# CHANGES IN TESTICULAR MORPHOLOGY, LEYDIG CELLS ULTRASTRUCTURE AND STEROIDOGENESIS IN RATS EXPOSED TO THE HERBICIDE ATRAZINE

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Atrazine (ATZ) is an herbicide widely used worldwide. Potential risks of this herbicide on health include several male reproductive disorders, such as promotion of hermaphroditism in frogs [1], change in sex ratio in fish [2], delayed sexual maturation [3, 4], reduction in number and motility of sperm [5], and reduction in weight of prostate and seminal vesicle [4, 5]. Several *in vitro* experiments showed that ATZ induces the expression and activity of the enzyme aromatase, which participates in the steroidogenic cascade promoting the bioconversion of androgens into estrogens [6]. However, the interference of ATZ in steroidogenic enzymes *in vivo* is not established. Studies using other organochlorine compounds, indicated that they cause inhibitory effects on testicular steroidogenesis by reducing the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) [7]. The 3β-HSD is responsible for various stages of the steroidogenic pathway, including synthesis of androstenedione, the testosterone precursor. Owing to evaluate the possible interference of ATZ in steroidogenesis, we analyzed the immunoexpression of the 3β-HSD, as well as the testosterone and estradiol levels, measured by radioimmunoassay and enzyme immunoassay. For this purpose, adult male rats were treated with ATZ at 300mg/Kg for 7 days, 200mg/Kg for 15 or 40 days and 50mg/kg for 15 days, by gavage. Control rats received vehicle at the same volume. The dosages were chosen based in previous studies showing adverse effects in the male genital system [1, 2]. Testis fixed with neutral buffer formalin were stained using standard methods for microwave antigen retrieval, before incubation with primary goat anti-human 3βHSD antibody. For negative controls, the sections received PBS. After incubation with a biotinylated secondary antibody (rabbit anti-goat, 1:100) and avidin-biotin complex, the reactivity was visualized using DAB chromogen. The radioimmunoassay and ELISA were performed using commercial kits according to instruction of the manufacturer. Morphophysiological changes in the testes of animals exposed to the ATZ were also investigated by light and transmission electron microscopy. Animals treated with ATZ at 200 and 300 mg/Kg, showed a decrease in Leydig cells 3β-HSD immunoexpression (Fig.1), confirmed by Western-blotting (Fig.2). Testosterone levels were also dramatically reduced in plasma (65% for 50mg and 85% for 200mg and 300mg) and testis (59% for 200mg) of treated animals (Fig.3 and 4). Conversely, plasma and testicular estradiol levels showed an increase of 33% and 38%, respectively, after ATZ treatment at 200mg (Fig.3 and 4). There were remarkable changes in the testicular structure, including dilatation of seminiferous tubules (at 200mg/Kg/15d and 300mg/Kg/7d) or testis atrophy (at 200mg/Kg/40d), characterized by reduction in the seminiferous tubule lumen and drastic reduction in the number of germ cells, resulting in Sertoli-only tubules (Fig.5). Changes in Leydig cells and macrophages ultrastructure as well as in the interaction between them were also observed in animals exposed to the ATZ (Fig.6). In treated animals, the Leydig cells were pleomorphic, presenting different sizes, shapes and irregular nuclei, sometimes with a greater amount of heterochromatin. On the other hand, macrophages of ATZ treated animals showed fewer electron dense granules and irregular nuclei; commonly with deep folds and prominent nucleoli. Leydig cells and macrophages did not show close interactions, as the spaces between these cells were larger and the cytoplasmic projections were shorter than in control animals. The extracellular matrix presented greater amounts of collagen fibers and fibroblasts. The present study extends the information about the ATZ as an endocrine disruptor by demonstrating that this herbicide affects the 3β-HSD expression, as well as testosterone and estradiol plasma and testicular levels, resulting in histopathological changes in the testis. Our results suggest that *in vivo* ATZ treatment might have the ability to interfere with testicular steroidogenesis by inducing lesions in Leydig cell. These results represent novel finding on ATZ toxicity suggesting decreasing in 3β-HSD as a possible mechanism of ATZ action.

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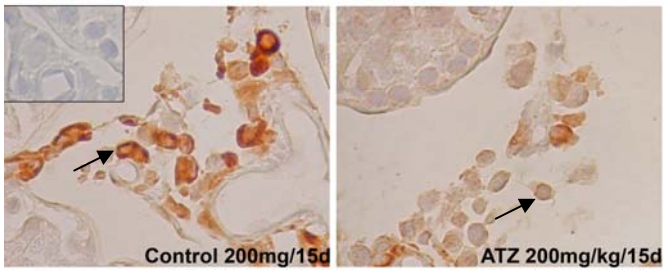


**Figure 1** - Immunoexpression of 3β-HSD. Rats treated with Atrazine showed a decrease in Leydig cells (arrow) 3β-HSD immunoexpression.

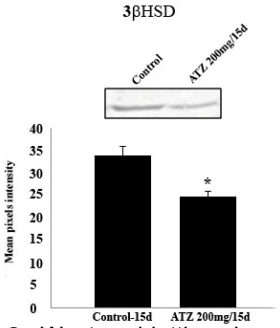


**Figure 2** –Western-blotting showed a decrease in Leydig cells 3β-HSD in treated animals.

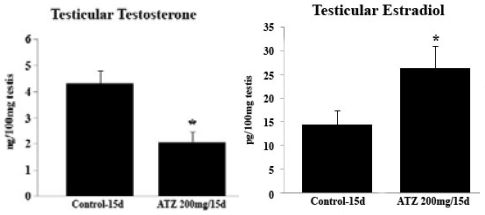


**Figure 3 -** Testicular testosterone and estradiol levels.

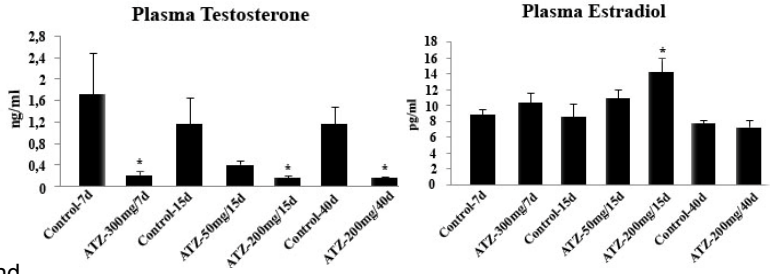


**Figure 4** - Plasma testosterone and estradiol levels.

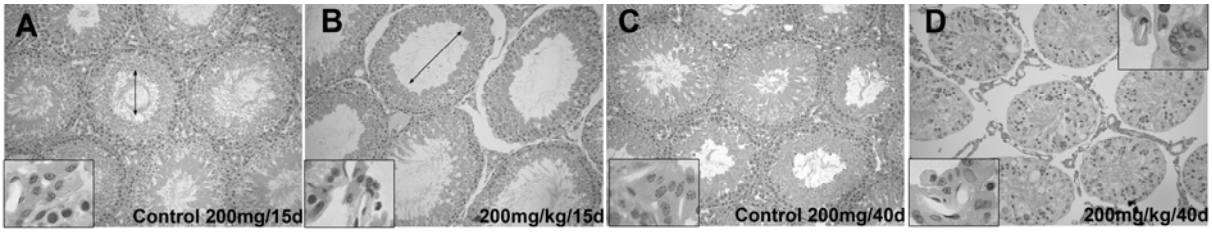


**Figure 5 –** Morphological alterations in testes Atrazine (ATZ)-treated. **(A and C)** Control rat; **B and D -** Atrazine treated rats showing dilatation of seminiferous tubules **(B)** and testis atrophy characterized by reduction in the seminiferous tubule lumen and drastic reduction in the number of germ cells **(D).**

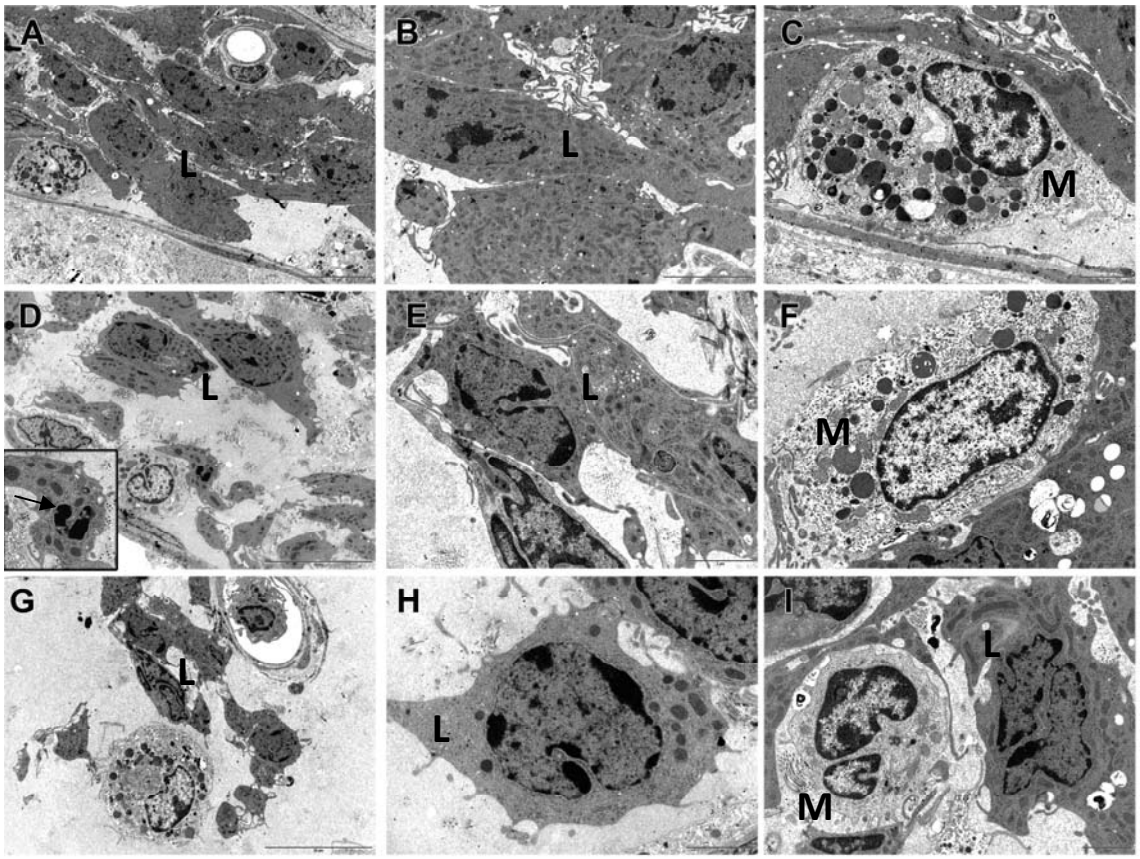


**Figure 6** - Ultrastructural testis alterations after atrazine (ATZ) treatment. **(A, B and C)** control rats showed Leydig cells **(L)** and macrophages **(M)** with close interactions. **(D – I)** treated ATZ rats showed pleomorphic Leydig cells **(L)** with irregular nuclei and a greater amount of heterochromatin (arrow). **F** and **I** macrophages **(M)** with fewer electron dense granules.