THINK INDIA JOURNAL

ISSN:0971-1260

Vol-22- Issue-14-December-2019

Induction Of Nuclear Abnormalities (Na) In Erythrocytes Of Fish, *Etroplus Suratensis* Following The Exposure Of Pyrethroid Insecticide, Lambda-Cyhalothrin

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ABSTRACT

Rapid industrialization, technological advancements and successful green revolution in both developed and developing countries have resulted in the introduction of a variety of toxic chemicals into the environment. Man-made toxic chemicals are released into the environment during production, transportation as well as utilization, and thus pose a threat to living biota. Insecticidal products containing pyrethroids have been widely used to control insect pests in agriculture, public health, homes and gardens. Pesticides affect not only the physiology and survival of aquatic organisms including fish but also can interact with their genetic material which may lead to the mutations or carcinogenesis. Genotoxicity not only reduces the 'fitness' (i.e. growth, fertility and fecundity) in wild fish populations, but also pose risk to human health via food chain. Hence, the present investigation has been made to detect the nuclear abnormalities (NA) in erythrocytes of fish, *Etroplussuratensis* under the influence of lambda (λ) -cyhalothrin. The blood samples obtained from control and different sub-lethal concentrations of lambda-cyhalothrin treated fishes at 24hrs, 48hrs, 72hrs and 96 hrs of exposure. From this investigation, different doses of pyrethroid insecticide lambda-cyhalothrin induced nuclear abnormalities (binucleated, lobed and notched nuclei) in *E.suratensis* after different exposure period. In the present study various nuclear abnormalities (binuclei, lobed and notched nuclei) were identified and it revealed that such identified abnormalities may be indicators of genotoxicity.

Keywords: *Etroplussuratensis*, Erythrocytes, Genotoxicity, λ-cyhalothrin, Nuclearabnormalities.

INTRODUCTION

In genotoxic pollution, the toxicants are mostly introduced into the water bodies through anthropogenic activities such as industrial, agricultural, domestic, and urban activities. However, due to ecological-level interactions, the health of the biota that depends on it is adversely compromised through contact with hazardous chemicals capable of damaging the DNA and perpetuating the

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irreversible effects evidenced by micronuclei formations. These micronuclei serve as useful marker for environmental biomonitoring of the aquatic chemical contaminants. This damage tends to be irreversible and continues manifesting in future generations through heredity. Pesticides affect not only the physiology and survival of aquatic organisms including fish but also can interact with their genetic material which may lead to the mutations and/or carcinogenesis. Genotoxicity not only reduces the 'fitness' (i.e. growth, fertility and fecundity) in wild fish populations, but also pose risk to human health via food chain. Besides, causing mortality, these pollutants can cause genotoxicity in aquatic organisms which can lead to severe consequences in fishes like development of tumors (Folmar,1993). The aquatic ecosystem is the greater part of natural environment which is facing the threat of shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Rahman*et al.*, 2002).

The formation of nuclear abnormalities (NA's) such as lobed, blebbed, and notched nuclei described by Carrasco *et al.* (1990) has been reported in fish erythrocytes as a consequence of exposure to environmental and chemical contaminants with cytotoxic, genotoxic, mutagenic or carcinogenic activity. Ayllon and Garcia-Vazquez (2000) reported the induction of nuclear abnormalities in erythrocytes of *Phoxinusphoxinus* after treatment with colchicines, mitomycin- C and cyclophosphamide. Cavas and Ergene-Gozukara (2003b) demonstrated the induction of NA in erythrocytes of *O.niloticus* exposed to a textile mill effluent as well as to cyclophosphamide. *In situ* analysis of nuclear abnormalities in fish was first reported by Carrasco *et al.* (1990), who reported that there were no significant differences in NA frequencies for fish collected in clean and polluted areas. On the other hand, a recent study performed by Bombail*et al.* (2001) found higher erythrocyte NA's frequencies in *Pholisgunellus* specimens captured from highly polluted areas than in those captured from clean areas.

Genotoxic chemicals such as insecticides have common chemicals and physical properties that enable them interact with genetic materials (Campana*et al.*, 1999; Cavas and Ergene-Gozukara, 2003; Candioti*et al.*, 2010; Dogan*et al.*, 2011). The mutation that may result from an interaction between a chemical and the genetic material is a heritable change in the cell genotype, and thus the error may be transferred to the daughter cell or the next generation.In the present investigation has been made to detect the nuclear abnormalities under the influence of lambda-cyhalothrinon *E.suratensis*.

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MATERIALS AND METHODS

The fishes exposed to lambda-cyhalothrin at different sub-lethal concentrations of LC_{50}value for a short-term exposure of 24 hrs, 48, 72 and 96 hrs. Nuclear abnormalities were estimated following the method of Carrasco*et al.* (1990).Briefly cells with two nuclei were considered as binucleated. Nuclei with invaginations larger than those in the blebbed nuclei, including those with several lobes, were classified as lobed nuclei. Nuclei with vacuoles or voids with appreciable depth into the nucleus were recorded as notched nuclei.

Statistical analysis was carried out by two-way ANOVA and significant difference in pesticide treated group from control group for various time of exposure on NA's were represented along with mean±SD.

RESULTS

The present study, tested doses of pyrethroid insecticide lambda-cyhalothrin induced nuclear abnormalities (binucleated, lobed and notched nuclei) in *E.suratensis* after different exposure period.

Binudeated erythrocytes:

In 24 hrs exposure, there was no occurance of binuclei in control group (0) of *E.suratensis*. The binucleated erythrocytes increased from 1.33 ± 0.47, 2.67 ± 0.94, 7 ± 0.82, 8 ± 0 and 11.67 ± 0.94 at 0.005 ppm, 0.006 ppm, 0.008 ppm, 0.013 ppm and 0.026 ppm respectively (Table 1). In 48 hrs, binucleated erythrocytes varied from0.33 ± 0.47 recorded in pesticide free medium, 3 ± 0.82 in 0.005 ppm followed by3.67 ± 0.94 in 0.006 ppm, 7.33 ± 1.25 in 0.008 ppm, 10.67 ± 0.94 in 0.013 ppm and 13.33 ± 3.3 in 0.026 ppm respectively (Table 1). After 72 hrs exposure, binuclei enhanced from 0.33 ± 0.47 recorded in control medium to 2.33 ± 0.47 at 0.005 ppm, 3.33 ± 0.47 at 0.006 ppm, 8.67 ± 0.47 at 0.008 ppm, 10.67 ± 1.25 at 0.013 ppm to 15 ± 3.56 at 0.026 ppm (Table 1). In 96 hrs, the binucleated erythrocytesin control medium was 0.33 ± 0.47 and binuclei varied from 5.67 ± 0.47 in 0.005 ppm, 5.67 ± 0.47 in 0.006 ppm, 10.33 ± 0.47 in 0.008 ppm, 13.67 ± 0.47 in 0.013 ppm, 19 ± 0.82 in 0.026 ppm respectively (Table 1).

Two-way ANOVA (Table 1.1) showed the significant difference between the exposure durations with respect to the binucleus ($P<0.01$) and difference due to concentrations were statistically highly significant variation ($P<0.0001$) compared with pesticide free medium. Figure 1 was shown the variations of binucleated erythrocytes in *E. suratensis* exposed to different concentrations of lambda-

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cyhalothrin on various exposure periods. During the period of study, the binucleated erythrocytes were identified in *E.suratensis* (Plate 1).

Lobed nuclei

The *E. suratensis* exposed for 24 hrs, the lobed nuclei varied from 0 recorded in control medium 1 ± 0.82 recorded in 0.005 ppm of lambda-cyhalothrin, 2 ± 0 recorded in 0.006 ppm, 6.33 ± 0.47 in 0.008 ppm, 8.67 ± 0.94 in 0.013 ppm and 13 ± 0 recorded in 0.026 ppm against none of micronuclei was identified in the control medium 0 (Table 2). In 48 hrs, number of lobed nuclei increased from 1.33 ± 1.25 followed by 3 ± 0.82, 8.67 ± 0.47, 9 ± 1.41 and 13 ± 2.83 when exposed to 0.005 ppm, 0.006 ppm, 0.008 ppm, 0.013 ppm and 0.026 ppm against the control value of 0 respectively (Table 2). In 72 hrs exposure, lobed nuclei varied from 0.33 ± 0.47 in control group, 2 ± 0.82 in 0.005 ppm, 3.33 ± 0.47 in 0.006 ppm, 8.67 ± 0.47 in 0.008 ppm, 11 ± 0 in 0. 013 ppm to 14.33 ± 3.3 in 0.026 ppm (Table 2). In 96 hrs exposure, the lobed nuclei 0.67 ± 0.94 was recorded at control group and increase to 3.67 ± 1.89 recorded at 0.005 ppm, 5 ± 1.41 in 0.006 ppm, 10 ± 0.82 in 0.008 ppm, 12.67 ± 0.47 in 0.013 ppm and 16 ± 0.82 recorded in 0.026 ppm (Table 2).

From the two-factor ANOVA (Table 2.1) that there was highly significant difference between the exposure durations with respect to the lobed nuclei ($P<0.001$) and variations due to concentrations were statistically highly significant difference ($P<0.0001$) compared with control group. Figure 2, was shown the variations of lobed nuclei in *E. suratensis* exposed to different concentrations of lambda-cyhalothrin on various exposure periods. Plate 2 was shown the lobed nuclei identified in *E.suratensis* during the study period.

Notched nuclei

In *E. suratensis* exposed to lambda-cyhalothrin for 24 hrs, the values of notched nuclei increased from 0.67 ± 0.94 at 0.005 ppm, 1.33 ± 0.94 in 0.006 ppm, 6.33 ± 0.47 in 0.008 ppm, 8.67 ± 0.94 at 0.013 ppm and 12.33 ± 0.94 in 0.026 ppm against no notched nuclei was identified in the control group (0) during the study period (Table 3). After 48 hrs, notched nuclei enhanced 1 ± 1.41 followed by 2.67 ± 0.47, 8.67 ± 0.47, 9.67 ± 0.47 and 13.33 ± 2.05 when exposed to 0.005 ppm, 0.006 ppm, 0.008 ppm, 0.013 ppm and 0.026 ppm against no notched nuclei was identified in the control medium 0 (Table 3). In 72 hrs exposure, notched nuclei number varied from 0.33 ± 0.47

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(control) to 2 ± 0.82 (0.005 ppm), 3.33 ± 0.47 (0.006 ppm), 8.67 ± 0.47 (0.008 ppm), 11.33 ± 0.47 (0. 013 ppm) 14.33 ± 3.68 (0.026 ppm) and were represented in Table 3. The 96 hrs exposure, notched nuclei increased from 0.67 ± 0.94 recorded at control group to 3 ± 0 recorded at 0.005 ppm, 5.33 ± 1.7 in 0.006 ppm, 10 ± 0 in 0.008 ppm, 12 ± 0.82 in 0.013 ppm and 17 ± 1.41 recorded in 0.026 ppm respectively (Table 3).

Two-way ANOVA (Table 3.1) indicated that, there were highly significant difference between the exposure durations with respect to the notched nuclei (P<0.001) and notched nuclei variations due to concentrations were statistically highly significant (P<0.0001) compared with control. Figure 3 was shown the variations of induction of notched nuclei formation in *E. suratensis* exposed to different concentrations of lambda-cyhalothrin on various exposure periods. The numbers of notched nuclei were identified in blood erythrocytes in *E.suratensis* (Plate 3).

Table 1:Binucleus in erythrocytes of *E.suratensis*exposed to different sub-lethal concentrations of lambda-cyhalothrin

Exposure period (hrs)	Control	Concentration of lambda-cyhalothrin (ppm)				
		0.005	**0.006**	**0.008**	**0.013**	**0.026**
24	0	1.33 ± 0.47	2.67 ± 0.94	7 ± 0.82	8 ± 0	11.67 ± 0.94
48	0.33 ± 0.47	3 ± 0.82	3.67 ± 0.94	7.33 ± 1.25	10.67 ± 0.94	13.33 ± 3.3
72	0.33± 0.47	2.33 ± 0.47	3.33 ± 0.47	8.67 ± 0.47	10.67 ± 1.25	15 ± 3.56
96	0.33 ± 0.47	5.67 ± 0.47	5.67 ± 0.47	10.33 ± 0.47	13.67 ± 0.47	19 ± 0.82

Values are expressed as mean ± SD

Table 1.1: Two-way ANOVA showed the binucleated erythrocytes on*E.suratensis*

Source of Variation	SS	df	MS	F	P-value
Variation due to exposure duration	48.68056	3	16.22685	13.38808	P<0.01
Variation due to concentration	588.6898	5	117.738	97.14057	P<0.0001
Error variance	18.18056	15	1.212037		
Total variance	655.5509	23			

SS - sum of squares **df** - degrees of freedom **MS** - mean of squares

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Table 2: Lobed nuclei in erythrocytes of *E.suratensis* exposed to different sub-lethal concentrations of lambda-cyhalothrin

Exposure period (hrs)	Control	Concentration of lambda-cyhalothrin (ppm)				
		0.005	0.006	0.008	0.013	0.026
24	0	1 ± 0.82	2 ±0	6.33 ± 0.47	8.67 ± 0.94	13 ± 0
48	0	1.33 ± 1.25	3 ± 0.82	8.67 ± 0.47	9 ± 1.41	13 ± 2.83
72	0.33 ± 0.47	2 ± 0.82	3.33 ± 0.47	8.67 ± 0.47	11± 0	14.33 ± 3.3
96	0.67 ± 0.94	3.67 ± 1.89	5 ± 1.41	10 ± 0.82	12.67 ± 0.47	16 ± 0.82

Values are expressed as mean ± SD

Table 2.1: Two-way ANOVA showed the lobed nuclei in erythrocytes of *E.suratensis*

Source of Variation	SS	df	MS	F	P-value
Variation due to exposure duration	26.68056	3	8.893519	22.182448	P<0.001
Variation due to concentration	580.6343	5	116.1269	289.64665	P<0.0001
Error variance	6.013889	15	0.400926		
Total variance	613.3287	23			

SS - sum of squares **df** - degrees of freedom **MS** - mean of squares

Table 3: Notched nuclei in erythrocytes of *E.suratensis* exposed to different sub-lethal concentrations of lambda-cyhalothrin

Exposure period (hrs)	Control	Concentration of lambda-cyhalothrin (ppm)				
		0.005	0.006	0.008	0.013	0.026
24	0	0.67 ± 0.94	1.33 ± 0.94	6.33 ± 0.47	8.67 ± 0.94	12.33 ± 0.94
48	0	1± 1.41	2.67 ± 0.47	8.67 ± 0.47	9.67 ± 0.47	13.33 ± 2.05
72	0.33 ± 0.47	2 ± 0.82	3.33 ± 0.47	8.67 ± 0.47	11.33 ± 0.47	14.33 ± 3.68

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96	0.67 ± 0.94	3 ± 0	5.33 ± 1.7	10 ± 0	12 ± 0.82	17 ± 1.41

Values are expressed as mean ± SD

Table 3.1: Two-way ANOVA showed the notched nuclei in erythrocytes of *E.suratensis*

Source of Variation	SS	df	MS	F	P-value
Variation due to exposure duration	31.01852	3	10.339506	22.214854	P<0.001
Variation due to concentration	609.9815	5	121.9963	262.11406	P<0.0001
Error variance	6.981481	15	0.4654321		
Total variance	647.9815	23			

SS - sum of squares **df** - degrees of freedom **MS** - mean of squares

Figure 1: Variations of binucleated erythrocytes in *E. suratensis* exposed to lambda-cyhalothrin for different exposure period

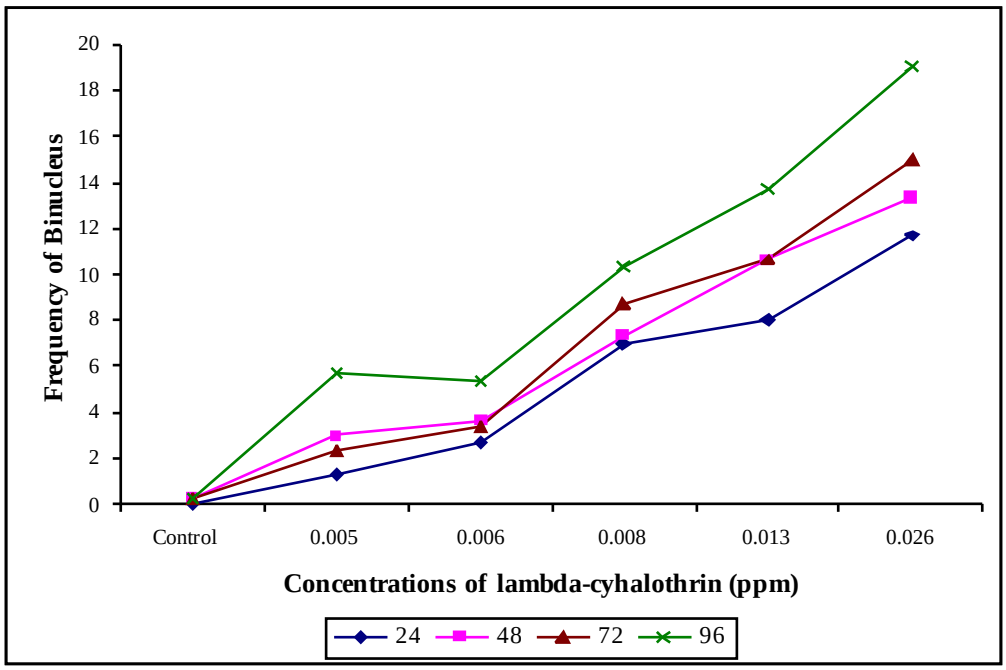

Plate 1: Binudeated erythrocytes observed in *E.suratensis* exposed to lambda-cyhalothrin

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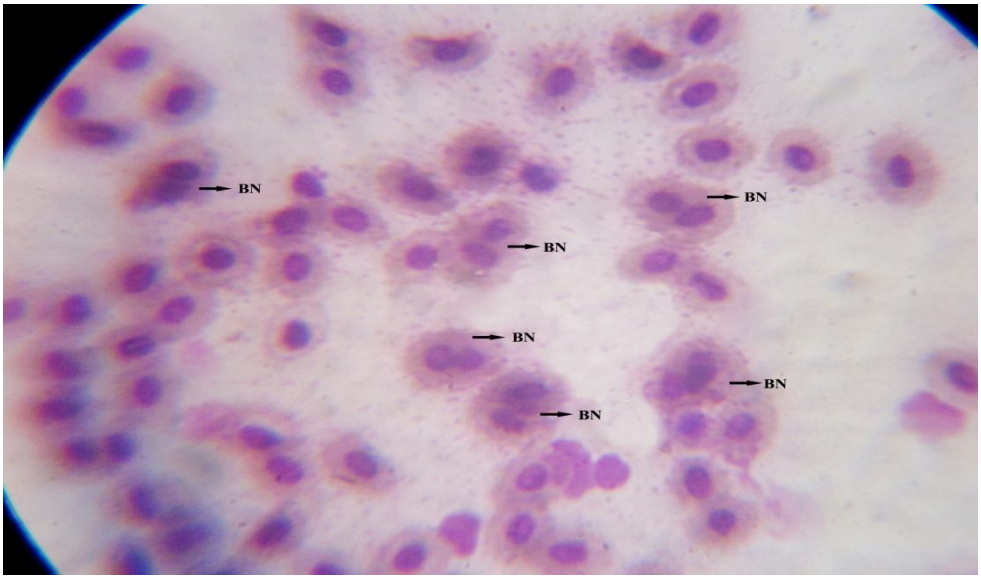

BN- Binucleated erythrocytes

Figure 2: Variations of lobed nuclei in *E. suratensis* exposed to lambda-cyhalothrin for different exposure period

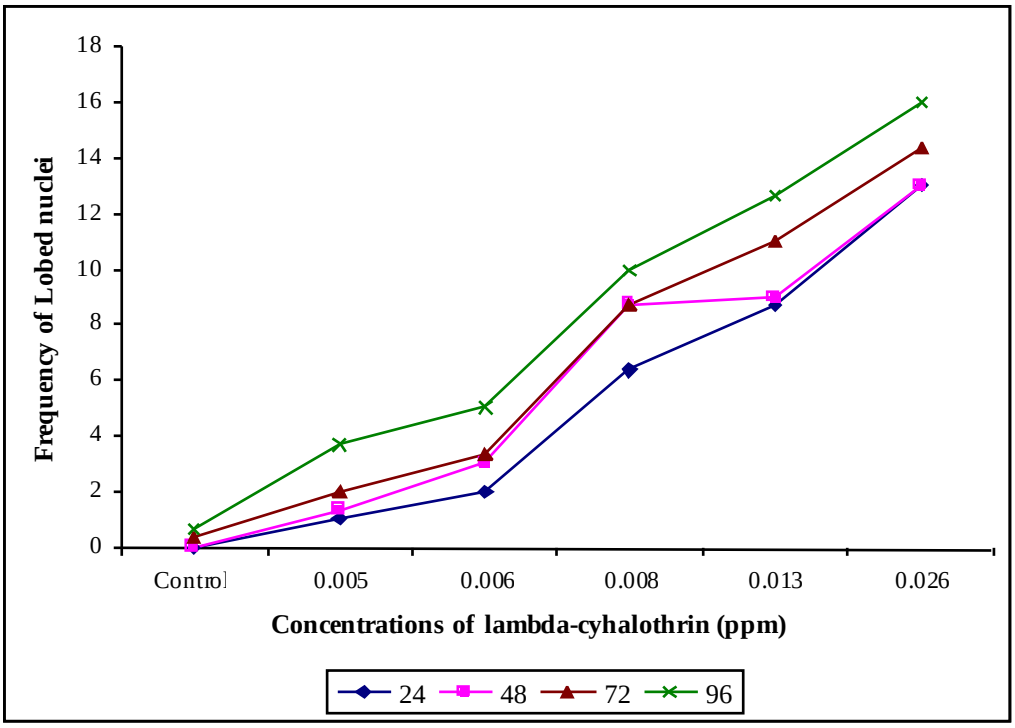

Plate 2: Lobed nucleiobserved in *E.suratensis*exposed to lambda-cyhalothrin

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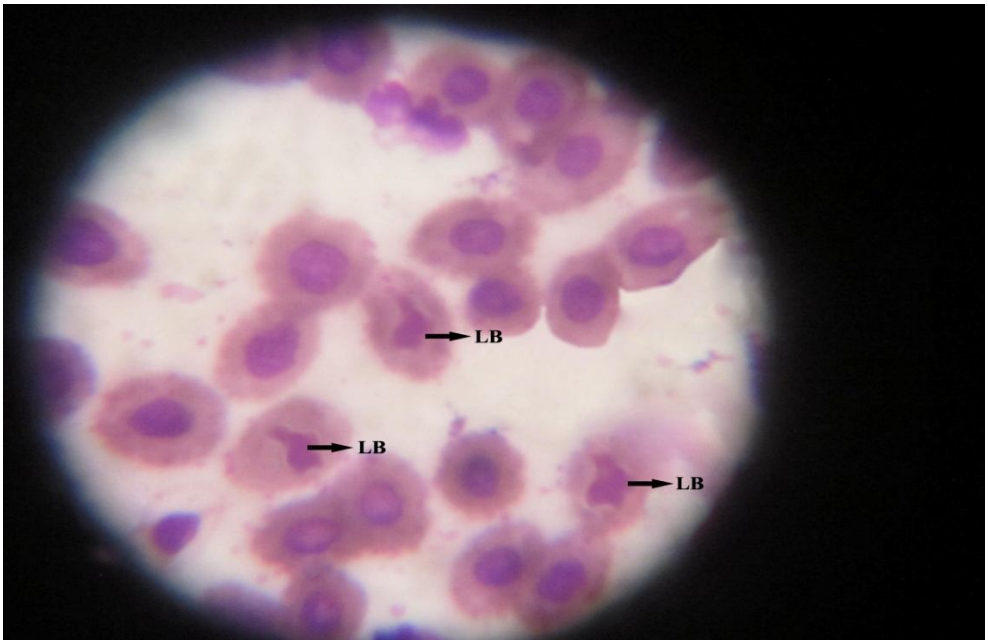

LB- Lobed nuclei

Figure 3: Variations of notched nuclei in *E. suratensis*exposed to lambda-cyhalothrin for different exposure period

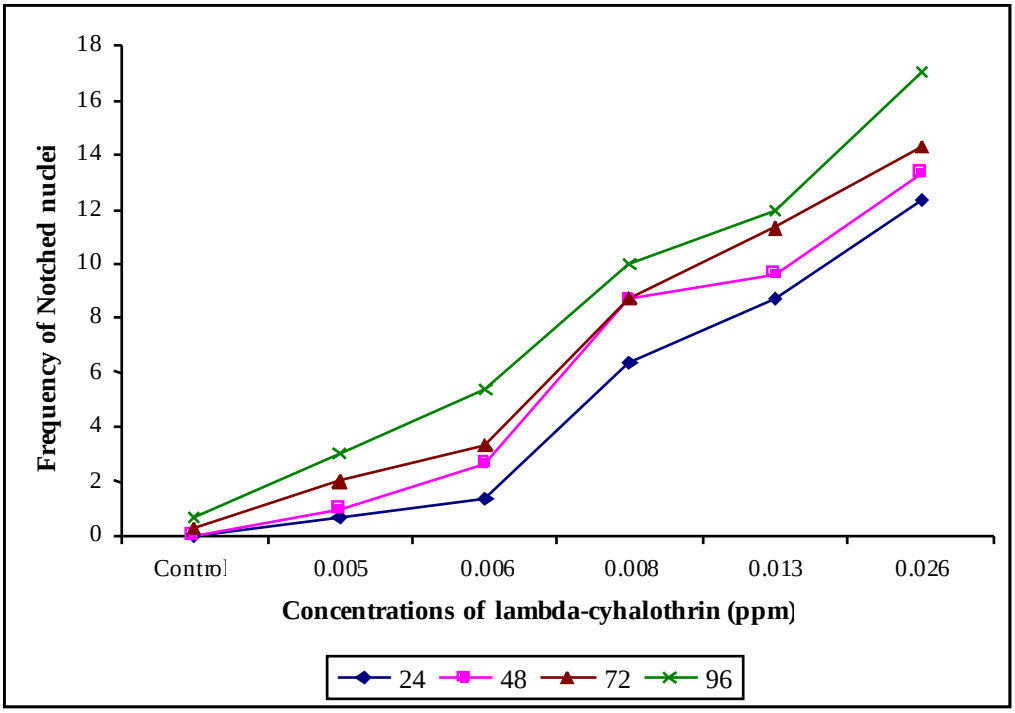

Plate 3: Notched nucleiobserved in *E.suratensis*exposed to lambda-cyhalothrin

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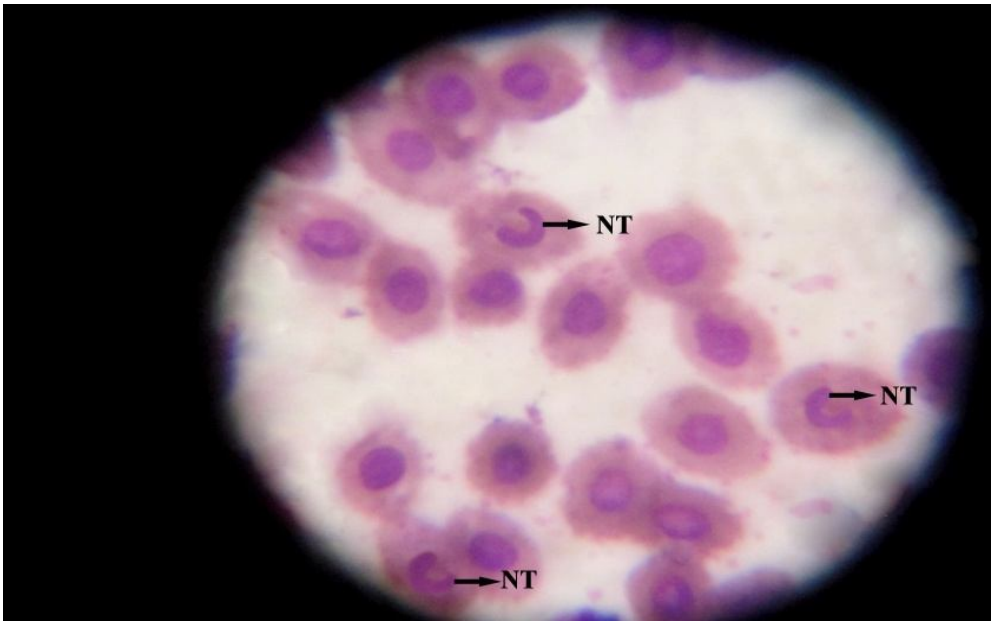

NT- Notched nuclei

DISCUSSION

The response of *E.suratensis* in micronuclei formation and nuclear abnormalities after treatment of lambda-cyhalothrin, showed increased from lowest concentration to highest concentration than the control. The results of this study coincide with the observations of Madhu (1995) who reported that the pesticides like methyl parathion, phosphamidon, dichlorvos, monocrotophos and malathion induced nuclear abnormalities formation in *Liza parsia* and *Mugilcephalus*.

Maximum numbers of nuclear abnormalities were observed in the *E.suratensis* exposed to highest dosage of lambda-cyhalothrin (0.026 ppm) for 96 hrs. In other,*E. suratensis* exposed to lower concentration (0.005 ppm) with 24 hrs exposure the nuclear abnormalities formation were higher than that of control animals. Hence it was concentration dependent manner. Increased NA's incidences after lambda-cyhalothrin exposure might be due to the inability of the fish to detoxity the poison and the existing unexcreated toxic metabolites effect leading to the loss in integrity of nuclear membrane and membrane bound enzymes by enhancing lipid peroxidation. The time dependent nuclear abnormalities might be due to the effect of lambda-cyhalothrin on erythropoietic cells rather than on the circulating erythrocytes.

Some of the changes such as binucleated erythrocytes (Plate 1), lobed nuclei (Plate 2) and notched nuclei (Plate 3) were identified on lambda-cyhalothrin treated *E. suratensis*. These morphological

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changes could be the result of oxidative damage to mitochondrion. This oxidative damage also pledges the apoptotic changes like production of fodrin proteins and cleavage of cytoskeleton gelsolin and increased caspase activated DNase (CAD) in the nucleus which is responsible for the degradation, breakdown and disintegration of nuclear lamins proteins (Fernandes*et al.*, 2007). These nuclear abnormalities could also be due to over generation of caspase activated DNAs which are responsible for the cleavage of cytoskeletal (gelsolin, fodrin andvimentin) and nuclear proteins (Banerjee*et al.*, 2001). It is alluring to speculate that blebbed, notched and lobed nuclei could result from aneuploidy, i.e., a process leading to formation of chromosomal abnormalities (Cavas and Ergene-Gozukara, 2005).

This observation was in support with the study of Bucker *et al.* (2012) statemented the benzene induced nuclear abnormalities such as binucleated, lobed and notched in Amazonian electric fish *Apteronotusbonapartii.*Various chemical exposures have shown morphological nuclear abnormalities in both human and fish cells (Fenech*et al.*, 1999; Cavas*et al.*, 2005).

The presence of variations in nuclear morphology of fish erythrocytes has been previously described in several studies, and in several cases, they have been interpreted as nuclear lesions analogous to micronuclei (Sanchez-Galan *et al.*, 1998; Ayllon and Garcia-Vasquez, 2000; Cavas and Ergene-Gozukara, 2005). Binucleated and budding cell nuclei are types of nuclear abnormalities that can also be considered indicative of genotoxic elements in the environment (Ayllon and Garcia-Vasquez, 2000; Serrano-Garcia and Montero-Montoya, 2001). Erythrocytes of fish in polluted waters often show micronuclei, but binucleated cells can be more common (Wirzinger*et al.*, 2007). Cavas and Ergene-Gozukara (2005) reported the higher number of micronuclei and nuclear abnormalities in *Mugilcephalus* from polluted areas than clean area. Sanchez-Galan *et al.* (1998),reported that binucleated cells are induced by unspecific pollution along with micronuclei in brown trout.

In the present study various nuclear abnormalities (binuclei, lobed and notched nuclei) were identified and it revealed that such identified abnormalities may be indicators of genotoxicity. The present work also agreed with various authors. Several authors have identified NA including blebbed, lobed, and notched nuclei and bi-nucleated cells as possible indicators of genotoxicity (Ayllon and Garcia-Vazquez, 2000; Cavas and Ergene-Gozukara, 2003; Cavas and Ergene-Gozukara, 2005; Da Silva Souza and Fontanetti, 2006).

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Genotoxins can induce changes in DNA that are passed on to future generations. The present study also demonstrated these as indicators of genotoxic effect in fishes. Nuclear abnormalities can also be used as an indicator for DNA damage in the cell. Such abnormalities may lead to changes in the genetic components and concern has been expressed about the genetic consequence of pollution to fish population which is exposed to low level of pollution over a prolonged period (Barker and Rackman, 1979). Kurelec (1993) suggested that genetic damage can cause the inhibition of growth, decrease the fecundity, and faster ageing. Therefore it is possible that these genetic damages may also decrease fecundity and faster ageing of *E.suratensis*.

The present study revealed that *E.suratensis*can be used as a good model to study the genotoxic effects of aquatic pollutants in fish.The high incidence of nuclear abnormalities in *E. suratensis* from higher concentrations showed that the aquatic animals in these areas are under serious threat of pollution. The results of the present study intensely showed that the lambda-cyhalothrin induced genetic damage in the form of nuclear abnormalities in *E.suratensis*.

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