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LIVER LESIONS IN BLEAK *(Alburnus alburnus alborella Filippi)* COLLECTED FROM SOME CONTAMINATED SITES OF LAKE OHRID. A HISTOPATHOLOGICAL EVIDENCE[1]

Danica ROGANOVIĆ-ZAFIROVA[1,2] & Maja JORDANOVA[1]

[1]Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje,
[2]Hydrobiological Institute, Ohrid, Macedonia

ABSTRACT

Roganović-Zafirova D. and Jordanova M. (1998/99). Liver lesions in bleak *(Alburnus alburnus alborella* Filippi) collected from some contaminated sites of Lake Ohrid. A histopathological evidence. Ekol.Zašt.Živ.Sred. Vol. 6, No 1/2, 11-18, Skopje.

Histopathological changes observed in the liver of bleak individuals collected from contaminated sites of Lake Ohrid were presented in this study. The possible correlation of these findings with the pollution-induced hepatic lesions in bleak was discussed. Particular attention was paid to the foci of cell alteration (FCA), tumor-like changes, and coagulative necrosis present in the investigated bleak liver tissue.

Key words: pollution, Lake Ohrid, bleak, liver, histopathology

ИЗВОД

Рогановиќ-Зафирова Д. и Јорданова М. (1998/99). Лезии во црниот дроб на плашицата *(Alburnus alburnus alborella Filippi)* изловена од контаминирани региони на Охридското Езеро. Хистопатолошка студија. Екол. Заšт. Живот. Сред. Том 6, Бр. 1/2, 11-18, Скопје.

Во оваа студија беа презентирани хистопатолошки наоди во црниот дроб на плашици од загадени региони на Охридското Езеро. Дискутирана е можната поврза ност на обсервираните промени со хепатотоксичното дејство на загадената средина, со посебен осврт на фокусите на клеточната алтерација, неопластичните лезии и фокалната коагулативна некроза, видени кај повеќе испитувани индиви дуи.

Клучни зборови: Загадување, Охридско Езеро, плашица, црн дроб, хистопатологија

INTRODUCTION

There is a growing body of evidence in the scientific literature during last two decades concerning pollution related fish diseases in marine and freshwater ecosystems. Various anthropological activities, like: discharge of industrial wastes, oil and gas drilling, leaching of fertilizers, pesticides and herbicides from agricultural areas, and others, may cause diseases in fish and shellfish populations

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(Dethlefsen & Tiews 1985; Brown et al. 1987, Dethlefsen 1988; Bucke 1991).

The liver is well known target organ of the toxic impact regarding its function in biotransformation and excretion of xenobiotics. After entering uptake, liver is the first organ to be exposed by portal circulation. Various pathological changes of fish liver has been reported in a number of species exposed to pollutants both in field and experimental studies (Baumann & Harsbarger 1985; Kranz & Dethlefsen 1990; Wester & Vos 1994).

Lake Ohrid receive various xenobiotic contaminants including pesticides and heavy metals primarily from agricultural watersheds, discharge of mines process water and effluence of industrial wastewater, both from Macedonian and Albanian parts (Basler & al. 1995). The aim of this investigation was to perform a pilot screening study of bleak liver response to present pollution using histopathological tools.

MATERIAL AND METHODS

Both male and female bleak individuals with total length from 110 to 162 mm, were collected from two sites of Lake Ohrid - Grasnica (77 individuals) and Studencista Channel (27 individuals). Bleak were cached by overnight nets (various mesh dimensions) in August, October and December, 1995. The fishes were sacrificed by severing spinal cord within 24 hours after catching. The livers were examined by hand lens for gross changes, excised, and fixed in 10 % buffered formalin. Specimens containing liver pieces from 2 to 7 individuals were sampled for paraffin embedding and histological processing. Blocks were sectioned at 5 μm and stained with hematoxylin and eosin (H&E), periodic acid - Schiff (PAS) and Feulgen nuclear reaction for DNA. A 173 liver sections were carefully screened for the presence of microscopically recognizable lesions. All observed lesions were registered and described and their occurrence in the investigated material approximately estimated. Analysis was performed on Reichert N°326971 and Leitzs Wetzlar light microscopes.

RESULTS

Gross specimen inspection showed normal liver appearance in most investigated individuals. Dark red or reddish-yellow and brown colored regions on the liver surface were registered in some cases. Liver specimens from 14 individuals were found to have small, pale, white-yellowish nodules.

Microscopic examination of liver specimens revealed a number of changes including necrosis, inflammation focuses, blood vessel dilatation, tumor-like proliferation and parasitic infestation. The histopathological findings of the investigated material are summarized in Tab. 1.

Severe congestion was registered in approximately 50% of the analyzed sections. Dilated and engorged central veins and sinuses filled with excessive amount of blood were observed as a regional or general change in the liver. In most cases, congestion did not associate degeneration or necrosis of surrounding parenchyma.

Hepatocytes of investigated bleak livers as a rule showed large euchromatic nucleus with prominent nucleolus and wide basophilic cytoplasm. Generalized or regional fatty change of parenchyma was a regular finding in approximately 20% of the analyzed sections (Fig. 1.). In some areas of fatty change, single large lipid droplets in hepatocyte cytoplasm showed tight perisinusoidal distribution. Hydropic degeneration (cellular swelling) of hepatocytes was present but less frequent observation in analyzed tissue.

The presence of foci of hepatocellular coagulative necrosis was a discrete but quite frequent finding. Necrotic hepatocytes with dark hyperchromatic nuclei, and deeply stained cytoplasm were found diffusely scattered over rather broad regions of hepatic tissue or focussed in small mostly perivascular areas. Tiny focuses

of coagulative necrosis consisting of dead hepatocytes with picnotic nucleuses and faintly stained depleted cytoplasm (Fig. 2) were found in some specimens. Most of these findings were not associated with inflammation. Bile duct epithelium necrosis was quite uncommon finding and, as a rule, associated with a local peribiliar inflammation.

Tab. 1 Histopathological survey of liver lesions registered in Lake Ohrid bleak individuals (*Alburnus alburnus arborella*) collected in Grasnica and Studencista Channel at various dates during 1995 year.

Таб. 1 Хистопатолошки преглед на лезиите во црниот дроб на единки од охридска плашица (*Alburnus alburnus arborella*) изловени во регионот на Грашница и Каналот Студенчишта во различни периоди од 1995 година.

SPECIMEN	CON	ХРГ	DEGENER.		FCA	NEKROSES			INFLAMATION					BP	TUMR	PARASITES		
			FCD	HD		IP	PV	PB/IB	IP	PV	IV	PB	MC			NP	MXS	HLM
G-17.08.95 8 individuals	+++	++	++	-	++	++	++	-	-	-	++	-	++	-	+++	-	-	-
G-31.08.95 32 individuals	++++	++	++	+++	+	++	++	+	-	++	+	+	+++	-	+	+		+
G-10.10.95 37 individuals	++++	+	++	+	+	+++	+++	++	+	+	+	+	+++	-	++	+	+	+
K - 6.12.95 27 individuals	++++	+	++	-	+	++++	-	-	+	-	-	-	+++	-	+	-	-	-

Legend: G -Grasnica , K –Studencista Channel , CON - congestion , HRG -hemorrhage , FD – fatty degeneration, HD – hydropic degeneration , FCA – foci of cell alteration, IP - intraparenchymal, PV –perivascular , PB -peribiliar, IB -intrabiliar, IV- intravascular, MC-macrophage centers, BP – bile duct proliferation, TUMR – tumors , NP- non identified protistan, MXS - mixosporean, HLM –helminths. Finding observed in percent of analyzed sections: 1-10% +; 10-20% ++; 20-30% +++; over 30% ++++

Легенда: G –Грашница , K – Канал Студенчишта, CON - конгестија, HRG -хеморагија, FD – масна дегенерација, HD – хидропсна дегенерација, FCA – фокуси на клеточна алтерација, IP - интрапаренхимално, PV –периваскуларно , PB -перибилиарно, IB - интрабилијарно, , IV- интаваскуларно, MC-макрофагни центри, BP – билијарна пролиферација, TUMR – тумори , NP- неидентификувани протисти, MXS - миксоспорозои, HLM –хелминти. Лезии видени во процент на анализирани пресеци: 1-10% +; 10-20% ++; 20-30% +++; преку 30% ++++

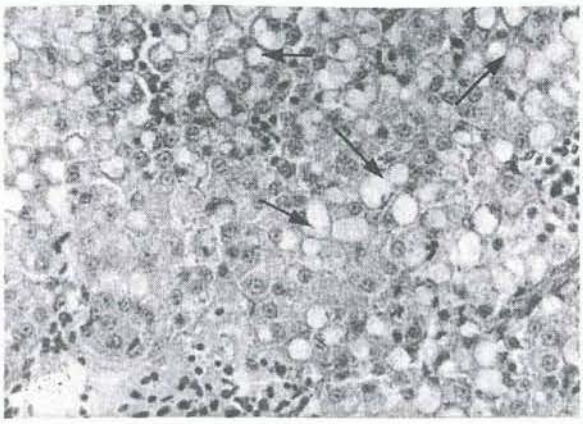

Fig 1. Section of liver from a bleak collected from Grasnica with regional diffuse fatty change of the parenchyma (arows). H&E x 400

Сл. 1. Пресек на црн дроб од плашица уловена во регионот на Грашница со регионална дифузна масна дегенерација (стрелки). H&E x 400

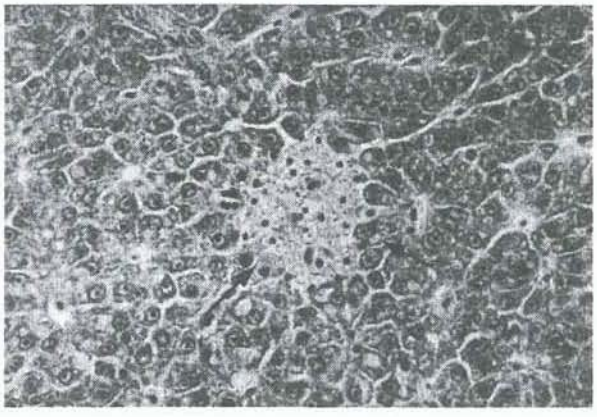

Fig. 2 Focal hepatocellular coagulative necrosis. Small group of dead hepatocytes with picnotic nuclei and pale cytoplasm (arrows). H&E x400

Сл. 2 Фокална хепатоцелуларна коагулативна некроза. Мала група некротични хепатоцити со пикнотични јадра и бледа цитоплазма. H&E x400

Some peculiar inconsistently found small lesions in the hepatic tissue were considered foci of cellular alteration (FCA). Lesions appeared as few cell groups of hepatocytes with clear "ground glass" cytoplasm, regularly surrounded by a collar of infiltrated lymphocytes (Fig. 3).

Inflammatory reaction, usually registered as small perivascular, intralobular or peribiliar granulomas was relatively rare finding in the bleak liver. Infiltrated areas consisted mostly of lymphocytes sometime accompanied by variable number of macrophages, plasma cells and fibroblasts. Lymphocytic infiltration in the bile duct epithelium was registered just in few cases. Increased presence of lymphocytes in the lumen of liver vessels was also observed in few sections.

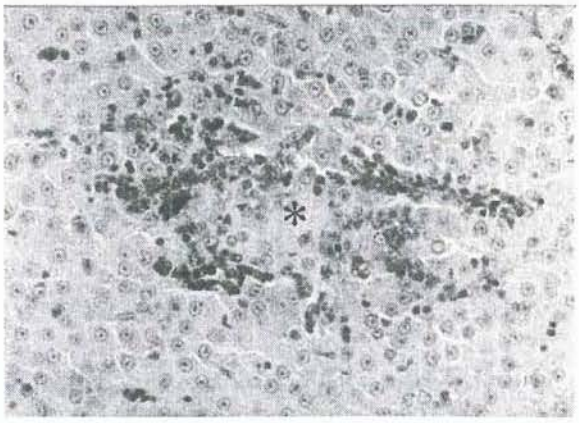

Fig. 3 A focus of cellular alteration (FCA) in bleak liver parenchyma (asterix) surrounded by inflammatory cells. H&E x400

Сл. 3 Фокус на клеточна алтерација во црнодробниот паренхим на плашицата (ѕвездичка) опкружен со инфламаторни клетки. H&E x400

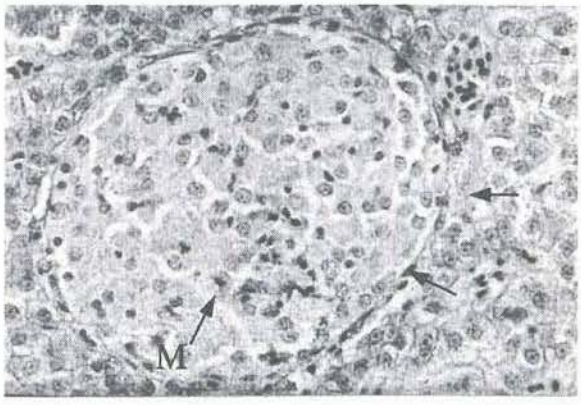

Fig. 4 Tumor-like nodule in bleak liver with large pale cells. Note sharp bordering with normal parenchymal tissue (arrows). M – mitotic figure. H&E x400

Сл. 4 Туморовиден нодулус во црниот дроб на плашицата со големи бледи клетки. Јасна разграниченост од нормалниот црнодробен паренхим (стрелки). M – митоза. H&E x400

Haemorhagic inflammatory lesions consisting of intraparenchymal exudates that contained accumulations of extravascular erythrocytes and inflammatory cells were noticed in a few sections. In most observed cases with inflammatory reaction, no causative agent - morphologically recognizable, was observed

except in some intraparenchymal infiltration surrounding small necrotic areas, probably remnants of dead parasites. Wide necrotic regions in the parenchyma infiltrated by inflammatory cells were considered the consequence of a similar process.

The occurrence of melanomacrophage centers was a consistent finding in all analyzed bleak livers. They showed various dimensions - from small, few cell groups to wide accumulations of macrophages with small, dark, bean shaped nuclei and a wide yellowish, strongly PAS positive cytoplasm. In some cases, a marked increase of the number and dimensions of melanomacrophage centers was found.

In approximately one tenth of investigated livers a peculiar, repeatedly occurring tumor-like lesion was observed, usually located near blood vessels (Fig. 4). It consisted of epithelial-like cells aggregated in spherical or oval formations, separated from the surrounding liver tissue by a delicate thin layer of fibroblasts. Extremely variable dimensions of this proliferation were observed: form few cell groups to enormous proliferated masses invading wide areas into the liver tissue. No microscopically recognizable blood vessels were found into any observed proliferation with no regard on its size. Cells had pale, wide, slightly PAS positive cytoplasm and large vesicular nuclei containing no nucleoli. A thin peripheral border of infiltrating lymphocytes was an often finding around tumor-like masses. The analyzed bleak liver tissue showed very scarce occurrence of helminths. Tapeworm larvae were occasionally observed in liver parenchyma with no local inflammation associated. An unknown protistan parasite colonizing vascular endothelium of liver tissue was almost permanently observed. Dump-shaped cells showed clear unstained cytoplasm surrounded by a thick eosinophilic pellicle and a heterochromatic peripherally located disc-shaped nucleus. A peculiar star-shaped structure located opposite to the nucleus was the only visible content in the cytoplasm. As a rule, these cells were found just beneath the endothelial layer of the large vessels. Various rates of infestation, from individual protistan-like cells to colonies forming small polyp-like protrusions into blood vessel lumen were observed in different sections. Relatively scarce finding of mixosporeans infestation associated with lymphocyte inflammation was observed primarily in the bile ducts. The binuclear, oval, slightly elongated mixosporeans spores with two polar capsules were strongly PAS positive.

DISCUSSION

The first histopathological screening of liver from bleak individuals living in some contaminated sites in Lake Ohrid revealed the presence of clear hepatic lesions including tumor-like formations.

An apparent, very frequently observed change in bleak liver was the severe congestion of central veins and sinuses. Vascular and sinusoidal congestion in fish liver has been reported as a possible hepatotoxic lesion after exposure to some pesticides (Couch 1975). In our material, the possibility of artificial agony-induced change was not excluded, so this evidence has to be interpreted with caution. This even more because of the collection method used, and the lack of necrosis in the surrounding parenchyma, which may suggests acute agony-induced congestion.

General or regional diffuse fatty change of the parenchyma was recurrently observed in the investigated material. This finding was reported after variety of hepatotoxic insults (Hinton et al. 1992). But, this condition was also observed in vitellogenic females, in fish that store abundant lipids (van Bohemen & al. 1981), and under influence of nutritional state (Segner & Moller 1984; Segner & Juario 1986; Segner & Braunbeck 1988).

Focal and diffuse hepatocellular coagulative necrotic lesions in bleak liver observed as a discrete but consistent finding may be taken as a more confident indication of hepatotoxicity. Currently, focal hepatocellular coagulative necrosis has been accepted as useful biomarker of anthropogenic toxicant exposure in the absence of evidence for parasitic infestation (e.g. fibrotic tracks or cystic spaces) or prior infection by microorganisms (Hinton et al. 1992). Most of registered necrotic lesions in Ohrid bleak liver were not associated with

inflammation and lacked morphological evidence for parasites or bacterial infection.

Inflammatory lesions in bleak liver were registered as relatively rare mild change, in some cases associated with the presence of mixosporeans. Melanomacrophage centers were markedly induced in few of investigated individuals. This finding may be interpreted, either or both, as a residual lesion of previous infection and parasitism, or injury effected by chemical toxicants.

Foci of cellular alteration including basophilic, eosinophilic, clear, and fat vacuolated were reported to be associated with experimental exposure to carcinogens in various fish species. Their environmental relevance has been confirmed in field investigations (Hinton & al. 1992). From this point of view bleak liver lesions appearing as few cell groups of hepatocytes with clear "ground glass" cytoplasm surrounded by lymphocyte infiltration deserves attention and further investigation as alterations that eventually precede neoplasm.

The incidence of at least tree cases with a neoplasm originating in a specific cell lineage in a defined geographic location is considered epizootic (Harshbarger & Clark 1990). In the investigated bleak liver material, the same type of tumor-like lesion was recurrently registered in many liver specimens from bleak collected in various periods of the year. In the past 30 years several dozens wild fish tumor epizootic were identified in North America. Epithelial tumors, and hepatocellular neoplasms in particular, are strongly correlated with exposure to chemical contaminants (Harshbarger & Clark 1990). Experimental dietary intake of chlorinated hydrocarbon DDT, for example, produced hepatomas, hepatocellular carcinomas and cholangiocarcinomas in rainbow trout (Halver et al. 1962). The increased prevalence of certain tumors may signal exposure to xenobiotic initiators and possibly promoters of carcinogenesis. However, other factors such as oncogenic viruses (Papas & al. 1976, 1977; Kimura et al. 1981a; Kimura & al. 1981b, Yoshimizu et al. 1987), parasite induced proliferation and immunosupression (Anderson 1990) may be suspected etiologic agents of tumor epizootics. The registered tumor-like lesion in bleak liver did not appear to be of hepatocellular or cholangiocellular origin. The finding deserves further study to define the lesion and its prevalence in contaminated opposing clean areas of Lake Ohrid.

Lesions associated with parasitic infestation were consistently registered in the bleak livers. Accurate determination of the found parasitic protistan and helminthes and its prevalence assessment was beyond the scope of this study.

CONCLUSIONS

Microscopic analysis of liver tissue from bleak individuals living in contaminated sites of Lake Ohrid littoral revealed a number of histopathological changes. A part of registered findings may be considered pollution-induced liver lesions. Discrete but frequently found foci of coagulative necroses suggest mild hepatotoxic effects in the investigated individuals. The presence of foci of cellular alteration and a peculiar tumor-like lesion recurrently occurring in collected bleak livers deserve particular attention and further investigation.

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ЛЕЗИИ ВО ЦРНИОТ ДРОБ НА ПЛАШИЦАТА *(Alburnus alburnus alborella Filippi)* ИЗЛОВЕНА ОД КОНТАМИНИРАНИ РЕГИОНИ НА ОХРИДСКОТО ЕЗЕРО. ХИСТОПАТОЛОШКА СТУДИЈА.

Даница РОГАНОВИЌ-ЗАФИРОВА[1,2] и Маја ЈОРДАНОВА[1]

[1]Институт за биологија, ПМФ, Скопје, [2]Хидробиолошки завод, Охрид

РЕЗИМЕ

Во трудот се презентирани хистопатолошки наоди на црн дроб од плашица *(Alburnus alburnus alborella* Filippi*)* изловена од контаминирани делови на литоралот од Охридското езеро. Микроскопската анализа кај вкупно 104 единки од двата пола покажа присуство на повеќе типови на хистопатолошки лезии во црнодробното ткиво вклучувајќи: конгестија, некроза, инфламаторни процеси, пролиферативни промени и паразитни инфестации (Таб. 1). Дискутирано е значењето на регистрираните хепатални промени во однос на биолошкиот одговор на овој организам на присутните контаминанти во околната средина. Од овој аспект посебно внимание заслужува појавата на фокална коагулативна некроза на хепаталниот паренхим без пропратни инфламаторни реакции (Сл. 2) што може да биде одраз на благи хепатотоксични ефекти. Регистрираните фокуси на клеточна алтерација укажуваат на пренеопластични промени во црнодробното ткиво кај дел од испитуваните единки (Сл. 3). Еден посебен вид на туморовидна пролиферативна лезија со неидентификувано потекло (Сл. 4.) се јавува со инциденција на епизоотија. Прикажаните наоди укажуваат на присутни хепатотоксични и други патогени ефекти на загадената средина врз популацијата на плашицата од Охридското езеро.