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European Journal of Experimental Biology, 2013, 3(3):572-575

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Histological changes of the lymphatic organs and white blood cell count following formaldehyde administration in the rainbow trout

Ali Louei Monfared[1*], Sahar Hamoon Naward[2], Zahra Bakhteyari[2], Hajar Azizian[3] and Sara Rahimi[3]

[1]*Department of Anatomy, Faculty of Para-Veterinary Medicine, University of Ilam, Ilam, Iran*
[2]*Department of Basic Sciences, Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran*
[3]*Faculty of Veterinary Medicine, University of Shahid Chamran Ahwaz, Ahwaz, Iran*

ABSTRACT

Formaldehyde (FA) is a major source of environmental pollution. FA has been wide applications in the various industries, hospitals and research centers. The aim of present study was to characterize the potential toxicity of formaldehyde of the lymphatic organs and white blood cell count in the rainbow trout after one month exposure.80 O. mykiss were randomly divided into four groups (n=20). Control group was kept in water without any add-on material, while experimental groups were exposed to concentration of 25, 50 and 100 mg/L of FA solution, respectively, for a month. At the end of the administration period, heparinzed blood samples were drawn by cardiac puncture and total white blood cells were counted. Tissue specimens were taken for histological evaluation and prepared sections were stained with Hematoxylin–Eosin (H&E). In the fish treated with formaldehyde; there was a significant reduction in the number of white blood cells count. The histological structure of the spleen of fish treated with formaldehyde revealed histological changes include: increasing in the thickness of the capsule, significant reduction in the lymphoid cell population and increasing in the number of macrophages as well as megakaryocytes in the spleen tissue when compared with controls. In addition, in the experimental groups necrosis and vacuolization of the skin cells in the different layers, impaired regulation of cell keratinocytes and cell dysplasia was seen in the basal layer of the skin. According to these results formaldehyde should be considered as an environmental hazard to fish especially immunosuppressive threaten and its releasing to water supplies would be prevented.

Keywords: Histology, formaldehyde, fish, spleen, skin.

INTRODUCTION

Formaldehyde (CH_2O) is a flammable colorless reactive gas, readily polymerized at normal room temperature and pressure, with a relative molecular mass of 30.03 and a pungent odor. Formaldehyde is soluble in water, ethanol and diethyl ether. It is also used in polymerized from as par formaldehyde [1].With human population growth, increases demand for protein consumption. Farmed fishes very important as the supply protein and fatty acids required by humans. FA wide applications in various industries, as well as hospitals and research centers, leads to aquatic exposure to contamination due to industrial effluents and sewage plants[2, 3].In previous studies, treatment with FA effects on different organs in fish and mammals has been studied. For instance Louei Monfared has been reported that administration of FA causes adverse changes in the histological structure of the placenta in mice [4].FA has different effects on biological systems: the exposure of experimental animals to formaldehyde results in its rapid metabolic incorporation into DNA, RNA and proteins [5,6]. FA and/or xylene may affect the systemic cellular immunity, as well local immunity in bronchus (BALT) particularly in young and adult rats [7].

Since there are little information on the possible side effects of these chemical materials on the histological structure of trout`s skin, spleen and peripheral white blood cell count; however, the aim of this study was to determine overall effect of formaldehyde on the histological properties of the major lymphoid organs in the rainbow trout.

MATERIALS AND METHODS

For this study, 80 *O. mykiss* with an average weight of 325 ± 44.5 g randomly divided into four groups: a control and three experimental groups. Control fish were kept in aquaria containing water without any additives, while the fish of the experimental group exposed to concentrations of 25, 50 and 100 mg/L of aquarium water solution of FA for a month. At the end of the administration period, heparinized blood samples were taken by cardiac puncture and total white blood cells were counted. For histological evaluation, spleen and skin samples were taken and washed with saline. The samples fixed in buffered formalin (10%), processed for sectioning (5-6 µm) and stained with H&E. The sections were examined with an Olympus BX60 microscope and visualized through the Color-View Camera (Olympus, Tokyo, Japan). All results were presented as mean ± SE. Data were analyzed by one-way ANOVA followed by Duncan's multiple comparisons test. Multiple comparisons tests were only applied when a significant difference was determined in the ANOVA analysis, $P < 0.05$. The SPSS 13.0 (Chicago, USA) was used for analyzing the results.

RESULTS

In fish treated with different concentrations of FA there was a significant reduction in the number of white blood cells (Table 1).

TABLE I: Total count of the white blood cells (means ± SE) in the control and treated rainbow trout (*O. mykiss*) with different concentrations of FA

Parameter/Groups	Control	25mg/L FA	50mg/L FA	100mg/L FA
Total count of white blood cells ($\times 10^3$ /ul)	19.7±1.8[a]*	8.2±5.6 [b]	5.2±2.7 [b]	5.5±1.1 [b]

** Means in the same row with different letters are significantly different (ANOVA, P<0.05).*

The histological structure of the spleen in the treated fish with different concentrations of FA, significant histological changes include increasing the thickness of the capsule, significant reduction in the lymphoid cell population and increased the number of megakaryocyte in spleen were seen when compared with the controls(Figures 1 to 4).

In addition, in the experimental group of fish the skin has been showed necrosis and vacuolization of the skin layers, impaired in the cell keratinocytes and cell dysplasia in the basal layer of the skin (Figure 5).

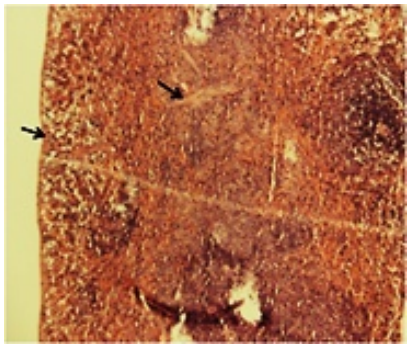

Fig. 1) Transverse sections through the spleen tissue of the 50 mg/L FA treated fish. The figure shows significant elevation in the splenic capsule thickness (arrows). (Haematoxylin and Eosine stain) (× 400).

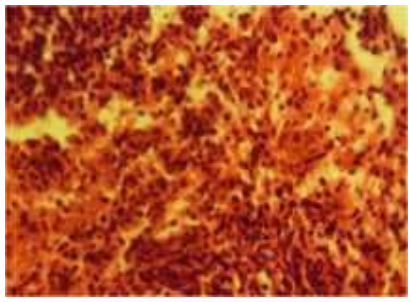

Fig. 2) Transverse sections through the spleen tissue of the 100 mg/L FA treated fish. The figure shows reduction in the lymphoid cell population[Stained with Hematoxylin - Eosin, magnification ×400]

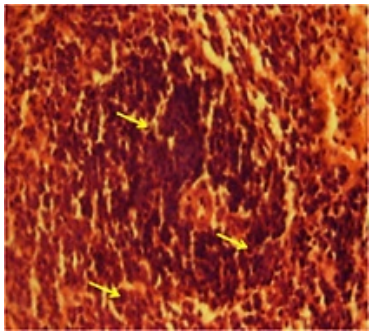

Fig. 3) Transverse sections through the spleen tissue of the 50 mg/L FA treated fish. The figure shows increased in the number of spleen`s macrophages (arrows) [Stained with Hematoxylin - Eosin, magnification ×400]

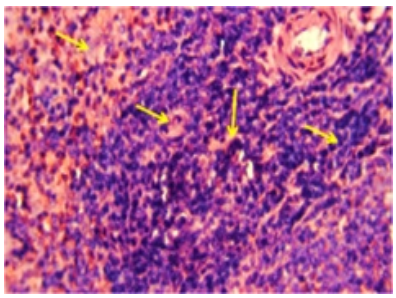

Fig. 4) Transverse sections through the spleen tissue of the 200 mg/L FA treated fish. The figure shows increased in the number of megakaryocyte (arrows) in the spleen`s tissue [Stained with Hematoxylin - Eosin, magnification ×400]

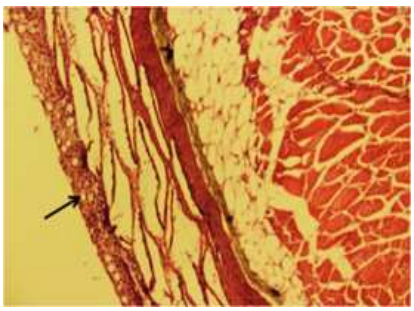

Fig. 5) Transverse sections through the skin tissue of the 100 mg/L FA treated fish. The figure shows necrosis and vacuolization of the skin layers, impaired regulation of cell keratinocytes and cell dysplasia in the basal layer of the skin (arrow). [Stained with Hematoxylin - Eosin, magnification ×400]

DISCUSSION

The results of this study in rainbow trout treated with different concentrations of FA indicated extensive histological changes in the spleen, skin, and also significant reduction in the levels of white blood cells count.

In the present work we found necrosis and vacuolization of the skin layers, impaired in the cell keratinocytes and cell dysplasia in the basal layer of the skin. Similarly, Pinkerton et al. (2004) had been examined the histopathological changes in the nasal cavity of rats following exposed to various concentrations of FA as a high dose of 145 ppm and a low dose of 15 ppm for 6 hours. They had been reported that FA has dose-dependent impacts on the skin tissue including hyperkeratosis epithelial spiny cells, cell dysplasia, and disruption of cell regulation and basal cell dysplasia [8]. These findings are in line with our results in the present study. Also, Monteiro-Riviere and (1986) reported similar results on the effects of formaldehyde on the respiratory epithelium tissue [9].

In the present study, significant histological changes include increasing the thickness of the capsule, significant reduction in the lymphoid cell population and increased the number of megakaryocyte in spleen were seen when compared with the controls. Previous researchers have shown that FA vapor can cause significant morphometric changes in the white pulp of the spleen in rats [10].

Although exact causative mechanism(s) and factors for FA induced immunological toxicity is not yet clear, but chromosomal damages by FA exposure in human peripheral blood cells has been stated to be occur [11,12]. Also, chromosomal damage leading to micro-nucleated lymphocytes was found to be more frequent in the highly exposed pathology and anatomy laboratories workers than in controls individuals. The difference was suggested to be due to a higher frequency of chromosome loss, suggesting FA-induced defects in the mitotic apparatus [13].

In the present work, histological study, histometrical analysis and peripheral blood cells count indicates the potential immunotoxic properties of FA exposure at different concentration in the trout. In line with these findings, it has been claimed that functions of both the immune and hematopoietic systems could be affected by chronic exposure to FA, phenol and organic chlorohydro carbons chemical substances [14].

According to these results, formaldehyde should be considered as an environmental hazard to fish and its releasing to water supplies would be prevented.

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