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Ethnyl estradiol administration affects serum estrogen level of male *Clarias batrachus.*

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Abstract- Dose dependent effect of ethnyl estradiol was studied on the serum estrogen level of male *Clarias batrachus*. Fishes weighing 150- 200 g were used for the study. They were divided into four different groups. Experimental groups were fed with ethnyl estadiol (5µg, 10µg and 20µg Kg^{-1} feed) in the diet supplemented through premarin (oral contraceptive pill). Control group fishes were fed normal fish diet. After treatment for 30 consecutive days blood was collected and serum estrogen assayed by Enzyme Immunoassay. Results showed significant increase in blood estrogen levels in ethnyl estradiol treated fishes. Serum estrogen values were 0.1 µg/ml (control), 0.6 µg/ml (5 µg ethnyl estradiol), 1.0 µg/ml (10 µg ethnyl estradiol), and 1.3 µg/ml (20 µg ethnyl estradiol). A dose dependent effect was evident. The present results indicate that ethnyl estradiol has feminization effect on the male fish and may cause ovarian follicle development. At juvenile stage exogenous administration of ethnyl estradiol may result in gonadal malfunctions and suppress testicular development leading to sterilization or feminization. This may cause reproductive failure and result in reduced fish population in aquatic environment.

Key words: Ethnyl estradiol, *Clarias batrachus*, Students't'-test, ANOVA.

INTRODUCTION

Ethnyl estradiol is a synthetic estrogen widely used in oral contraceptive pills. Generally oral contraceptive pills contain 0.02 to 0.1 mg (20-100 µg) of ethnyl estradiol.[1] Ethnyl estradiol is partially metabolized and removed by urine and drains out to the sewage treatment plants and other aquatic environments. In surface water and sewage treatment plants, ethnyl estradiol frequently occurs in concentrations ranging between 1ng/l to 200-300 ng/l.[2,3] It has been reported that in response to low concentrations of ethnyl estradiol the fish synthesizes vitellogenin.[4,5] Aquatic organisms are highly threatened due to the harmful effects of endocrine disrupting chemicals, EDC.[6] Ethnyl estradiol is an EDC which causes vitellogenin induction in males, intersex gonads and reduced fertility in fish populations by reduction in the growth of gonads.[7] Many studies have documented that estrogens cause feminizing effects on masculine traits, like spermatogenesis.[8] It also affects the male secondary sexual characters as well as the male reproductive behaviour.[9] Reports show that ethnyl estradiol reduces the reproductive capacity in the Japanese medaka (*Oryzias latipes*), Zebra fish (*Danio rerio*) and in the rainbow trout (*Oncorhynchus mykiss*).[10,11] Ethnyl estradiol significantly reduces the reproduction of fishes at concentration of 10 ng/l. The exposure of estrogen during the period of fetal development, leads to irreversible changes in the tissue organization and this causes effects on fertility and

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behavior of the animal.[12,13] In fish, the effects of ethnyl estradiol on gonadal development, fertility and reproduction have been observed in laboratory experiments and also in wild fish.[14,15]

In Japanese medaka (*Oryzias latipes*), the developmental exposure to ethnyl estradiol during the first generation caused reduction in fertility and survival of embryo during the next two and three generations.[16] Estrogens are receptor mediated sex hormones. Ethnyl estradiol is a synthetic hormone that hinders reproductive function in humans.[17] The mode of its action is mediated via estrogen receptors. The mode of action of ethnyl estradiol in aquatic organisms is also expected to be via estrogen receptors, just like in mammalian systems.[17]

The Indian catfish magur (*Clarias batrachus*) is very hardy in nature[18] and can survive in adverse conditions. Considering the fact that the wastewater effluent and the rivers receiving the effluent have synthetic steroid hormones and their metabolites[19] and that most of the work has centered on fishes belonging to temperate zones the present study was aimed at observing the effects of ethnyl estradiol on the serum estrogen level in a tropical zone fish, *Clarias batrachus.*

MATERIALS & METHODS

Experimental animal: The study was performed on the males of the catfish, *Clarias batrachus*. Healthy males of *Clarias batrachus* weighing 150- 200g (14 ± 0.1 cm-18 ± 0.3 cm) were purchased from local breeders in the month of August. The fishes were transported in oxygenated bag and brought to the laboratory. The fishes were kept in glass aquaria under normal aquatic conditions at a temperature of 28±5°C. The fish were acclimatized for a period of 3 days.

Treatment and selection of dose: A toxicity bioassay was conducted to determine the LD_{50} for ethnyl estradiol according to the standard method opted by earlier worker.[20] LD_{50} of ethnyl estradiol was found to be 55µg kg^{-1} feed. The fish diet with increasing dose of ethnyl estradiol supplemented through premarin (Oral contraceptive pill), were provided daily to the fishes of different groups.

Experimental Design: The fishes were divided randomly into four different groups. Stocking density maintained for the experiment was 10 fish per aquarium. They were maintained in 4 separate glass aquaria. Control group fishes were fed normal fish diet. Experimental groups were fed with diets containing 5 µg, 10 µg and 20 µg of ethnyl estradiol per kg feed supplemented through premarin. The experiment was conducted for a period of 30 days. All the aquaria were aerated with air blower. The water exchange was done on daily basis.

The experiment was performed according to guidelines accepted by the "Local Animal Ethical Committee" for investigation on animals.

Biochemical Study: After treatment for thirty consecutive days, the blood was collected by puncturing the caudal vein using a medical syringe, which was previously rinsed with 2.7% EDTA solution and shaken gently in order to prevent hemolysis of blood. After collection of blood sample from fishes of four different groups, serum estrogen was assayed by Enzyme Immunoassay.[21]

Data Analysis: The data obtained was analyzed by Students't'-test and analysis of variance, ANOVA.[22]

RESULT

Results are presented in Table 1 and Figure 1. A dose dependent stimulatory effect of ethnyl estradiol was observed on the serum estrogen level of the fish. Fishes treated with ethnyl estradiol indicated significant ($p < 0.005$) increase in the serum estrogen level as compared to the control group fishes. ANOVA indicated a significant variation in serum estrogen level with respect to ethnyl estradiol dose ($F=23.41$, $p< 0.001$).

DISCUSSION

Results of the present study revealed that the level of serum estrogen is affected by exposure to synthetic hormone ethnyl estradiol. A dose dependent effect of ethnyl estradiol was evident. This finding is similar to the earlier reports in zebrafish where ethnyl estradiol alters sex differentiation and may even lead to sex reversal in males.[23] Exposure of male fishes to a high dose of 20 µg kg^{-1} feed of ethnyl estradiol resulted in significantly higher serum estrogen level (1.45 ± 0.06 ng/ml) as compared to that of control fishes (0.15 ± 0.02 ng/ml). The present results indicate that the exogenous administration of ethnyl estradiol on juvenile fish may cause gonadal malfunction and suppress the testicular development. It may cause development of ovarian follicle leading to sterilization or feminization.[24] Numerous effects of ethnyl estradiol on fish have been reported from all over the world such as deformities in the larvae of fish from North sea.[25]

Table-1 Serum estrogen levels (ng/ml) in male *Clarias batrachus* after treatment with different doses (µg kg^{-1} feed) of ethnyl estradiol supplemented through premarin (oral contraceptive pill) for thirty consecutive days

Dose of ethnyl estradiol	Serum estrogen
Control	0.15 ± 0.02
5 µg kg^{-1} feed	0.60 ± 0.01[a]
10 µg kg^{-1} feed	1.02 ± 0.04[a]
20 µg kg^{-1} feed	1.45 ± 0.06[a]

Data are expressed as mean ± SE for 10 fishes. Significance of difference from control: a, $p < 0.005$.

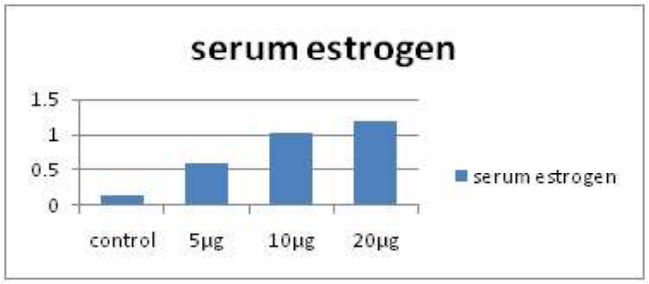

Figure 1- Dose of ethnyl estradiol (µg kg^{-1} feed) and the level of serum estrogen (ng/ml). Data indicated significant ($p< 0.005$) increase in the serum estrogen levels in fishes treated with ethnyl estradiol through premarin at the end of 30 days of experiment.

In United Kingdom, feminization was observed in male fish near outlets of sewage treatment.[26] A significant reversal of the steroidal system has been reported in carps from the Ebro River.[27] However, reports relating to fishes inhabiting tropical waters are lacking. The present results are new in the case of the freshwater Indian catfish, *Clarias batrachus*.

Ethnyl estradiol is the main active ingredient in contraceptive pill and it is excreted by women which then reaches the sewage treatment plants.[28] In women pharmaceutical estrogens, in oral contraceptives add upto the normal endogenous estrogen estradiol. It is possible that if the human fetus in utero is exposed to high doses of ethnyl estradiol, it may produce adverse developmental effects since in certain cases pregnancies do occur even after the use of oral contraceptives.[29] Ethnyl estradiol is a highly effective estrogen and it induces vitellogenin in male rainbow trout.[30] Intersex of gonads has been observed due to exposure of ethnyl estradiol in fathead minnow[31] and Japanese medaka.[32] Exposure to estrogen causes disrupted gonadal development and presence of intersex.[31,33] There are reports of arrest in development of testis and presence of ova- testis having primary stage oocyte after ethnyl estradiol exposure. In fish, this gender – bending effect has been identified. These results are in agreement with those of our present study with *Clarias batrachus* which exhibited a negative impact of ethnyl estradiol on its testes as evidenced by increase in serum estrogen levels. In the present work *Clarias batrachus* was exposed to ethnyl estradiol for duration of thirty days and this led to a significant change in the serum estrogen level of the fish. It may be opined that ethnyl estradiol exposure for a longer duration may cause adverse effects on the gonads of this fish. This requires further investigation. In tilapia (*Oreochromis niloticus*) it has been shown that exposure to high concentrations of ethnyl estradiol during the early life has long term consequences on growth, and gonads.[34] In the same study it has been reported that ethnyl estradiol exposure to tilapia influences serum levels of IGF-I and its expression in the liver and gonads. Review of literature suggests that in the aquatic toxicity base set fishes are the only taxa which have estrogen receptors.[35] Thus, ethnyl estradiol has a mode of action that binds to the estrogen receptors.

The present results clearly showed an elevation in serum estrogen level of *Clarias batrachus* after exposure to ethnyl estradiol for a period of thirty days. It is suggested that this compound affects the reproduction of the fish. Further studies are required to elucidate the complete role of ethnyl estradiol in the sexual differentiation as well as gonad development in this fish.

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