

GENOTOXIC EFFECTS OF BUTATAF HERBICIDE ON NILE TILAPIA

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Abstract

This study was carried out to assess the capability of butataf herbicide in inducing cytogenetic damage of Nile tilapia, *Oreochromis niloticus*. The 96 hours half lethal concentration (96 hr LC₅₀) of the butataf herbicide determined for the adults *O. niloticus* was 0.2 ppm. The field concentration of this herbicide was 0.004 ppm which corresponds to 1/50 of the LC₅₀. The present experimental assay was carried out on the 30 day of exposure to the 1/100, 1/50, 1/25 LC₅₀ and 1/10 LC₅₀ which were used to study the effect of butataf on the rate of cell proliferation as well as the possible chromosomal aberrations of Nile tilapia genome. The results revealed that the butataf herbicide decrease of the rate of cell proliferation (low mitotic index). The rate of cell proliferation reached 1.1% in treated Nile tilapia with the highest dose 0.02 ppm of butataf which compared with the control groups (6.7%). In addition, different types of chromosomal aberrations; i.e., fragments, chromatid breaks. Stickiness, hypo and hyperpolyploidy were observed. The study concluded that butataf may have a genotoxic effect on tilapia fish.

Keywords: genotoxicity, Nile tilapia, butataf herbicide, mitotic index, chromosome aberrations.

INTRODUCTION

In order to detect genotoxic activity in an aquatic environment, a cytogenetic investigation on fish was carried out by several authors. Cytogenetic observations were primarily based on chromosomal aberration analysis, although such tests exhibit some disadvantages

such as low mitotic activity, the difficulty of finding a sufficient number of metaphases for scoring chromosome aberrations and the limitation of a suitable fish karyotypes (Kligerman, *et al.*, 1975; Hooftman, 1981; Al-Sabti, 1991). The study of toxicity should be more concerned with sublethal effects and the sublethal study has been forced because of need to find the safe concentration of the pollutants (Johnson, 1968). Tooby (1971) recorded many differences in the toxicities of different herbicides even among different forms of the same herbicide. Butataf herbicide is used to eliminate noxious weeds in the rice fields; unsuspected side effects on fish are to be expected (Svobodova *et al.*, 1993). Generally, genotoxic effects of some mutagenic and/or carcinogenic chemicals on fish cells should be done in a dose-response manner. It was proven that there exists a species-response between different species of the same family (Cyprinids) to the same dose of the same chemical (Al-Sabti 1985, 1986). Negative and positive controls must also be carried out to ensure reliable results about the genotoxicity of the chemicals investigated. Since many types of DNA damage caused by mutagens present in water induce alteration in chromosomes, the measurement of chromosomes aberrations offers an acceptable parameter for monitoring mutagenic substances in water. Moreover, chromosomes aberrations selectively count only the primary DNA lesions that are not repaired by the machinery of the cell (Evans 1977 & Obe *et al.* 1982). Because of lack of suitable karyotypes in most of fish, testing the genotoxic potential of an agent on fish has to be mainly extrapolated from cytogenetic bioassays which carried out only on some particular fish species having suitable karyotypes. Therefore, it could be added benefit to find toxicological endpoints other than the conventional ones that could help determine genotoxicity, particularly in those fish that have unsuitable karyotypes for genotoxicity assessment. Meanwhile, evaluation of additional parameters such as a

qualitative assay of protein profiles was taken up in two fish models, *O. mossambicus* and *A. testudineus* (Guha and Khuda-Bukhsh, 2002). In this regards, Guha and Khuda-Bukhsh (2003) studied the genotoxic effects in fish *Oreochromis mossambicus* exposed to different doses of β -carotene (0.02, 0.05 and 0.1 %) separately and conjointly with 0.2% ethylmethane sulphonate during five different time periods. The relative efficacy of three doses of 0.02, 0.05 and 0.1%, β -carotene in ameliorating genotoxic effects of 0.2% EMS was also tested after a treatment period of 48hr. The results indicated that ethylmethane sulphonate caused chromosomes aberrations and an apparent alteration of protein synthesis in various tissues. Some of these genotoxic effects of ethylmethane sulphonate appeared to be improved by all three doses of β -carotene, of which the 0.02% dose showed a marginally better efficacy.

In this work one bioassay was employed. Which is: chromosomal aberrations (CA) in Nile tilapia fish which were treated with butataf herbicide.

MATERIALS AND METHODS

Apparently healthy Nile tilapia, *Oreochromis niloticus* specimens with an average body weight of 100 ± 5 g were obtained from Abbassa Fish Farm, Abbassa, Abou-Hammad, Sharkia and they were acclimated in laboratory conditions for two weeks prior to start the experiment. The 96 hour half lethal concentration (96 hr LC₅₀) of the butataf herbicide (N-Butoxymethyl-2-chloro-2, 6-diethylacetanilide) was determined according to Behreus and Karbeur (1953) as 0.2 ppm and was applied to field with a concentration of 0.004 ppm. Five groups of fish were kept for 30 days to evaluate the effect of different sublethal concentrations for butataf herbicide on cytogenetic behavior. Each group consisted of 30 fish was divided into three replicates of ten fish. Then maintained in glass aquaria supplied

with de-chlorinated aerated water at a temperature of 26 ± 2 °C, pH 7.2 ± 0.2 and dissolved oxygen 5.5 ± 0.5 mg/l. The first group was kept as control. Other groups of applied fish were exposed to the 1/10, 1/25, 1/50 and 1/100 LC₅₀ (0.02, 0.008, 0.004 & 0.002 ppm), respectively. The feeding rate was 3% of the live body weight daily using 30% of protein feed mix.

Cytogenetic analysis

1- Mitotic index was calculated according to Brusick (1980) as in following equation:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Number of total examined cells}} \times 100$$

2- Chromosomal aberrations

Chromosomes preparations were made at the end of the experiment from the gills by modified method of Al-Sabti *et al.* (1983). Fish were injected i.p. (intraperitoneal) with doses of 0.01 ml/g body weight of 0.6% solution of colchicines for 3hr and then gill filament tissues were removed and placed in hypotonic 0.005 N KCl solutions for 20 min. The tissues were snipped and fixed in fresh and cold fixed solution methanol/ glacial acetic acid (3:1) for 40 min. By pastier pipette dropping on a clean and cold (-5 °C) slide was done. The slides were dried by alcohol lamp and left at room temperature for 3 hr before staining. Slides were stained with 10% Giemsa in phosphate buffer pH 6.8 for 15 min. About 100 well-spread, metaphase figures of control and each of the five treated groups were microscopically examined using oil immersion lens to calculate the number of selected aberrant metaphase and the normal ones.

Statistical analysis

All statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) program for windows (ver. 10.0).

RESULTS AND DISCUSSION

After exposure of Nile tilapia; *Oreochromis niloticus* to different concentrations of the butataf herbicide, the 96 hour half lethal concentrations (96 hr LC₅₀) was 0.2 ppm, and field concentration in nature (0.004 ppm) equal to 1/50 of the half lethal concentration are given in Table (1). Consequently, according to Bathe *et al.* (1974) the butataf is considered as highly toxic herbicide to *O. niloticus* fish. During determination of the 96-hr LC₅₀, fish exhibited erratic swimming movements. The skin had a spotted appearance and mucus secretion increased and accumulated on the gills, so the fish exhibited a different respiratory manifestation such as surfaced swimming, opening their mouth with rapid and frequent respiratory movement. The erratic swimming and surfaced fairly frequently movements may be due to hyper-contraction of the muscles which due to cholinesterase inhibition as previously reported by Ferguson (1989) while Atallah, *et al.* (1997) attributed such changes to the extraordinary need for the oxygen which due to the thick coating of the gills with profuse mucus together with congestion and hyperplastic epithelium of the secondary lamellae. The mottled appearance of skin and heavy mucus secretion are due to the melanosis (aggregation of melanocytes) happened by the stimulation of α -melanocyte stimulating hormone (α -MSH) secretion in fish which was subjected to stressors and irritation of the gills which subjected to pollutants (Satchell, 1984 and Ferguson, 1989).

Table (1): Different sublethal concentrations in ppm of butataf herbicide for Nile tilapia.

Concentrations Fish species	96 hr LC ₅₀ (ppm)	¹ / ₁₀₀ LC ₅₀ (ppm)	¹ / ₅₀ LC ₅₀ (ppm)	¹ / ₂₅ LC ₅₀ (ppm)	¹ / ₁₀ LC ₅₀ (ppm)
<i>Oreochromis niloticus</i>	0.20	0.002	0.004	0.008	0.02

Cytogenetic analysis

1- Mitotic activity

Cell proliferation in gills of Nile tilapia treated with different concentrations of butataf herbicide as represented by mitotic indices is presented in Table (2). Mitotic indices of the control group showed to be higher than that of treated *O. niloticus*. The results showed a gradual decline in mitotic indices with increasing of the dose of butataf herbicide. This decrease ranged from 3.7 to 1.1 % at all doses of butataf herbicide when compared with that of the control group (6.7%). These results are in agreement with (Shaban, 1999) who obtained low mitotic index in both Nile tilapia and common carp after treatment with other toxic compound (Aflatoxin β_1).

2- Chromosome aberrations

As presented in Figure (1 a) of this study, metaphase of Nile tilapia, *O. niloticus* consists of 22 pairs with no morphologically distinct sex chromosomes (Majumdar and McAndrew, 1986). Tables (2 & 3) and Figures (1 b to d) show the different types of structural and numerical chromosomal aberrations which were obtained after exposure of Nile tilapia to butataf herbicide. The statistical analysis revealed highly significant ($p < 0.01$) effect of butataf herbicide in inducing chromosomal aberrations. The total aberrant metaphases in

fishes ranged from 18 to 42% after exposure to butataf as compared to the control group (4%). Different types of structural aberrant were represented by chromosomal deletion, chromosomal fragments, chromosomal breaks and chromosomal stickiness, while the numerical aberrations were represented by hyper and hypoploidy. The number of aberrant metaphase was increased with increasing of the dose of butataf herbicide. The most frequent type of aberrations induced at 1/10 dose level was stickiness 15 %. These results nearly are in agreement with (Shaban, 1999) who found that the most frequent type of aberrations increased with increasing the dose of AF β ₁.

The use of fish chromosomes in genotoxic investigations has been little practiced, in spite of the fact that the fishes make up the largest and most diverse group of vertebrates. Fish have been proven to be important animals in experimental laboratory work not only for cytotoxicological and different genetic studies, but also for biochemical and physiological research. The use of fish cells for the study of genotoxic effects has only recently been explored. Unfortunately, only a small number of fish species are suited for cytogenetic investigations because of their large number of chromosomes and /or their small size; moreover the mitotic index in fish is too low when compared to that in mammals (Al-Sabti 1991).

As fish may act as 'sentinel' organisms for indicating aquatic pollution, several species have been successfully used as test materials for detecting genotoxic activity in the aquatic environment, (Hooftman, 1981; Kligerman, *et al.*, 1975; Manna & Mukherjee, 1989). Analysis of metaphase chromosomes in fish for the occurrence of chromosome aberrations and sister-chromatid exchange (SCE) in order to detect as well as quantify the extent of genotoxicity or point mutation induced by an agent has proven to be useful only in a few fish models (Kligerman, 1982; Manna, 1984). The majority of fish species

are not suitable for the chromosomal assay because of their small size and large diploid number of chromosomes without any marked variation in size. Therefore, assay of the peripheral erythrocytes in fish for the occurrence of micronuclei (MN) and different types of nuclear abnormalities has been adopted as a good substitute for the chromosomal assay, and based on information on the frequency of occurrence of micronuclei or nuclei with abnormal shape, a monitoring system for potential genotoxicity of an agent has been proposed (Hooftman & de Raat, 1982; Manna, *et al.*, 1985; Guha & Khuda-Buksh, 2001 and Ramadan, 2005).

Table (2): Mitotic indices of gills cells of Nile tilapia after treatment with butataf herbicide.

Concentrations	No. of divid. cells	No. of non-divid. cells	Total No. of cells	Mitotic indices	Chi square value
Control	30	416	446	6.7	
$1/100$ LC ₅₀	16	418	434	3.7	4.1*
$1/50$ LC ₅₀	12	430	442	2.7	7.9*
$1/25$ LC ₅₀	6	442	448	1.3	16.8***
$1/10$ LC ₅₀	5	454	459	1.1	19.3***

* Significant at $p < 0.05$

***highly significant at $p < 0.01$

Table (3): Chromosome aberrations in gills cells of Nile tilapia after treatment with butataf herbicide.

Concentrations	No. of examined metaphase	Total No. of aberration metaphase	Structural chromosomes aberrations				Numerical chromosomes aberrations	
			D	F	B	S	Hyperpoly ploidy	Hypopoly ploidy
Control	100	4	0	1	1	2	0	0
$1/100$ LC ₅₀	100	18	2	4	3	6	2	1
Chi square value		8.1***	1.98	1.75	0.98	1.9	1.98	0.99
$1/50$ LC ₅₀	100	26	3	5	4	9	3	2
Chi square value		14.6***	2.95	2.59	1.75	4.2*	2.95	1.98
$1/25$ LC ₅₀	100	35	4	6	5	13	3	4
Chi square value		20.97***	3.9*	3.45*	2.59	7.5**	2.95	3.9*
$1/10$ LC ₅₀	100	42	5	7	6	15	4	5
Chi square value		26.2***	4.88*	4.33*	3.45*	9.2***	3.9*	4.88*

* Significant at $p < 0.05$ ***highly significant at $p < 0.01$ D = Chromosome deletion
 F = Chromosome fragments B = Chromosome breaks S = Chromosome stickiness

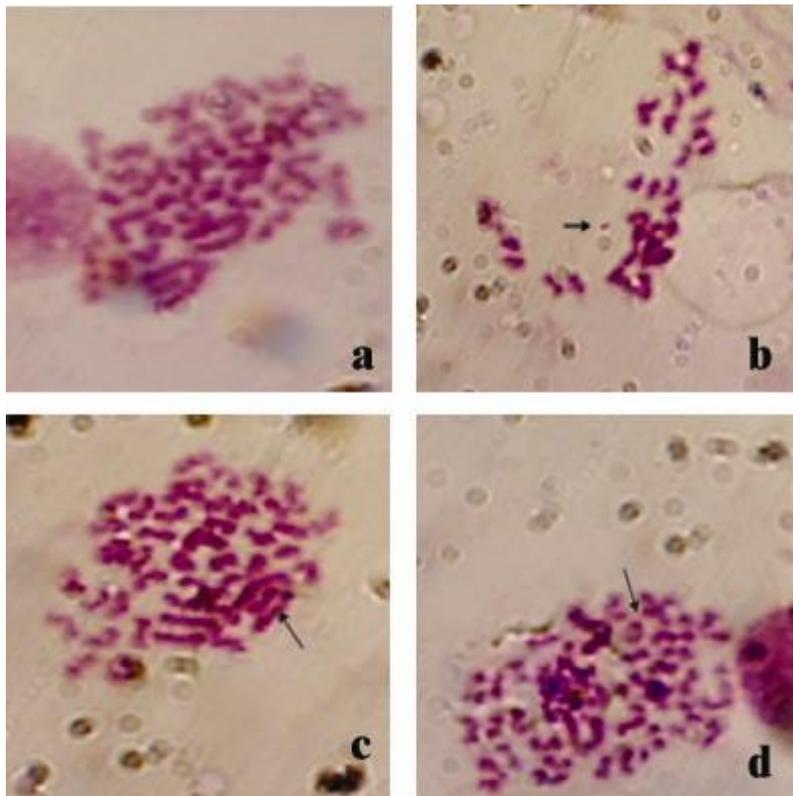


Figure (1): Photomicrograph slides of gills cells showing normal metaphase (a); chromosome fragments (b); chromosome stickiness (c) and ring chromosome (d) of Nile tilapia.

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تأثير السمية الوراثية لمبيد الحشائش البيوتاتاف على البلطي النيلي

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الملخص العربي

نظراً لاستخدام البيوتاتاف كمبيد عشبي لمقاومة الحشائش في حقول الأرز كان لابد من دراسة خطورته على الأسماك التي تعيش في مياهها. ودفعنا ذلك لدراسة مدى خطورة هذا المبيد على أسماك البلطي النيلي و التي تعد واحدة من أهم الأسماك النيلية في مصر. وقد استهدف البحث دراسة التغيرات السيتولوجية من استخدام هذا المبيد على أسماك البلطي النيلي لمدة ٣٠ يوم. حيث تم تعيين الجرعة النصف مميتة خلال ٩٦ ساعة فوجدت أنها تساوي ٠.٢ جزء في المليون. وعرضت مجموعات من الأسماك محل الدر اسه إلى ١٠٠/١ و ٥٠/١ و ٢٥/١ و ١٠/١ جزء في المليون من الجرعه النصف مميتة وتمت المقارنة مع المجموعة الضابطة. و قد أظهرت نتائج البحث انخفاض معدل الانقسام الميتوزي بزيادة التركيز من ٦.٧ إلى ١٠.١ ٪. وارتفاع معدل التشوهات الكروموسومية و إن بدت أعلاها في صورة فجوات و كسور ونقص والتصاقات.

وبهذا فان النتائج تشير إلى أن مبيد الحشائش " البيوتاتاف " يمثل خطراً كبيراً في إحداث تغيرات وراثية (تشوهات كروموسومية) مشيراً إلى خطورته على الجهاز الوراثةي للأسماك التي تعيش في المياه الملوثة بهذا المبيد. لذلك يجب البحث عن بدائل أخرى أكثر أماناً وسلامة منه للحفاظ على الثروة القومية والصحة العامة للمواطنين.