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"THE EFFECT OF THE EXTREMELY LOWE FREQUENCY (ELF) ELECTROMAGNETIC FIELD (EMF) ON DNA AND RNA CONTENTS IN MUSCLES OF TILAPIA, *OREOCHROMIS SPILURUS*"

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SUMMARY

The impact of the extremely low frequency (ELF), electromagnetic field (EMF) on muscles RNA and DNA contents in the muscles of the tilapia, Oreochromis spilurus were investigated. A significant variations in the muscles RNA and DNA contents in response to 5kV/m2 and 10kV/m2 EMF field strength were reported. Both field strengths resulted in a significant decline in the DNA and RNA contents of the fish muscles.

INTRODUCTION

The extremely low electromagnetic fields (ELF) have been found to affect animals. ELF was found to exert significant effects on RNA and transcription at the cellular level and also affect RNA transcription and protein synthesis (Goodman and Henderson, 1986, 1988; Goodman et al., 1989), and transformation of myotic into non-myotic cells (Rodemann et al., 1989). It has been, also, found to promote and may have association with cancer and other diseases (Wertheimer, and Leeper, 1979; Anderson, 1990; Silverman, 1990; Wood, 1993).

Studies on the impact of the extremely low electromagnetic fields on fish are rare. Studies about the effects of 50-100 Hz EMF on animal embryos (fish, chick, fly, sea urchin, rat and mouse) showed that early embryonic stages are responsive to fluctuation in magnetic fields (Zimmerman et al., 1990; Cameron, et al., 1993). They reported that studies on sea urchin embryos indicated that exposure to rotating 60 Hz EMF, which are similar to those in our environment, interfered with cell proliferation at the morula stage.

The impacts of extremely low frequency (ELF), electromagnetic field (EMF) (5kV/$m^2$and 10kV/m^2) respectively on the nucleic acid (DNA and

RNA) contents in the muscles of the, Oreochromis spilurus, were the aim of this study. In this study, the effects of two different field intensities were investigated.

MATERIALS AND METHODS

Experimental Fish

The marine, Oreochromis spilurus, was used as the experimental species. Juvenile fish were obtained from King Abdulaziz University hatchery.

Fish Exposure Set up

The fish were kept in glass aquaria (50 x 40 x 40 cm). They were kept under the same experimental conditions with continuous aeration and at room temperature. One aquaria (50 x 45 x 25 cm) served as the control group and another one served as the exposure tank. Two 46 X 46 aluminum sheets were put along the length of the exposure aquarium from each side. They were connected to the field generator. For safety, the two aluminum sheets were raped carefully inside a plastic material to prevent any contact with water. The fish were fed 30% protein fish diet (RADWA, Jeddah, KSA).

The electromagnetic field was derived from a 50 Hz stabilized power supply that had a maximum 6000V output (CENCO, CAT# 87208, 115 Volts-60 cycles). The power supply input voltage was adjusted by using a Variac Lab Volt (Model # 187 AP, Buck Eng. Co., Inc, Farmingdale, N. J., USA).

Electromagnetic Field (EMF)

In this study, the impact of the extremely low frequency (ELF), electromagnetic field (EMF) on Oreochromis spilurus, were studied. The following biophysical characteristics were taken into consideration. Constant frequency of 50Hz was used. Two field strengths were used which are the internationally permissible field strength (5KV/m2) and higher field strength of 10KV/m2. The intensity was fixed during the study period and exposure time was for two weeks.

Experimental Groups

Two experimental treatments were studied. One is the internationally permissible field strength (5KV/m2) and the other is higher field strength of 10KV/m2. The direct effect and the late effect of the EMF were estimated among the exposed groups as follows:

Control Group: Each treatment was assigned a control group which received no treatments.

Direct Effect Estimation Group: The fish were monitored directly (D) two weeks after exposure in order to measure the various parameters stated bellow.

Late Effect Estimation Groups: The fish were sacrificed two (L1) and four (L2) weeks after the termination of exposure in order to assess DNA and RNA contents.

The fish were sacrificed directly after exposure in order to measure the various parameters stated bellow.

The fish were sacrificed after two and four weeks after the termination of exposure, in order to measure the different parameters stated bellow.

Nucleic Acid (RNA and DNA) Estimation

Fish from the various experimental groups were sacrificed directly after 2, 4 and 6 weeks from the initiation of the study.

DNA and RNA were estimated using the method suggested by Giles and Myres (1965) and Ceriotti (1955).

To prepare the sample for analysis, the fish were sacrificed and kept in ice. Then, 0.2 gm of the muscle tissue was wighted accurately and homogenized with 4 ml chilled distilled water using a glass homogenizer and then left for 10 minutes. Three ml of 5% perchloric acid (PCA) were added to the homogenate. The precipitate was separated from the supernatant by centrifugation at 5000 rpm. The supernatant, then, was drained off and the precipitate was washed with 3 ml of 5% PCA. The later was heated at 90 oC for 20 minutes. After cooling the solution for 10 minutes, it was centrifuged and the supernatant was used for nucleic acid estimation.

Statistical Analysis:

Statistical analysis was conducted by using the SPSS statistical package. A one way between groups analysis of variance was conducted to explore the impact of exposing fish to electromagnetic field (EMF) of 60 Hz and field strengths of 5kV/m2and 10kV/m2 on DNA and also on RNA contents. The strength of association which indicates the relative magnitude of the differences between means was calculated as described by Cohen (1988); and Pallant (2001). It was calculated as described in the following equation:

Eta squared = SS between groups ˜ Total SS

The post hoc LSD multiple comparisons test was conducted to test the difference among the means of the various exposure groups.

RESULTS

Among the 5 kV/m2directly exposed fish [D5], a decrease in muscles mean DNA contents (M=0.27375, SD=0.0638 gm/100gm fish muscles) has been shown after two weeks of exposure when compared to the control group (M=0.32975, SD=0.0354 gm/100gm fish muscles). Two weeks after cessation of the EMF exposure [L5.1], muscles DNA contents jumped up to (M=0.31655, SD=0.0235 gm/100gm fish muscles) which is almost near the control group levels. Muscles DNA content increased slightly 4 weeks after the end of

the exposure [L5.2] to (M=0.32925, SD=0.0564 gm/100gm fish muscles) which almost resemble the control levels (See Table2).

A one way between groups analysis of variance was conducted to explore the impact of exposing fish to electromagnetic field (EMF) of 60 Hz and field strength of 5kV/m^2 on the muscles DNA contents. There was a statistically significant difference at the $p<0.05$ level in muscles DNA contents for the four experimental groups [F (3, 56) =4.572, p=0.006] A large effect size (effect size=0.1958) was detected by calculating the eta squared which indicates that the actual difference in mean scores between the groups. Post hoc comparisons using the LSD test indicated that the mean muscles DNA contents for the D5 (M=0.27375, SD=0.0638 gm/100gm fish muscles) group was significantly different from C5 (M=0.32975, SD=0.0354 gm/100gm fish muscles), L5.1 (M=0.31655, SD=0.0235 gm/100gm fish muscles) and L5.2 (M=0.32925, SD=0.0564 gm/100gm fish muscles) groups (See Table1 and Table2).

A slightly different trend were observed among the 10 kV/m^2exposed fish where the muscles DNA contents decreased to about (M=0.28500, SD=0.0354 gm/100gm fish muscles) after two week of exposure [D10 group], when compared to the control group (M=0.32975, SD=0.0354 gm/100gm fish muscles). Two weeks after stopping the EMF exposure [L10.1], the muscles DNA contents kept decreasing down to (M=0.24950, SD=0.0116 gm/100gm fish muscles) and stayed near this level (M=0.24300, SD=0.0138 gm/100gm fish muscles) at the termination of the experiment [L10.2] (See Table4).

A one way between groups analysis of variance was conducted to explore the impact of exposing fish to electromagnetic field (EMF) of 60 Hz and field strength of 10kV/m^2on the muscles DNA contents. There was a statistically significant difference at the $p<0.05$ level in muscles DNA contents for the four experimental groups [F (3, 56) =38.649, p=0.000] A large effect size (effect size=0.672) was detected by calculating the eta squared which indicates that the actual difference in mean scores between the groups. Post hoc comparisons using the LSD test indicated that the mean muscles DNA contents for the D5 (M=0.28500, SD=0.0354 gm/100gm fish muscles) group was significantly different from C10 (M=0.32975, SD=0.0354 gm/100gm fish muscles), L10.1 (M=0.24950, SD=0.0116 gm/100gm fish muscles) and L10.2 (M=0.24300, SD=0.0138 gm/100gm fish muscles) groups (See Table3 and Table4).

Table (1). Analysis of variance for DNA and RNA contents (gm/100g fish muscles for the different experimental groups at the 5KV/m2 field strength experiment.

Source of Variation	df	Mean Squares	
		DNA contents	RNA contents
Groups	3	0.009**	0.007**
Error	56	0.002	0.001

** Significant at 0.01 level of probability.

Table (2). Means for DNA and RNA contents (gm/100gm fish muscles) for the Different Experimental Groups at the 5KV/m^2 field strength Experiment.

Groups	N	Mean DNA contents gm/100gm fish muscles	Mean RNA contents gm/100gm fish muscles
Control [C]	24	0.329750 a	0.213712a
Direct effect [D5]	12	0.273750 b	0.171275 b
First late effect [L5.1]	12	0.316550 a	0.179575 b
Second late effect [L5.2]	12	0.329250 a	0.181150 b

* Means followed by the same letter are not significantly different according to LSD multiple comparisons test at 0.05 level of probability.

Table (3). Analysis of variance for DNA and RNA contents (gm/100g fish muscles for the different experimental groups at the 10KV/m2 field strength experiment.

Source of Variation	Df	Mean Squares	
		DNA contents	RNA contents
Groups	3	0.028**	0.001**
Error	56	0.001	0.001

** Significant at 0.01 level of probability.

Table (4). Means for DNA and RNA contents (gm/100gm fish muscles) for the Different Experimental Groups at the 10KV/m^2 field strength Experiment.

Groups	N	Mean DNA contents gm/100gm fish muscles	Mean RNA contents gm/100gm fish muscles
Control [C]	24	0.329750 a	0.213712a
Direct effect [D10]	12	0.285000 b	0.197675 a
First late effect [L10.1]	12	0.249500 c	0.211621 a
Second late effect [L10.2]	12	0.243000 c	0.194400 a

* Means followed by the same letter are not significantly different according to LSD multiple comparisons test at 0.05 level of probability.

RNA contents

Among the 5 kV/m2directly exposed fish [D5], a decrease in muscles mean RNA contents (M=0.17128, SD=0.0158 gm/100gm fish muscles) has been shown after two week of exposure when compared to the control group (M=0.21371, SD=0.0376 gm/100gm fish muscles). Two weeks after cessation of the EMF exposure [L5.1], muscles RNA contents stayed almost in the same level for the D5 group and increased slightly to (M=0.17958, SD=0.0221 gm/100gm fish muscles). Muscles RNA contents increased slightly 4 weeks after the end of the exposure [L5.2] to (M=0.18115, SD=0.0324 gm/100gm fish muscles) which resembles the control levels (See Table2).

A one way between groups analysis of variance was conducted to explore the impact of exposing fish to electromagnetic field (EMF) of 60 Hz and field strength of 5kV/m2on the muscles RNA contents. There was a statistically significant difference at the $p<0.05$ level in muscles RNA contents for the four experimental groups [$F(3, 56) = 8.75$, $p=0.000$]. A large effect size (effect size=0.3226) was detected by calculating the eta squared which indicates that the actual difference in mean scores between the groups. Post hoc comparisons using the LSD test indicated that the mean muscles RNA contents for the C5 (M=0.21371, SD=0.0376 gm/100gm fish muscles) group was significantly different from D5 (M=0.17128, SD=0.0158 gm/100gm fish muscles), L5.1 (M=0.17958, SD=0.0221 gm/100gm fish muscles) and L5.2 (M=0.18115, SD=0.0324 gm/100gm fish muscles) groups (see Table1 and Table2).

A different trend were observed among the 10 kV/m2exposed fish where the muscles RNA contents decreased to about (M=0.19768, SD=0.0256 gm/100gm fish muscles) after two week of exposure [D10 group], when compared to the control group [C10] (M=0.21371, SD=0.0376 gm/100gm fish muscles). Two weeks after stopping the EMF exposure [L10.1], the muscles RNA contents increased up to (M=0.21162, SD=0.0176 gm/100gm fish muscles) and decreased again to (M=0.19440, SD=0.01923 gm/100gm fish muscles) at the termination of the experiment [L10.2] (See Table4).

A one way between groups analysis of variance was conducted to explore the impact of exposing fish to electromagnetic field (EMF) of 60 Hz and field strength of 10kV/m2on the muscles RNA contents. There was a no statistically significant difference at the $p<0.05$ level in muscles RNA contents for the four experimental groups [$F(3, 56) = 1.68$, $p=0.182$] as seen in table 2. A medium effect size (effect size=0.078) was detected by calculating the eta squared which indicates that the actual difference in mean scores between the groups. Post hoc comparisons using the LSD test

indicated that there is no significant difference among the mean muscles RNA contents for the four experimental groups [D5 (M=0.28500, SD=0.0354), C10 (M=0.32975, SD=0.0354), L10.1 (M=0.24950, SD=0.0116) and L10.2 (M=0.24300, SD=0.0e138)] (see Table3 and Table4).

DISCUSSION

The results of this study indicated that there is a significant effect for EMF on the nucleic acids (See Tables 1-4). The two field strengths which have been tested showed significant effects on the DNA and RNA contents of Oreochromis spilurus muscles. A decline of the DNA and RNA contents in the muscles of the tilapia has been shown in this study. Among the exposed fish DNA and RNA contents in the muscles decreased after two weeks of continuous exposure (D5) using the internationally permissible field strength (5kV/m2). The concentrations, then, increased almost to the normal levels after two weeks (L5.1) and four weeks (L5.2). Similar pattern has been shown among the albumin and total protein contents in the plasma of mice exposed to 5kV/m2field strength (Kumosani and El-Mashak, 1997).

The DNA and RNA contents have also declined after two weeks of exposure (D10) when 10kV/m2 field strength was used. In contrast to the pattern was shown among the fish treated with 5kV/m2, the concentrations of DNA stayed at low levels and did not recover to the normal levels after two and four weeks from the cessation of the treatment. On the other hand, RNA concentrations increased after two weeks from the cessation of the treatments (L10.1). Four weeks after the cessation of the treatment (L10.2) the RNA concentration was decreased slightly but not significantly.

Electromagnetic fields are characterized as non-ionizing radiation which can only "excite" molecules but not ionize them. Studies showed that not only ionizing radiation causes biological alterations in life matter; the non-ionizing radiation can also cause some effects which can be harmful to human health (Bassett et al., 1977; Wertheimer and Leeper, 1979; Savitz et al., 1988; Fadel et al., 1994; El-Mashak et al., 1992; El-Mashak and El-Gebaly, 1994). A great interest has increased to test the effects of such EMF on various bio-systems. It has been found that the mechanism by which the EMFs can affect bio-systems depends on the electric and magnetic properties of that system (Fam, 1980; El-Mashak, et al., 1990). Reports showed that 50 and 60 Hz EMFs may be harmful to human health (Wertheimer and Leeper, 1979; Tomenius, 1986; Savitz et al., 1988). Other reports showed some relationship between EMF's exposure and childhood cancer (Wertheimer and Leeper, 1979; Tomenius, 1986; Spitz and Johnson, 1985). It is believed that EMF could be

cancer promoter but not initiator (Stuchly et al., 1991; 1992; Beniashvili et al., 1991).

The organization of DNA of giant chromosomes of insects (larvae of Acricotopus lucidus) is affected by electromagnetic fields (Kremer et al., 1988). Radio frequency exposures of different types are not genotoxic. The types of evidence to be discussed include the induction of DNA strand breaks, chromosome aberrations, micronuclei formation, DNA repair synthesis, sister chromatid exchange, and phenotypic mutation.. (Meltz, 1995; and 2003). DNA single and double strand breaks from RF exposure (2450 MHz) (Lai, 1995). In another study, Digital cell phone very low intensities direct DNA damage and the rate at which DNA is repaired. It is equal to about 800 μW/cm2 power densities (Phillips et al, 1998). Maes et al. (1995) reported that whole blood exposed to the radiating antenna of a GSM base station showed increased chromosome aberrations when placed within a distance of 5 cm or less with two hour exposures. Combined effects of 954 MHz radio frequency radiation and the chemical mutagen mitomycin C were studied by the same authors using human lymphocytes. Blood samples were exposed to AM radiation from a GSM base station at an estimated SAR of 1.5 W/Kg. Microwave exposure enhanced the harmful effect of the chemical mutagen and showed a clear increase in a form of chromosome aberration. Single strand DNA breaks were also reported. Lai and Singh (1995) first reported DNA strand breaks from microwave RFR at low intensity levels. A dose-dependent increase in DNA single- and double-strand breaks in brain cells exposed at 0.6 W/Kg and 1.2 W/Kg whole body specific absorption rate (SAR) was found after two hours of exposure to 2450 MHz RFR. Phillips et al. (1998) reported DNA single-strand DNA breaks exposed to cellular telephone frequencies 813.5 MHz and 836.5 MHz at low SAR (average 2.4 and 24 μW/g-1). Phillips postulated that DNA-repair rates may be affected by exposure to RFR. He postulates that ELF magnetic field exposure can affect both DNA damage and repair processes, and lead to cell death (apoptosis). Blank and Goodman (1997) postulate that the mechanism of EMF signals transduction in the cell membrane may be explained by direct interaction of electric and magnetic fields with mobile charges within enzymes. Recent studies on DNA show that large electron flows are possible within the staked base pairs of the double helix of DNA molecules. Therefore gene activation by magnetic fields could be due to a direct interaction with moving electrons within DNA. Electric fields as well as magnetic fields stimulate gene transcription and both fields could interact with DNA directly.

The extremely low frequency (ELF) magnetic field has been found to effect RNA transcription and protein synthesis (Goodman and Henderson,

1986; 1988 and Goodman et al. 1989) and transformation of myotic into non-myotic cells (Rodemann et al., 1989). Several studies reported the biological impact of the extremely low-frequency EMF. Electric currents in the range of (7-18 μA) are induced to the body for every 1 kV/m2of (50 Hz) electric field strength and currents up to (250 μA) are produced by the handheld home appliances (Deno, 1978; Deno, 1987 and Deno et al., 1982).

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