

ENDOSULFAN TARGETS GONADAL DIFFERENTIATION IN THE ASIAN CATFISH, *CLARIAS BATRACHUS*

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Introduction:

Hypothalamo-hypophyseal-axis is highly conserved among vertebrates which regulates early gonadal development and reproductive cycle in most of the annually spawning teleosts. With growing concern over the use of endosulfan in India and other world nations, in the present study we probed the effect of endosulfan as an endocrine disruptor, which is known to target gonadal differentiation by acting at the level of gonad and/or brain. To ascertain this, we analyzed the effects of endosulfan and flutamide (as a reference) on catfish gonadotropin-releasing hormone (cfGnRH)-tryptophan hydroxylase (Tph)-gonadal axis. Earlier studies often used adult fish and the impact of these compounds

during gonadal differentiation/development was never analyzed in depth at molecular level.

Methods:

Treatment was done by adding endosulfan and flutamide, alone and in combination to the aquarium tanks holding 50 days post hatch catfish juveniles for 50 days. Fishes were then sacrificed to dissect out the gonads and brain for total RNA preparation and histology. Prior to sacrifice, blood was collected, spun and it was used for estimation of estradiol-17 β . The quantitative real-time PCR was carried out for the control and treated samples following total RNA isolation and first strand synthesis. All the gene expression patterns were analyzed by relative qRT-PCR

Fig.1A,B. Changes in the expression of various transcripts of gonads/brain after the treatment of endosulfan and flutamide, alone and in combination, in juvenile catfish. *denotes significant difference from control, P < 0.05.

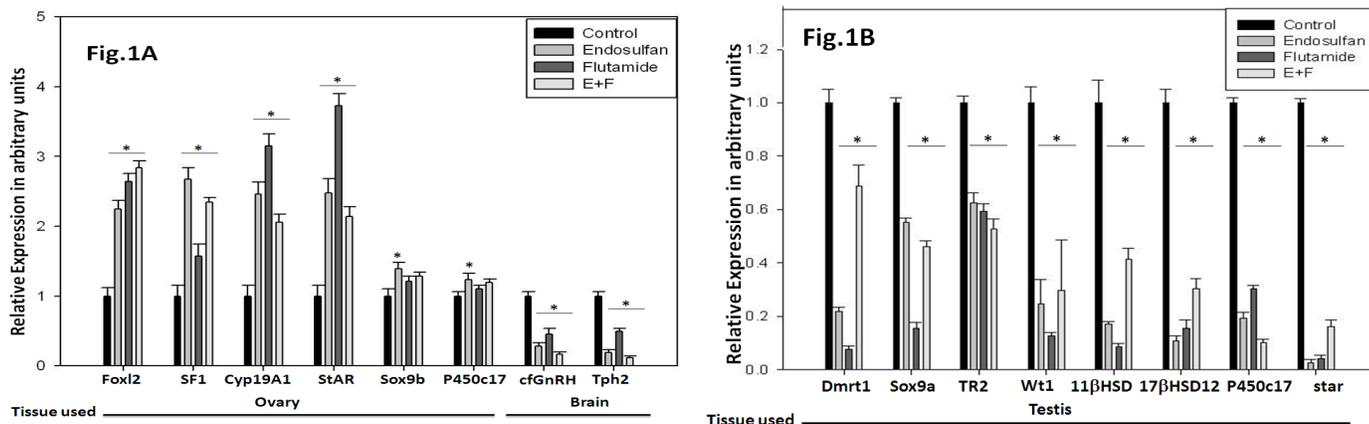
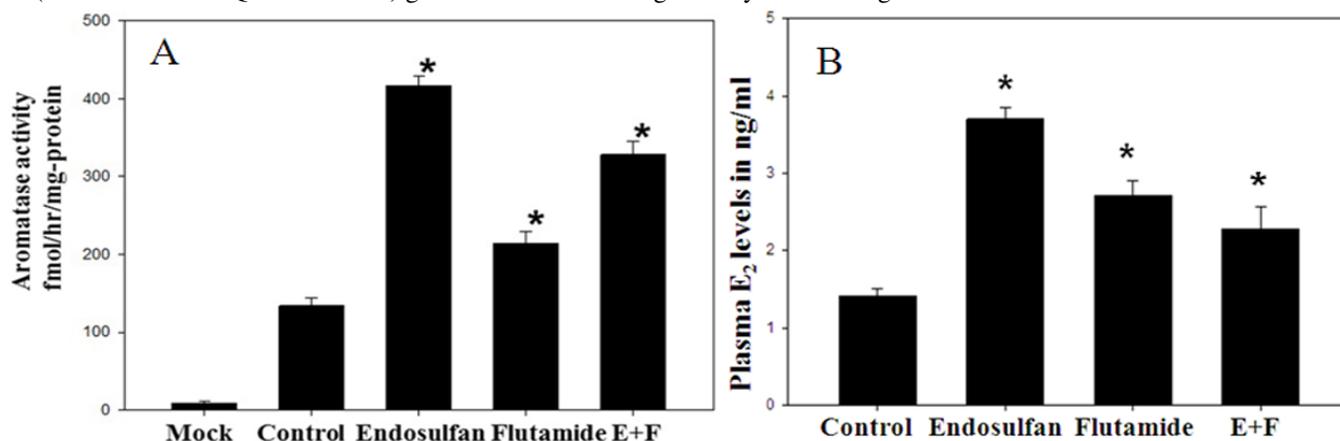


Fig. 2A,B. Aromatase activity and levels of estradiol-17 β after the treatment of endosulfan and flutamide, alone and in combination, in juvenile catfish. *denotes significant difference from control, P < 0.05. Financial support from DBT (BT/PR11263/AAQ/03/419/2008) grant awarded to BS is gratefully acknowledged.





using SYBR Green detection method except for Tph2 where Taqman probes were used. The real-time PCR specific primers for all the target genes and internal control β -actin were designed such that, at least one of the primers spanned the intron-exon boundary to exclude the amplification from genomic DNA. Radiometric assay was done to estimate aromatase activity in ovary. Immunocytochemistry for tryptophan hydroxylase-2 (Tph) localization was done in catfish brain while hematoxylin and eosin staining was done for gonadal sections.

Results and Discussion:

The results were depicted in figures 1 and 2. Treatment with 2.5ppb endosulfan and 33ppb flutamide, alone and in combination for 50 days enhanced the expression of transcription factors such as *sox9b*, *foxl2* and *Ad4BP/SF-1* in the ovary while down regulated *sox9a*, *dmrt1*, *wtl* and orphan nuclear receptor, *NR2C2* (TR2) in the testis. In the case of females, the expression of *CYP19A1* and *StAR* were increased while *P450c17*

expression elevated only in the endosulfan group. Conversely, expression of *P450c17*, *StAR*, *11 β -HSD* and *17 β -HSD12* was decreased in all the treated males. The expression of Tph and cfGnRH in the brain declined in all the treated females and the impact being maximal in endosulfan-treated fish. Significant reduction of Tph immunoreactivity in the telencephalon-preoptic-hypothalamus region of female brain substantiated our Tph transcript quantification results. The treatment had impact on these correlates in males as well. Histological analysis confirmed modulation of oocyte growth in the treated females and crumpling of lumen in treated males compared to control fishes. Increase in plasma E_2 levels and ovarian aromatase activity in the endosulfan treated females was found to be higher than other groups. These results together demonstrate that the exposure of endosulfan and flutamide modulate the cfGnRH-Tph-gonadal axis, either directly or indirectly.