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**MORPHOMETRIC STUDY OF THE BRANCHIAL
TISSUE RESPONSE OF TILAPIA, OREOCHROMIS
SPILURUS SPILURUS (GUNTER) EXPOSED TO
ALKYLBENZENE SULPHONATE UNDER
HYPER SALINE WATER CONDITION**

(With 4 Tables and 4 Figures)

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دراسة مورفومترية لإستجابة النسيج الخياشيمي لأسماك البلطي
المعرضة تجريبياً لمادة الأكايل بترين سلفونيت تحت ظروف الملوحة الزائدة

صلاح عفيفي

تم في هذه الدراسة تقييم إستجابة النسيج الخياشيمي لأسماك البلطي وذلك باستخدام المؤشرات المورفومترية حيث تم تعريض 30 سمكة بلطي للجرعة تحت المميتة من مادة الأكايل بترين سلفونيت يومياً لمدة 21 يوماً ، بينما إستمرت 20 شملته فقط تحت ظروف الملوحة الزائدة كمجموعة ضابطة ، أخذت العينات من خياشيم الأسماك (عشرة في كل مرة) بعد 7 ، 14 ، 21 يوماً من التعرض للمادة بينما أخذت خياشيم المجموعة الضابطة في بداية ونهاية التجربة فقط. تم تثبيت جميع العينات في محلول 10% فورمالين وتم إعدادها للفحص بالميكروسكوب الضوئي. وبالفحص الميكروسكوبي لوحظ إنخفاض في مؤشر عدد الخلايا المخاطية وخلايا الكلوريد في الأسبوع الأول والثاني بعد التعرض بينما ارتفع عدد هذه الخلايا بنهاية الأسبوع الثالث وذلك مقارنة بعدد هذه الخلايا في المجموعة الضابطة. كان معدل التكاثر الطبقي في الخياشيم 25.5% ، 63.4% ، 66.7% في الأسبوع الأول والثاني والثالث بعد التعرض على التوالي ، بينما كان هذا المعدل 8.66% في المجموعة الضابطة. كان معدل الإندماج الطبقي 23.4% ، 40.4% ، 66.7% في الأسماك المعاملة ، بينما كان هذا المعدل 0.1% في المجموعة الضابطة.

SUMMARY

Evaluation of the branchial tissue response in tilapia, Oreochromis spilurus spilurus (Gunter) was made using morphometric indices. Thirty fingerlings of tilapia were exposed to a sublethal dose of 5.46 mg/l¹ of alkybenzene sulphonate (ABS) daily for 21 days. Twenty fingerlings were kept under the same conditions but without adding the

detergent and served as control. The gills of the treated fish (ten fish per interval) were sampled at 7, 14, and 21 days post-exposure. While, the gills of control fish were taken at the start and end of the experiment. The second left gill arches of both treated and control fish were fixed immediately in 10% formalin and processed for light microscopy. The mucus and chloride cells indices were decreased by the first and second week and then increased by the third week post-exposure in treated fish compared to the control. The lamellar hyperplasia index was 25.5 %, 63.4, and 66.7% at 7, 14, 21 days post - exposure, respectively compared to the control (8.66%). While, the lamellar fusion index was 33.4%, 50.4%, and 66.7% in treated fish compared to the control (0.1%). Lamellitis neither thrombi indices in both treated and control fish were not evident.

Key words: *Branchial tissue response, Tilapia, Oreochromis spilurus spilurus*

INTRODUCTION

Our previous report on the acute toxicity of alkylbenzene sulphonate (ABS) in tilapia, *Oreochromis spilurus spilurus* (Gunter) determined the 96h-LC₅₀ to be 21.87 mg/l⁻¹ under hypersaline water condition. Moreover, the study described the alterations in the gills, skin, and brain in the acutely exposed fish and concluded that ABS is toxic to tilapia (Afifi et al., In press).

Teleost branchial tissue responds in a fairly restricted fashion to diverse environmental toxins (Mallatt, 1985). Common lamellar changes, including edema, fusion; epithelial necrosis; hyperplasia and altered ratios of mucus and chloride cells, were reported (Mitchell & Cech 1983; Mallatt, 1985 and Ewing et al., 1994). Sanchez et al. (1997) stated that rainbow trout, *Oncorhynchus mykiss* (Walbaum) treated with chloramine-T had a significant increased number of mucus cells on lamellae, but no effect on chloride cell, lamellar fusion, lamellar edema and lamellitis were noticed. Several studies have evaluated branchial tissue response to different toxins using morphometric indices such as chloride and mucus cell numbers, lamellar fusion, lamellar edema and lamellitis (Powell et al., 1995; Ewing et al., 1994; Sanchez et al., 1997).

The present study reports the assessment of branchial tissue response of tilapia, *Oreochromis spilurus spilurus* (Gunter) exposed to a

sublethal dose of ABS detergent under hypersaline water condition using some morphometric indices.

MATERIALS and METHODS

Fifty tilapia, *Oreochromis spilurus spilurus* (Gunter) fingerlings were collected from the outdoor tanks facility of the Fish Farming Center and acclimated to the indoor tanks for one week. Fish were randomized into two groups in two separate tanks containing 300L static seawater (42g/L⁻¹, salinity). Fish were fed on commercial crumbles of 36% protein, 4mm diameter once a day up to satiation during the acclimation and experiment.

Treated group (thirty fish) were exposed daily by adding ABS in a sublethal dose of 5.46 mg/l⁻¹. While, the control group (Twenty fish) was kept under the same conditions but without detergent. The second left gill arches of the treated fish were taken at 7, 14, and 21 days post-exposure (Ten fish each interval). While, the gill arches of control group were sampled at the start and end of the experiment. Samples were taken immediately, fixed in 10% formalin, dehydrated, sectioned at 4-6 μ m, stained by H. & E. and examined by light microscopy.

Slides were coded and examined without knowledge of the treatment allocation. Three filaments, centre filament, and a ventral and dorsal filament at a distance of 0.5 cm from the centre filament from the second left gill arch from every fish were observed. Morphometric indices based on a method of proportional morphometry developed for branchial tissue which were used by Speare and Ferguson (1989). Specifically, each selected filament was evaluated for the following indices:

- (1) Mucus cell numbers: the number of all mucous cells was assessed on the lamellae and dividing this value by the number of lamellae counted.
- (2) Chloride cell number: The number of chloride cells was assessed by counting those chloride cells whose apical-base axis was perpendicular to the long axis of the lamellae.
- (3) Lamellar hyperplasia: The percentage of lamellae with one or more points of thickened areas of epithelial tissue.
- (4) Lamellar fusion: The percentage of lamellae that were fused to adjacent lamellae.
- (5) Lamellitis: The percentage of lamellae that had one or more clusters (>5 cells) of inflammatory cells located in the regions between the pillar cells and the outer layer of lamellar epithelium.

(6) "Thrombi": The percentage of lamellae with one or more foci of accumulated cells and fibrin in the vascular channels of the lamella.

RESULTS

Subacute exposure of ABS detergent using a dose of 5.46 mg/l¹ daily in a static seawater (42g/L¹) resulted in reduced growth of fish compared to the control by the end of experiment (Table 1).

Table 1: Effect of subacute exposure of ABS detergent on the growth (per gram body weight) of *O. spilurus* under hypersaline water condition.

Control		Treated	
Start	End	Start	End
8.3±2.62	13.34±1.9	7.54±1.89	8.37±2.0

Table 2 showed a decrease in the mean number of mucus cells per lamella in treated fish at 7; 14 days post-exposure followed by an increased number at 21 days post-exposure compared to the control.

Table 2: Mean number of mucus cells (X40) per lamella in control and treated fish.

	Control X̄	Treated		
		1 st week	2 nd week	3 rd week
Dorsal	15.34	8.67	12.34	25.00
Central	10.67	3.67	10.00	16.34
Ventral	9.00	3.00	6.00	18.67
Total mean / 3 filaments	11.67	5.11	9.44	20.00

The chloride cell numbers showed a similar pattern to the mucus cell number (Table 3 & Fig. 1).

Table 3: Mean number of chloride cells (X40) per lamella in control and treated fish.

	Control X̄	Treated		
		1 st week	2 nd week	3 rd week
Dorsal	7.67	8.33	5.00	22.67
Central	2.67	1.33	0.34	7.00
Ventral	3.33	0.00	0.00	1.67
Total mean / 3 filaments	4.56	3.56	1.78	10.44

Treated fish with ABS showed an increase in the percentage of lamellar hyperplasia and lamellar fusion indices during the course of experiment compared to the control. There was no evidence for lamellitis neither thrombi indices (Table 4; Figs. 2,3,4).

Table 4: Mean percentage of gill lamellae with specific microscopic changes.

	Control	Treated		
		1 st Week	2 nd Week	3 rd Week
Lamellar hyperplasia	8.66	25.5	63.4	66.6
Lamellar fusion	0.10	33.4	50.7	54.7
Lamellitis	0.00	00.0	00.0	00.0
Thrombi	0.00	00.0	00.0	00.0

DISCUSSION

The present study describes the morphometry of the tilapia gills after being exposed daily to a sublethal dose of 5.46 mg/l¹ in a static seawater (42g/L¹, salinity). Decreased growth of treated fish compared to control by the end of experiment in this study was noticed. It is known that gill damage induced by some toxicants may affect feed conversion and somatic growth (Snachez *et al.*, 1997).

Branchial epithelium is comparatively vulnerable to environmental toxins because it is in direct contact with the medium in which toxins are delivered (Wendelaar & Vander Meij, 1989). Many waterborne toxins, gill diseases, and some therapeutic agents such as formalin, chloramine-T produce a hyperplastic reaction of the branchial mucus cells (Ferguson *et al.*, 1992 and Speare *et al.*, 1997). Moreover, mucus is a stereotypical response of fish gills to water-borne toxins (Mallatt, 1985). While, chloride cells are known to play a key role in absorption and excretion of ions as well as acclimation of tilapia species to seawater salinity (Jonassen *et al.*, 1997). The present study showed a decrease in the numbers of both mucus and chloride cells at the first and second week post-exposure followed by an increase at the third week post-exposure. These results suggest that ABS had a delayed rather than immediate effect on the mucus and chloride cell indices.

The present study shows an increase in the mean percentage of lamellar hyperplasia and lamellar fusion in treated fish with ABS compared to the control. These results suggest that ABS induced such specific microscopic changes in a time-dependent trend. Previous studies

have been reported lamellar hyperplasia, and lamellar hypertrophy in response to different types of toxins (Smith, 1972 and Klontz et al., 1984; Albassam et al., 1987). While lamellitis and thrombi indices were not evident in fish exposed to ABS. This is in agreement with previous reports on the use of chemicals or toxins induced gill lesions (Mallatt, 1985).

In conclusion, the present study shows that morphometry can be a reliable tool for assessment of branchial tissue response to the ABS detergent in tilapia.

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LEGENDS OF FIGURE

- Fig. 1:** Gills of *Oreochromis spilurus spilurus* exposed to a 5.46 mg/l^{-1} ABS daily for 3 weeks showing the chloride cells (c) having eosinophilic cytoplasm, rounded nuclei and located perpendicular at the lamellar trough. H. & E. X 132
- Fig. 2:** Gills of *Oreochromis spilurus spilurus* exposed to a 5.46 mg/l^{-1} ABS daily for 3 weeks showing lamellar fusion (F). H. & E. X 132.
- Fig. 3:** Gills of *Oreochromis spilurus spilurus* exposed to a 5.46 mg/l^{-1} ABS daily for 3 weeks showing Lamellar hyperplasia (H); mucus cells (m). H. & E. X 132.
- Fig. 4:** Control Gills of *Oreochromis spilurus spilurus* showing the normal architecture of the gill tissue. H. & E. X 132.

