plant extract treated fish was an usual finding in the normal condition i.e. before challenge with live bacteria. The results seems in agreement with Pritchard et al. (1996), who observed hepatocellular vacuolation with varying sizes from little to extreme in normal common carp livers.



**Figure1.** Hepatopancreas showing normal histological appearance with mild vacuolation of hepatic cells in control fish (H & E, left. 40 X and right. 100 X)



**Figure2.** Hepatopancreas showing normal histological appearance with mild vacuolation of hepatic cells in *M. piperita* (L.) treated T<sub>3</sub> group (H & E, left. 40 X and right. 100 X)

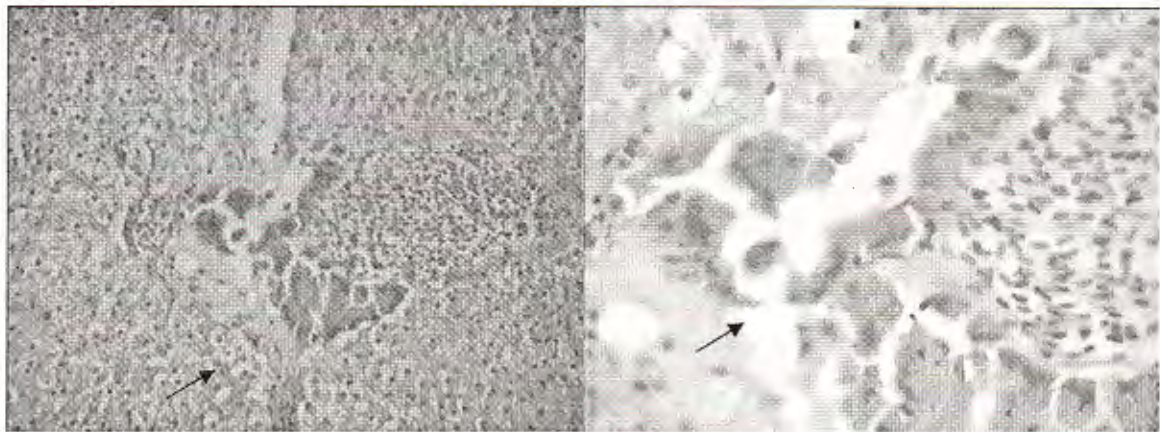
***b. After challenge with A. hydrophila***

After challenge with live *A. hydrophila* suspension, the histological appearance of liver tissues of the moribund fish sampled from the control and treated groups has been depicted in the Figures 3 and 4. The liver tissues in *A. hydrophila* infected fish showed identical histopathological changes. In case of severe infection, the liver tissues exhibited severe congestion and multiple granulomatous lesions. Focal necrosis of the hepatocytes and pancreatic arcinar cells were also observed in some places of the liver tissues. Liver tissues in *A. hydrophila* infected control fish showed multiple granulomatous lesions with necrotic mass surrounded by fibrous tissue capsule (Figures 3 and 4). Focal necrosis of hepatocytes is always associated with dilation of sinusoidal spaces in the liver tissue of rohu fingerlings infected with *A. hydrophila* (Das and Mukherjee, 1998). Liver enlargement and hypertrophy of the hepatocytes was observed in the Japanese flounder, *Paralichthys olivaceus* infected with *E. tarda* (Miwa and Mano, 2000).





**Figure3.** Liver tissues showing granulomatous lesions (arrow) in *A. hydrophila* infected control fish (H & E, left. 40 X and right. 100 X)



**Figure4.** Liver tissues showing congestion and marked cellular infiltration around pancreatic cells (arrow) in *A. hydrophila* infected *M. piperita* (L) treated T<sub>3</sub> group (H & E, left. 40 X and right. 100 X)

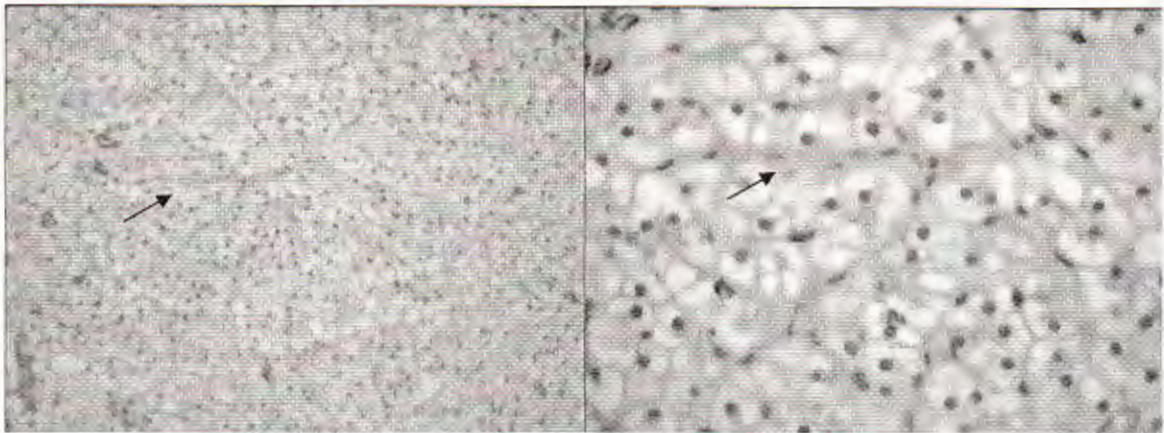
*c. After two weeks of challenge*

After two weeks of challenge with live *A. hydrophila* suspension, the histological appearance of liver tissues of the fish those survived has been depicted in the Figures 5 and 6. In the liver tissues, cord-like structures constituted by fibrous tissues were frequently seen in the hepatocytes of both the control and treated survivors.

Information on the regenerative capacity of damaged pancreatic arcinar tissue in fish is limited (McLoughlin et al., 1995). However, it is well known that the liver of the mouse or rat is capable of rapid restoration if part of it has been damaged by any physical or biological assault. In eel, carbon-tetraoxide causes widespread necrosis in the hepatic cells about one week after injection, but regeneration begins 4 or 5 days later, and is complete



at the end of about one month. The cord-like structures are characteristic of this regeneration. However, they remain incomplete and are rather glandular in appearance (Hibiya, 1982). In the present study, after two weeks of challenge, cord-like structures was frequently seen in the hepatic parenchyma of the survivors, which were sampled from all the groups. The results indicated the regeneration of liver tissues in the survivors. However, hemolysed blood was observed frequently in the liver tissues. The results suggested that the survived fish were not completely returning to normal condition but were in the process of recovery.



**Figure 5.** Liver parenchyma showing mild vacuolation and cord-like structures (arrow) in survival control fish after two weeks of challenge with *A. hydrophila* (H & E, left. 40 X and right. 100 X)



**Figure6.** Liver parenchyma showing mild vacuolation and cord-like structures (arrow) in survival *M. piperita* (L.) treated fish after two weeks of challenge with *A. hydrophila* (H & E, left. 40 X and right. 100 X)

## 2. Challenge study

Disease resistance of the experimental fish was showed in Table 1. The higher relative percent survival (RPS) to *A. hydrophila* in the treated groups compared to that in the control group indicated that the *M. piperita* (L.) extract had positive effect on the disease resistance to *A. hydrophila* infection. Enhanced nonspecific immune responses and resistance to disease were found in rainbow trout *O. mykiss* fed a  $\beta$ -glucan product and *S. cerevisiae* (Siwicki et al,1994). Practically, oral application of immunostimulants appears to be the route of choice in aquaculture (Sahoo and Mukherjee, 1999; 2001; Philip et al., 2001). The mechanisms of the metabolism of immunostimulants in the gastrointestinal tract are not known. However, the intestinal uptake and organ distribution of a similar form of  $\beta$ -glucan has been found in Atlantic salmon *Salmo salar* (Sveinbjornsson et al., 1995). It is probable that undigested and possibly digested  $\beta$ -glucans would be processed by mononuclear phagocyte system and thus stimulates an immune response (Duncan and Klesius, 1996).

**Table1.** Relative percent survival of Rohu (*Labeo rohita* Ham.) after challenge with *A. hydrophila*

Groups	Number of fish	Daily mortality of fish after challenge							% RPS
		1	2	3	4	5	6	7	
N	10 x2	8	6	0	0	0	0	0	-
Control	10 x2	4	6	0	0	0	0	0	28.57
T <sub>1</sub>	10 x2	1	4	0	3	0	0	0	42.86
T <sub>2</sub>	10 x2	2	2	0	4	0	0	0	42.86
T <sub>3</sub>	10 x2	2	2	0	2	0	0	0	57.14

N: Normal saline injected fish

## IV. CONCLUSIONS

The results indicated that there was no histological alteration in liver tissues of *M. piperita* (L.) extract treated fish. However, *A. hydrophila* infected fish showed histopathological alteration such as degenerative hepatocytes and congestion of liver tissues. Disease resistance against *A. hydrophila* increased in the *M. piperita* (L.) extract treated groups with the highest in the T<sub>3</sub> treated group.



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# ẢNH HƯỞNG CỦA THỨC ĂN CHỨA DỊCH CHIẾT CÂY BẠC HÀ MENTHA PIPERITA (L.) LÊN CẤU TRÚC TẾ BÀO GAN VÀ SỨC KHÁNG KHUẨN AEROMONAS HYDROPHILA Ở CÁ ROHU (LABEO ROHITA HAM.)

NGUYỄN THỊ THANH THỦY

**Tóm tắt:** Cá Rohu (còn gọi là cá Trời Ấn Độ) với khối lượng trung bình  $35 \pm 2g$  được chia ngẫu nhiên làm 4 lô riêng biệt (13 cá thể/bể, với hai lần lặp lại), lô đối chứng (ăn thức ăn bình thường) và ba lô xử lý  $T_1$ ,  $T_2$  và  $T_3$  ăn thức ăn có chứa dịch chiết cây Bạc Hà *Mentha piperita* (L.) với các nồng độ khác nhau 10, 20 và 30 ml/100g thức ăn. Sau 3 tuần xử lý, tất cả các lô cá đều được thu mẫu gan (3 cá/bể) để làm tiêu bản kiểm tra sự biến đổi cấu trúc tế bào. Sau đó tất cả các lô cá được tiêm chủng dưới màng bụng một liều  $10^6$  tế bào/cá dung dịch vi khuẩn *Aeromonas hydrophila* đã qua xử lý bằng formol. Sau hai tuần tiêm chủng, tất cả các lô cá được gây cảm nhiễm với một liều  $10^6$  tế bào/cá dung dịch vi khuẩn *Aeromonas hydrophila* sống. Sau khi gây cảm nhiễm, tất cả các lô cá đều được ăn thức ăn bình thường và theo dõi tỷ lệ tử vong lũy tích hàng ngày. Những cá thể bị nhiễm sắp chết và những cá còn sống sót sau hai tuần cảm nhiễm được thu mẫu gan để làm tiêu bản kiểm tra sự biến đổi cấu trúc tế bào. Kết quả cho thấy không có sự biến đổi về cấu trúc tế bào ở những lô cá được ăn thức ăn có chứa dịch chiết cây Bạc Hà *Mentha piperita* (L.). Tuy nhiên, những cá thể bị nhiễm khuẩn *A. hydrophila* đều có sự biến đổi cấu trúc tế bào gan như tụ máu và suy thoái tế bào gan. Sức kháng khuẩn *A. hydrophila* ở những lô cá xử lý dịch chiết cây Bạc Hà *M. piperita* (L.) đều tăng so với lô đối chứng và tăng rõ nhất ở lô xử lý  $T_3$ .

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