ABSTRACT

The effects of LF-EMF on the behavior of freshwater crab, *Barytelphusa cunicularis* were studied. It was observed that the crabs showed a total aggregation near the power supply after 60-90 minutes. No significant changes in the feeding behavior were observed in LF-EMF exposed crabs. However, the eyestalk ablated crabs showed a voracious feeding behavior. The fecal matter after 24 hours was higher compared to control crabs. Similarly a high degree of aggressiveness was found for about 1 hour thereafter the crabs became mild and showed less aggressiveness.

The serotonin is reported to cause aggregation and aggressive behavior. In the present study the crabs were injected with selective serotonin reuptake inhibitor drugs viz. setralin, citalopram and fluoxetine. A maximum aggregation was found in the order of fluoxetine>setralin> citalopram. Similarly, the aggressive behavior was observed in the order of fluoxetine>setralin> citalopram.

The effects of LF-EMF on the protein of neuroendocrine glands were studied after exposure to short and long time course. The protein content in the brain of crabs exposed to short time course viz. 24, 48, 72 and 96 hours showed a significant (F (4,10) = 3.5, P < 0.05) decrease initially, at 24 and 48 hours and later showed an increase at 72 and 96 hours. A maximum increase was observed at 96 hours. In long time course viz. 30,60, and 90 days exposure study the brain showed a significant (F (3,8) = 47.7, P < 0.05) decrease in brain protein concentration, all through the long time course study.

The protein concentration in thoracic ganglion showed a significant (F (4, 10) = 7.18, P < 0.05) decrease from 24 to 96 hours. The least concentration was found at 24 and 72 hours. The thoracic ganglion showed a significant decrease (F (3, 8) = 18.54, P < 0.05) in protein concentration during the entire long time course study. A significant decrease in protein was observed for 30, 60 and 90 days as compared to control.

The eyestalk protein showed a significant (F (4, 10) = 115.23, P < 0.05) increase during short time course studies. A significant increase was observed at 24 and 96 hours. At 72 hours a decrease in protein was recorded. On long term exposure
a significant \( F(3, 8) = 62.55, P < 0.05 \) increase in protein concentration was observed, with maximum increase at 30 days.

The eyestalk ablated brain of freshwater crab, showed a significant \( F(4, 10) = 17.32, P < 0.05 \) decrease in brain protein concentration during short time course studies. Thoracic ganglion of eyestalk ablated crab showed a statistically significant \( F(4, 10) = 18.89, P < 0.05 \) decrease in protein concentration. A significant decrease in protein concentration was observed at 24, 48, 72 and 96 hours as compared to control.

When the crabs were exposed to short time course viz. 24, 48, 72 and 96 hours, it showed a significant \( F(4,10) = 40.88, P < 0.05 \) decrease in AChE. An initial decrease in acetylcholinesterase was observed during 24 and 48 hours, thereafter an increase was observed. On long term exposure a significant \( F(3, 8) = 806.25, P < 0.05 \) decrease in acetylcholinesterase was noted. The thoracic ganglion after exposure to LF-EMF for short time course viz. for 24, 48, 72 and 96 hours showed a significant \( F(4, 10) = 714.73, P < 0.05 \); decrease and later at 72 and 96 hour an increase was observed. A similar increase in acetylcholinesterase was observed in thoracic ganglion when crabs were exposed to 30, 60 and 90 days with a statistical difference \( F(3, 8) = 407.08, P < 0.05 \). A gradual increase in acetylcholinesterase was observed from 30 days.

The biochemical analysis of hemolymph and tissues viz. gills, muscles and hepatopancreas were carried out. The following biochemical tissues were analyzed, i.e. DNA, RNA, protein, amino acid, carbohydrates, glycogen, amylase and lipid. Muscles showed a significant \( F(4, 10) =44.64, P<0.05 \) decrease in DNA concentration with time. Similar results were seen in hepatopancreas with a significant \( F(4, 10) =579.95, P<0.05 \) decrease. However muscles and hepatopancreas to long term exposure showed a statistically significant \( F(3, 8) =124.44, P<0.05 \); \( F(3, 8) =133.49, P<0.05 \) increase. No statistical \( F(4,10)=1.86,P<0.05 \) difference was seen in RNA concentration in muscles during the short term exposure however long term exposure showed significant \( F(3,8)=32.12,P<0.05 \) increase. Hepatopancreas showed significant \( F(4,10)=50.34,P<0.05 \) increase in RNA for short exposure and also a significant \( F(3,8)=77.42,P<0.05 \) increase at long term exposure.

The protein from hemolymph, gills, muscles and hepatopancreas were studied after short and long term exposure. The hemolymph protein showed a significant \( F(3,8)=62.55, P < 0.05 \) increase in protein concentration was observed, with maximum increase at 30 days.
The gills showed significant \( F(4, 10) = 52.33, P< 0.05 \) increase. Muscle showed a significant \( F(4, 10) = 43.27, P < 0.05 \) decrease. Hepatopancreas showed a highly significant \( F(4, 10) = 26.39, P < 0.05 \) decrease in protein. The long term exposure of hemolymph and gill protein showed an increase at 30 days, thereafter a continuous \( F(3, 8) =3255.25, P<0.05 \); \( F(3, 8) =1955.3, P<0.05 \) decrease. The hepatopancreas and muscle protein showed significant \( F(3, 8) =14.77, P<0.05 \); \( F(3, 8) =37.6, P<0.05 \) increase. Eyestalk ablated crabs showed statistically significant \( F(4,10)=1351.79,P<0.05 \); \( F(4,10)=45.23,P<0.05 \); \( F(4,10)=457.06,P<0.05 \) decrease respectively. Whereas, gills showed a decrease till 48 hours and thereafter an \( F(4, 10) =1088.95, P<0.05 \) increase. The free amino acid in hemolymph showed a significant \( F(4, 10) =3238.75, P<0.05 \); \( F(3, 8) =14.27, P<0.05 \) decrease during short and long term exposure. The eyestalk ablated crab also showed a \( F(4, 10) =2055.80, P<0.05 \) decrease compared to control.

Carbohydrates in hemolymph and gills exposed to short term showed a significant \( F(4, 10) = 72.08, P < 0.05 \); \( F(4, 10) = 936.93, P < 0.05 \) increase compared to control respectively. On long term exposure a significant \( F(3, 8) = 4.61, P < 0.05 \); \( F(3, 8) = 656.25, P < 0.05 \) decrease was observed. Eyestalk ablated crab showed a significant \( F(4, 10) =206.44, P<0.05 \) increase at 24 hours and thereafter decrease in hemolymph. Eyestalk ablated gills showed significant \( F(4, 10) =109.88, P<0.05 \) decrease. A significant statistical \( F(4,10)=1231.19,P<0.05 \); \( F(4,10)=219.76,P<0.05 \) increase in glycogen concentration was observed in muscles and hepatopancreas on short term exposure. A statistical significant \( F(3,8)=12870.6,P<0.05 \) decrease in muscle glycogen was observed on long term exposure and a statistical \( F(3,8)=169.74,P<0.05 \) increase in hepatopancreas was observed on long term exposure. Eyestalk ablated muscles and hepatopancreas showed significant \( F(4, 10) =84.98, P<0.05 \); \( F(4, 10) =1620.78, P<0.05 \) increase.

Hemolymph showed a significant \( F(4, 10) = 536.76, P < 0.05 \); \( F(3, 8) = 533.79, P < 0.05 \) decrease in amylase concentration for short and long term exposure. Eyestalk ablated crab showed a significant \( F(4, 10) =80.007, P<0.05 \) increase up to 48 hours and thereafter decrease.

Muscle lipids showed a steady decrease from 24 to 96 hours. Long term exposure also showed decrease in lipid concentration but eyestalk ablated crabs
showed significant increase in lipid concentration at 24 hours and later decrease. There was significant decrease in lipid concentration in hepatopancreas both in short and long term exposure. However at 24 hours there was significant increase in lipid concentration in eyestalk ablated crabs. The lipid percentage difference in muscles showed 20% decrease at 24 hours and 60 days, 30% decrease at 48 hours, 60% decrease at 72 hours, 96 hours and 90 days, however a 10% increase was observed at 30 days when compared to control. The lipid percentage difference showed a decrease in hepatopancreas for 24 hours (-25%), 48 hours (-46.43%), 72 hours (-71.43%), 96 hours (-89.29%), 30 days (-46.43%), 60 days (-57.14%) and 90 days (-71.43%) respectively. Eyestalk ablated crabs showed an increase of 160% and 100% in lipid percentage at 24 and 48 hours in muscles, however at 72 and 96 hours it showed decrease of 20% and 60%. In eyestalk ablated hepatopancreas the increase in lipid percentage was 89.28% at 24 hours, 0% at 48 hours and a decrease of 35.71% at 72 hours and 57.14% at 96 hours.

The effect of LF-EMF on the gills and hepatopancreas were studied. After a short time course the gills showed observable changes. After 24 hours the gill lamellae was found to be thinned. The gill mid lamellae showed the presence of hemocytes. The gill caps became thin and a single covering was observed. Obliteration and damage to gill lamellae was also observed. After 48 hours of exposure the mid lamellae of gills were found to be vacuolated. It also showed the presence of large number of hemocytes. The gill lamellae showed a prominent breakage and damage to mid lamellae. After 72 hours the gills showed a median obliteration in mid gill lamellae. The mid gill lamellae also showed a clumping of hemocytes. The gill lamellae showed breakage and rupture of gill cap. After 96 hours the gills showed hemocytes in the gill lamellae. The gill cap also showed a rupture. During long time study i.e. 30, 60 and 90 days the following changes were seen. After 30 days the gill showed thickened gill lamellae with hemocytes present in the lamellae. The gill cap was found to be elongated and at places broken. The gill cap showed a thick covering. After 60 days the gills showed a thinning of gill lamellae. An important observation observed was the separation of gill lamellae near the gill cap (exposed gill lamellae). After 90 days the gill cap showed obliteration. The gill lamella was thick and it showed the presence of hemocytes. The median lamellae were completely damaged.
After 24 hours the hepatopancreas showed the nucleus towards the lumen. The lumen was reduced. The cells showed hypertrophy of cells. The connective tissues were seen in between the hepatopancreatic lobules. After 48 hours the hepatopancreatic lumen was highly reduced (LR) the basal membrane are obliterated. After 72 hours the hepatopancreatic lumen was highly reduced with absorptive cells facing the lumen. The basement membrane is obliterated. After 96 hours a highly damaged hepatopancreatic lobules were seen. The cells also showed vacuolization. The cells also showed pycnotic nucleus. The cellular exudates were seen in lumen. After 30 days the hepatopancreas showed a highly reduced lumen. The secretory cells are seen with many pycnotic nuclei. After 60 days hepatopancreas, showed a complete obliteration of basal membrane. The secretory cells showed vacuolization (VSC). The lumen was also ruptured. After 90 days the basal membrane is highly obliterated with few pycnotic nuclei. The cells showed vacuolization and lumen diameter was more.

The effect of LF-EMF was studied on reproduction after exposure to short time and long time course study. For short time course crabs were exposed to LF-EMF for 24, 48, 72 and 96 hours. A statistically significant (ANOVA) increase in protein concentration was observed in the ovary, \( F(4, 10) = 400.51, P < 0.05 \). The ovary showed a mean increase in protein percentage in all exposure period. At 24 hours the percentage increase was 457.35% as compared to control. 48 hours showed 305.66% increase, 72 hours 303.68% increase and 96 hours showed an increase of 387.01%. A significant increase was observed at 24 hours. On long time exposure, a significant ANOVA difference in protein concentration was observed \( F(4, 10) = 381.81, P < 0.05 \); when crabs were exposed to electromagnetic for 10, 30, 60 and 90 days. Ovary showed maximum protein percentage at 10 days and thereafter a slight decrease till 90 days. However, the decrease was higher when compared to control. The percentage protein difference for 10 days exposure showed 525.90 %, 30 days showed 342.57%, 60 days showed 361.11% and 90 days showed 348.13% of protein percentage.

Eyestalk ablated crab showed an increase in protein concentration for 24, 48, 72, and 96 hours as compared to control. A statistically significant (ANOVA) increase in protein concentration was observed \( F(4, 10) = 507.93, P < 0.05 \). Eye stalk ablated ovary showed a mean percentage increase in protein at 24, 48, 72, and 96 hours respectively. At 24 hours it showed 225.9% protein content, 48 hours 229.58%,
72 hours 229.58% and at 96 hours 242.57% of protein percentage as compared to control.

The histological studies after 24 hours of LF-EMF exposure showed the ovarian follicles compact, nuclei and nucleolus are clearly visible. The basal membranes are well established and are in contact with ovarian follicles. The ovary shows presence of yolk globules. The 48 hours showed ovarian degeneration of nucleus (DN). The basal membrane was obliterated. The ovary also showed degenerating ovary (DO). The ovary showed the presence of vacuoles through out. The ovarian phagocytic cells are seen and thus showing the regression of the ovarian follicle. In 27 hours the basement membrane showed a complete separation. The ovary shows degenerating oocytes (DGO) and ovarian phagocytic cells. The cytoplasm of the ovary showed numerous yolk globules (YG). The 96 hours ovary shows a highly damaged condition. The nucleus showed a separation from cytoplasm. Prominent vacuolization in ovary was seen (OV). The basement membrane was highly obliterated. The ovarian phagocytic cells were largely intruded. The cytoplasm showed vacuolization. In long time course study of 30 days exposed ovary showed vacuolization in nucleus. The nucleus showed a degeneration of nuclear membrane (DNM). Separation of basal membrane and vacuolization was seen in the cytoplasm. There is a slight separation in the basement membrane. The germinal layer also showed vacuolization. The 30 days exposed ovary showed a degeneration of nucleus. The basal membrane is obliterated. The cytoplasm showed yolk globules. The 60 days exposure the nucleus was condensed and separated from the cytoplasm. The cytoplasm is completely separated from the basement membrane and the basement membrane is totally obliterated in 90 days. The cytoplasm showed vacuolization. This vacuolization spreads in to the entire ovary. In some nucleus and nucleolus is seen. Fatty droplets and pycnotic nuclei are also seen. The separation in basal membrane is very prominent.