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# Histological Changes in the Liver of the Zebrafish, (*Danio Rerio*) after Exposure to Poly(2-Ethyl-2-Oxazoline)

Merve Abar[1], Cansu Akbulut[1], Nazan Deniz Yön[1], Güllü Kaymak[1], Figen Esin Kayhan[2], Selma Helli[1] and Uğursoy Olgun[3]

[1]Sakarya University Faculty of Science and Arts, Department of Biology, Sakarya, Turkey.

[2]Marmara University Faculty of Science and Arts, Department of Biology, Istanbul, Turkey.

[3]Sakarya University Faculty of Science and Arts, Department of Chemistry, Sakarya, Turkey.

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**ABSTRACT**

Poly(2-oxazoline)s have widely been used in biomedical applications for the last years. They can mimick natural systems and they generally used as liposomes, drug and gene deliver and pseudopeptides. In this study investigating histological effects of different doses (10 mg/L, 50mg/L) of poly(2-ethyl-2oxazoline) (PEtOx) on liver tissue of zebrafish was aimed. Adult zebrafish individuals were exposed to different doses of PEtOxfor 5 days and after that they were dissected and liver tissues removed. Histological changes at liver tissue were investigated at light microscope after hematoksilen and eosin staining. In the experimental groups, contraction in vacuoles at hepatocyte cytoplasm, hypertrophy and increase in the number of hepatocyte cells and kuppfer cells were detected.



## Introduction

Poly (2-oxazoline)s have been used since 1960. They can mimic natural systems and they generally used as liposomes, drug and gene deliver and pseudopeptides. Poly (2-oxazoline)s participate in membrane structures. The living cationic ring-opening polymerization of 2-oxazolines provides easy and direct access to a wide variety of well-defined polymers, in which the end-group functionality can be controlled during the initiation and termination steps. Furthermore, the properties of can be tuned simply by varying the side chain of the 2-oxazoline monomer [1].

As poly(2-oxazoline)s have been used as antimicrobials and drug carriers in biomedical applications they show similarity to poly (ethylene glycol). Also poly (2-oxazoline)s increase solubility of insoluble drugs or low aqueous solubility drugs with potentially, provides against degredation and deactivation during advanced farmacogenetic transport and improved circulation, reduces antigenic activity. They can combined with contrast agents of drugs and can create functional components [2,3].

Even though poly(2-oxazoline)-functionalized liposomes were demonstrated to have similar beneficial properties as PEGylated liposome [4,5] drug delivery using such poly(2-oxazoline) liposomes has, surprisingly, not been reported to date. Nonetheless, drug loading and release from micellar drug carriers formed from poly(2-oxazoline) block copolymers have been reported [6]. Studied the use of PEtOx-*block*-poly(ε-caprolactone) micelles for the loading of paclitaxel, an anticancer drug with poor aqueous solubilityblock copolymer micelles showed low cytotoxicity. In addition, loaded micelles showed a comparable in vitro inhibition of the proliferation of KB human epidermoid carcinoma cells as the current clinical formulation, while avoiding the side effects such as hypersensitivity and neurotoxicity [7].

Poly(2-oxazoline)s can be regarded as analogues of polypeptides and also of polypeptoids [2]. So, it is considered as pseudopeptides, thus bioinspired polymers, due to their structural relationship to polypeptides [8].

Zebrafish is a well known vertebrate model for reproduction and development studies. The zebrafish (*Danio rerio*) naturally lives in Pakistan and India and is a small fish about 6 cm in length, characterized by a series of five pigmented stripes running the entire length of each side of its body. The zebrafish's hardiness makes them excellent stress test subjects, as they can survive fairly severe environmental changes without succumbing, surviving long enough to show developmental defects.Finally, zebrafish are easy and inexpensive to raise, requiring only filtered water, and a minimal investment in fish food, making them an ideal animal model for research laboratories with limited funding. All of these characteristics have contributed to making zebrafish the model of choice in this study. Consequently, the objective of this study was to identify histological changes in the liver tissue after variousdoses(10 mg/L, 50mg/L)of poly(2-ethyl-2oxazoline) exposure.

## Materials and Methods

### Test Animal

Zebrafish are maintained at standardized conditions of 27 °C ± 1 °C temperature, 95 % dissolved oxygen saturation, 61% moisture and photoperiod (12:12-h light:dark). Zebrafish were fed twice in a day (Tetra Pro)

### Experimental Design

Following the prelaminary experiment, all determinations were repeated three times. In our study we created one control and two experiment groups (n=15) concerning their different PEtOx doses (Group I: 10 mg/L PEtOx, Group II: 50 mg/L PEtOx, Group III: control group). After 48 h adaptation, different concentrations of PEtOx were added to the experimental aquaria. Mortality was controlled during the test and it was observed that no fish died. After exposure, on the fifth day of the study fishes were anaesthetized with ice water and ovary tissues were dissected immediately.

**Tele:**

**E-mail addresses: cansua@sakarya.edu.tr**

**Histological Study**

Tissues were fixed in neutral formaldehyde for 24 h. After fixation, tissues were dehydrated in ascending concentrations of ethanol, equilibrated in xylene. The tissues were then embedded in paraffin wax and cut into 5-7 µm sections on a Leica microtome. The sections were mounted on glass slides and stained with haematoxylin and eosin before examination under a Olympus light microscope.

**Results and Discussion**

In control group normal liver histology was monitored. Parenchyma cells, hepatocytes and their cytoplasm were observed clearly. Sinusiodis and nuclei wereeasily monitored. Sinusoids were connected to each other in the form of lattice to communicate with each other (Figure 1a, 1b).

In experiment groups,histological changes noticed at liver tissue of zebrafish when compared with control group. The liverparenchyma of fish showed varied histopathological alterations depending on the dose.In the 10 mg/L experimental group, degeneration, contraction at vacuoles in hepatocyte cytoplasm, hypertrophy and increase in the number of hepatocyte cells were detected (Figure 2a). Furthermore some Kupffer cells were observed more elliptical form.Dilatation of sinusoids and increase in the number of Kuppfer cells were monitored at liver tissue(Figure 2b). In 50 mg/L experimental group, vacuoles in hepatocyte cytoplasm were reduced to almost nothing (Figure 3a) Hypertrophy and increase in the number of hepatocyte cells were also monitored at this group too. Decrease in the number of Kuppfer cells were observed. Also changes in cell shape, vascular and sinusoidal degeneration and steatozis were observed (Figure 3b).

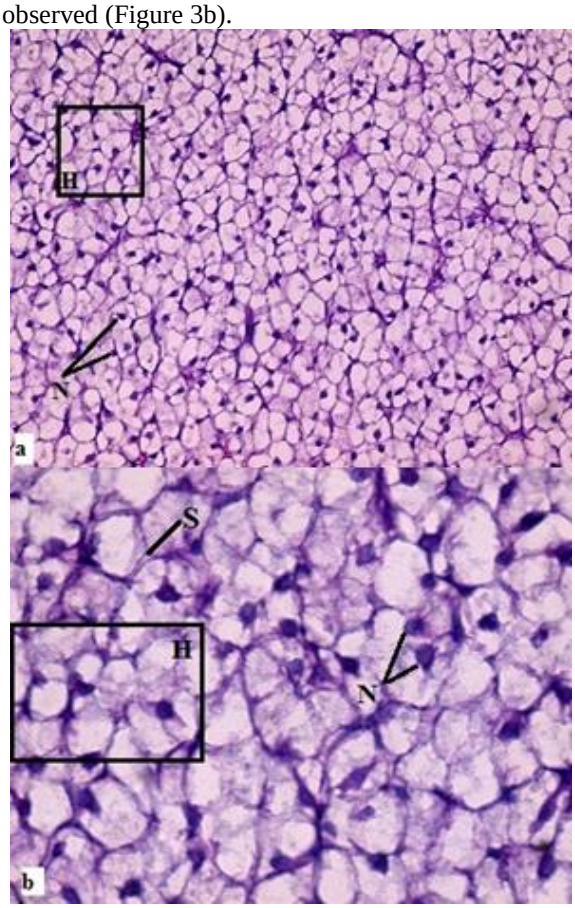


**Figure 1. Liver histology at control group,Hepatocytes (H), nuclei (N) and sinusoids (S), Stain: Hematoxylin and eosin, Magnification: a-x40 b-x100**

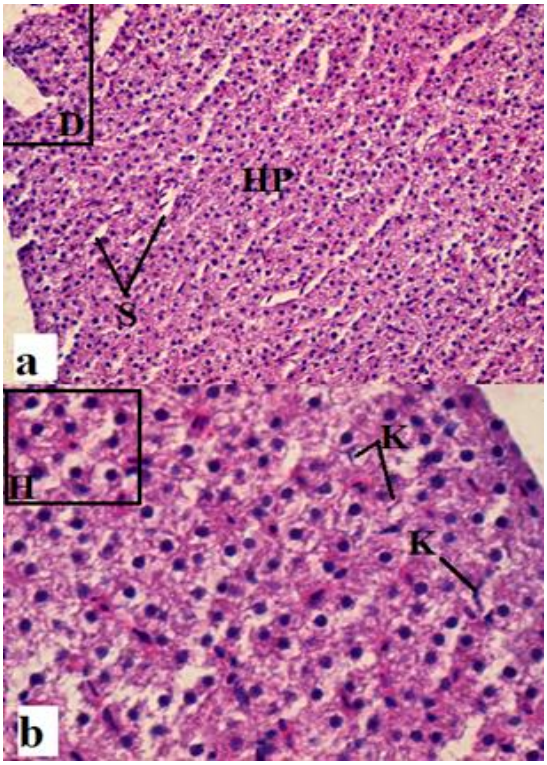


**Figure 2. 10 mg/L poly(2-ethyl-2oxazoline) exposure group,a) degeneration at liver tissue, hypertrophy and increase in the number of hepatocyte cells, b) increase in the number of hepatocyte and eliptical kuppfer cells, hypertrophy (HP), degeneration (D),sinusoids (S),hepatocyte (H), kuppfer cells (K). Stain: Hematoxylin and eosin, Magnification: a-x40 b-x100**

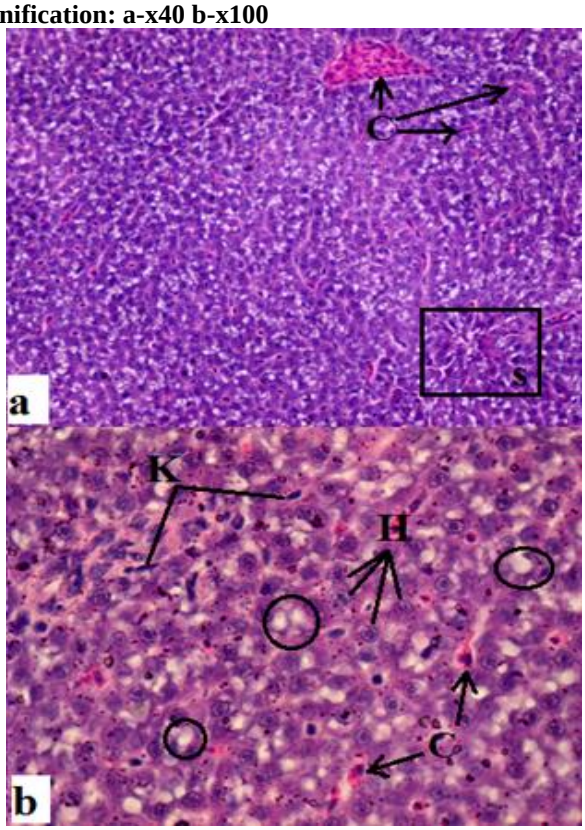


**Figure 3. 50 mg/L poly(2-ethyl-2oxazoline) experimental group a) Sinusoidal congestion, b) vascular and sinusoidal degeneration and steatozis (circle), sinusoids (S), sinusoidal congestion (C), Hepatocyte cells (H), Kuppfer cells (K),Stain: Hematoxylin and eosin stain Magnification:a-x40, b-x10**

The use area of poly(2-oxazoline)s have been developed in biomedical applications for the recent years, as a result of their biocompatibility as well as their stealth behaviour [2,9]. The biocompatibility of poly(2-methyl-2-oxazoline) (POx) was demonstrated in 1989. Labeled polymers were found to be excreted from mice without significant accumulation in organs, although some, presumably high-molecular-weight, polymer was found in skin and muscle tissue [10]. In Zalipsky and co-workers' study it was proved that PEtOx liposomes are faster than POx in rats and mice and revealed enhanced circulation times, with similar blood-clearance rates in vitro studies [4]. It was also proved that the liposomes accumulated mainly in the liver, kidney, and spleen [4,5].

Other vertebrates such as fish liver was carried out in key organs that control many vital functionsand tasks in the catabolism and anabolism is writing both internal an important place in the physiology of the fish [11]. The alterations in liver due to toxicity impact are often associated with a degenerative necrotic condition [12,13,14]. In the present study we elucidated acute toxicity effects of poly(2-ethyl-2oxazoline) on histopathology of liver of zebrafish.

Exposed to cypermethrin showed hydropic degeneration, vacuolar degeneration, aging and hemorrhage, cellular necrosis, infiltration of inflammatory cells in the interstitial tissue and fibroblastic proliferation at hepatocytes in the rainbow trout liver [15]. Similarly at this study proliferation at hepatocytes in the zebrafish liver. Yön et al. [16] investigated the histopathological changes in liver tissue of the swordtail fish *Xiphophorus helleri* exposed to deltamethrin. As the result of this study, hypertrophy of hepatocytes and dilatation of sinusoids were monitored. The cell membranes of hepatocytes were disintegrated and the nuclei were irregularly scattered forming a syncytial mass. The cytoplasm of the hepatocytes was highly vacuolated in the perilobularThese findings are consistent with our study.Cengiz and Unlu [17] reported hypertrophy of hepatocytes, increase of kupffer cells, circulatory disturbance, narrowing of sinusoids, pycnosis position of nuclei, fatty degeneration and focal necrosis in the liver of *G. affinis* exposed to deltamethrin. These findings aren't consistent with our study because decrease in the number of Kuppfer cells were observed our study.

Chronic cyanide poisoning of rainbow trout causes necrosis of liver cells [18]. In flatfish, pollutants, tumor and degenerative lesions in the liver creates [19]. Contaminated with DDT in the liver of Atlantic salmon fed baits have seen shrinks [20].Ammonium of rainbow trout puppies were observed in liver sinus blockage [21]. Field investigations have confirmed the use of preneoplasia (FOCI) and neoplasia as relevant biomarkers in teleosts [22,23,24,25].Distinctively in our study hypertrophy and increase in the number of hepatocyte cells, dilatation of sinusoids were monitored, vascular and sinusoidal degeneration and steatozis at liver tissue.

As a result, poly(2-oxazoline)snanoparticles in studies caused to deformations in the various tissues of the fish. All the histopathological observation indicated that exposure to of poly(2-oxazoline)s caused destructive effect in the liver tissue of zebrafish. In our study decrease in the number of Kuppfer cells were observed. Also changes in cell shape, vascular and sinusoidal degeneration and steatozis were observed. Degeneration, contraction in vacuoles at hepatocyte cytoplasm, hypertrophy and increase in the number of hepatocyte cells and Kuppfer cells were detected. Elliptical looking Kuppfer cells have been found to become more oval looking. Dilatation of sinusoids were monitored at liver tissue. Liver histopathological alterations, such as those observed in this study and findings from previous studies, may result in severe physiological problems, ultimately leading to the death of fish.

**References**

[1]Kobayashi, S. 1990. Ethylenimine Polymers. *Progress in Polymer Science*, 15: 751–823.

[2]Hoogenboom, R., Schlaad, H. (2011) Bioinspired Poly(2-oxazoline)s. Polymers 3, 467-488.

[3]David, G., Simionescu, B.C. & Albertsson, A.C. 2008. Rapiddes welling response of poly(N-isopropylacrylamide) / poly(2-alkyl-2-oxazoline) / poly(2-hydroxyethyl-1-methacrylate) hydogels. *Biomacromolecules*, 9: 1678-1683.

[4]Zalipsky, S., Hansen, C.B., Oaks, J.M. & Allen, T.M. 1996. Evaluation of blood clearance rates and biodistribution of poly(2-oxazoline)-grafted liposomes. *Journal of Pharmaceutical Science*, 85: 133 – 137.

[5]Woodle, M.C., Engbers, C.M. & Zalipsky, S. 1994. New amphipatic polymer-lipid conjugates forming long-circulating reticuloendothelial system-evading liposomes. *Bioconjugate Chemistry*, 5: 493–496.

[6]Lee, S.C., Kim, C., Kwon, I.C., Chung, H.S. & Jeong, Y.J. 2003.Polymeric micelles of poly(2-ethyl-2-oxazoline)-block-poly(epsilon-caprolactone) copolymer as a carrier for paclitaxel. *Controlled Release*,89: 437–446.

[7]Wang, C.H.,& Hsiue, G.H. 2003. New amphiphilic poly(2-ethyl-2-oxazoline)/ poly(L-lactide) triblock copolymers. *Biomacromolecules*, 4: 1487–1490.

[8]Aoi, K., Suzuki, H.& Okada, M. 1992. Architectural control of sugar-containing polymers by living polymerization: Ring-opening polymerization of 2-oxazolines initiated with carbohydrate derivatives. *Macromolecules*25: 7073-7075.

[9]Adams, N. & Schubert, U.S. 2007.Poly(2-oxazolines) in biologicalandbiomedical application contexts. *Advanced Drug Delivery Reviews* 59: 1504-1520.

[10]Goddard, P., Hutchinson, L.E., Brown, J., Brookman, L. J. (1989) Soluble polymeric carriers for drug delivery. Part 2. Preparation and in vivo behaviour of N-acylethylenimine copolymers. Journal of Controlled Release 10, 5 – 16.

[11]Brusl, J. & Anadon, G. 1996. The structure and function of fish liver. In: Munshi, J.S.D., Dutta, H.M. (Eds.), Fish Morphology. Science Publishers Inc., USA, pp. 77-93.

[12]Arellano, J.M., Storch, V. & Sarasquete, C. 1999. Histological changes and copper accumulation in liverand gills of the Senegales sole, *Solea senegalensis*. *Ecotoxicology and Environmental Safety* 44: 62–72.

[13]Olojo, E.A.A., Olurin, K.B., Mbaka, G. & Oluwemimo, A.D. 2005. Histopathology of the gill and liver tissues of the African catfish, *Clarias gariepinus* exposed to lead. *African Journal of Biotechnology*, 4 (1): 117–122.

[14]Figueiredo-Fernandes, A., Ferreira-Cardoso, J.V., Garcia-Santos, S., Monteiro, S.M., Carrola, J., Matos,P. & Fontainhas-Fernandes, A. 2007. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. *Pesquisa Veterinária Brasileira*, 27 (3): 103–109.

[15]Atamanalp, M. 2000. Bir Sentetik Piretroit İnsektisitinin Subletal Dozlarının Gökkuşağı Alabalığı (*Oncorhynchus mykiss* W.,1792)'na Makroskobik, Histopatolojik, Hematolojik ve Biyokimyasal Etkileri. Ph.D. Thesis, Atatürk University, Institute of Natural Sciences, Erzurum.

[16]Yön, N.D., Akbulut, C., Abar, M., Kayhan, F.E. & Kaymak, G. (2014) Histological Changes in the Liver of The Swordtail Fish, Xiphophorus helleri (Pisces: Poecilidae) After Exposure To Deltamethrin. *European International Journal of Applied Science and Technology*, 1(3): 27-38.

[17]Cengiz, E.I. & Unlu, E. 2005. Sublethal effect of commercial deltamehrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia afunis*: a microscopic study. *Environmental Toxicology and Pharmacology*, 21: 246-253.
[18]Dixon, D.G. & Gerard, L. 1981. Chronic cyanide poisoning of rainbow trout and its effects on growth, respiration and liver histopathology. *Archives of Environmental Contamination and Toxicology*, 10: 117-131.
[19]Köhler, A., Wahl E. & Söffker, K. 2002. Functional and morphological changes of lysosomes as prognostic biomarkers of toxic liver injury in a marine flatfish. *Environmental Toxicology and Chemistry*, 21: 2434 - 2444.
[20]Murty, A.S. & Priyamvada Devi, A. 1982. The effect of endosulfan and its isomers on tissue protein, glycogen and lipids in fish *(Channa punctatus). Pesticide Biochemistry and Physiology*, 17: 280-286.
[21]Smith, C.E. and Piper R.G., (1972). Lesions associated with chronic exposure to ammonia in The Pathology of Fishes Edt. By W. E. Ribelin, G. Migaki. The University of Wisconsin pres., 497-567.
[22]Murchelano, R.A. & Wolke, R.E. 1985. Epizootic carcinoma in the winter flounder *Pseudopleuronectes americanus. Science*, 228: 587-589.
[23]Kranz, H. & Dethlefsen, V. 1990. Liver anomalies in dab *(Limanda limanda)* from the southern North Sea with special consideration given to neoplastic lesions. *Diseases of Aquatic Organisms*, 9: 171-185.
[24]Hayes, M.A., Smith, I.R., Rushmore, T.H., Crane, T.L., Thorn, C., Kocal, T.E. and Ferguson, H.W.,(1990). Pathogenesis of skin and liver neoplasms in white suckers from industrially polluted areas in Lake Ontario. Sci. Total Environ, 94: 105-123.
[25]Myers, MS., Stehr, C.M., Olson, O.P., Johnson, L.L., McCain, B.B., Chan, S.-L. & Varanasi, V. 1994. Relationship between toxicopathic hepatic lesions and exposure to chemical contaminants in English sole *(Pleuronectes vetulus)*, starry flounder *(Platichthys stellatus)*, and white croaker (Genyone*mus lineatus)* from selected marine sites on the Pacific Coast, USA. Environmental Health Perspectives, 102: 200-215.