



Immunohistochemical Estimation of Apoptotic Activity and Testosterone Level in the Testes of Rats after Chronic Exposure to Uranium Acetate

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Abstract | Disorders in sex steroid genesis can be brought on by exposure to toxicants in the environment during fetal gonad development. The current study measures the amount of testosterone and apoptotic activity in rats following long-term exposure to uranyl acetate (UN). Twenty albino male rats were divided randomly into two groups as the following: GI: 10 rat received orally distal water for 90 days GII: 10 rats received orally (40 mg/kg.bw) UN for 90 days. The animals in both groups were sacrificed at the end of the study to determine the level of testosterone hormone and the histological and immunohistochemical effects of UN on the testes. The result showed degenerative changes in spermatogonia, interstitial thickening with hyalinization, severe necrosis (nuclear changes) in the germinal cells and apoptosis. The immunohistochemical result showed increased the apoptotic activity after exposure to UN. As for testosterone level was notably declined to (1.92 ± 0.03) compared with control group. Accordingly, to the current results we concluded that the chronic exposure to UN can induce reproductive toxicity in male rats, mainly in the spermatogonia by increased the apoptotic activity, also decreased the testosterone level.

Keywords | Apoptotic activity, Immunohistochemistry, Uranium acetate

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INTRODUCTION

One of the intensive nature occurring elements and valued for its radioactive properties is Uranium (Brugge, *et al.*, 2007; Keith and Faroon, 2022), because of the radioactive and chemical properties, uranium is distinguished as an element with a toxic effect (Brugge *et al.*, 2005). One of the targeted organs of chronic DU exposure is reproductive organs (Lu *et al.*, 2021). Uranyl acetate were administrate

to female mice by using gavage through gestation, parturition, and nursing had an enhance number of dead young per-litter Rodent reproduction and development can be adversely affect by natural uranium when It is probably exposure to the high doses of Uranium so in these research led to reproductive toxicity provide as an additional evidence of effect of Uranium approved (Grison *et al.*, 2022). Uranium has indicated to cause many effects like testicular toxicity, maternal toxicity, fetotoxic elevated developmental

ESTIMATION OF APOPTOTIC ACTIVITY

The apoptosis activity in paraffin embedded tissue section was determined by using a tunnel assay kit from novous biotechne brand.

MEASUREMENT OF TESTOSTERONE LEVEL

- The MILE card of the kit is placed in its place in the Mini-Vida's system, the machine automatically recognizes the test so that the system can show the result and then print it.
- A single STR and SPR strip was used for each sample of serum, control and standard solution and placed in a designated place in the system.
- 200 microns are pulled from the serum sample and placed in their own well On the STR strip as well as for control and standard solution.
- Follow the steps for the machine to start the calibration process automatically, which takes about 40 minutes.
- After the calibration, results were printed.

STATISTICAL ANALYSIS

SAS was used to examine the data (Statistical Analysis System - version 9.1). P 0.001 was regarded as statistically significant and Least Significant Differences (LSD) were performed to increased significant differences among means (Duncan, 1955).

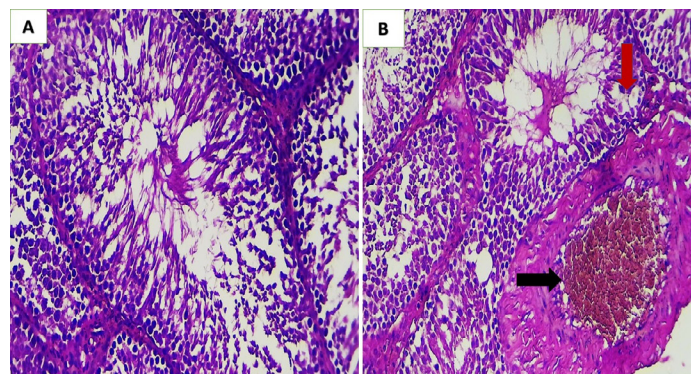


Figure 1: Histopathological section of rat testis; **A:** for GI group showing normal histological structures, **B:** for GII group showing severe thickening in blood vessels wall and congestion in their lumen (black arrow). Also vacuolation in the germinal cells (red arrow) (H and E, stains, 200x).

RESULTS

HISTOPATHOLOGICAL STUDY

Histopathological examination of testes sections was showed normal histological structures in GI group (Figure 1A). Whereas the GII group showed severe thickening in blood vessel wall and congestion in their lumen. Also, vacuolation in the germinal cells (Figure 1B), also interstitial thickening with hyalinization (Figure 2A) and severe necrosis (nuclear changes) in the germinal cells and apoptosis also seen (Figure 2B).

difference, and growth impediment independent of maternal toxic response when dosing to rodents (Domingo 2001; Hindin *et al.*, 2005). Uranium, was given as uranyl nitrate, postponement development of mouse embryos in culture and was correlated with lower embryo cell numbers through the hatched blastocyst stage, suggested there are severe alterations in DNA synthesis due to exposure to uranium (Kundt *et al.*, 2000; Yellowhair *et al.*, 2018). In the chronic exposure, there were some reports on rodents clarify a negative impact of uranium on male reproductive function, including a reduction in male fertility and in the spermatic number per-testis with a few histopathological effects on the seminiferous tubules and interstitium (Linares *et al.*, 2005). As well as the damage of DNA can be as consequence due to apoptosis cell death, which can be caused by metals either indirectly through the generation of free radicals, or by direct interaction between the metal and DNA (Ce'line, *et al.*, 2007). Rodents chronically exposed to 10–80 mg/kg/ day of uranium approaching few testicular histopathological abnormalities and minimized in spermatic number and pregnancy rate (Linares *et al.*, 2005).

EXPERIMENTAL DESIGN

EXPERIMENTAL ANIMALS: Twenty albino male rats aged 10–15 weeks and weighted 150 –200 gm were gained from (Animal house colony of National Center for Drug Control and Research) Ministry of health/ Baghdad, housed in plastic cages 50×30×10 cm and placed in the room for 10 days for adaptation. The temperature of the room was preserved at 21 ± 3 °C, by using exhausted fan the air of the room was changed continuously. The bedding of the cages was replaced every 7 days. The animals accommodate in animal house of Veterinary Medicine College at Al-qasim Green University and were fed on pellet ad libitum.

CHEMICALS: Uranyl acetate (UA) ($[\text{UO}_2 (\text{CH}_3\text{CO}_2)_2 \cdot \text{H}_2\text{O}] \cdot \text{H}_2\text{O}$) is yellow to green crystals) was obtained from SPI-Chem (U.S.A).

DOSE CALCULATION FOR URANYL ACETATE

The dose was calculated depending on the value (LD50 1/5) 40 mg/kg.bw (Domingo *et al.*, 1987). Melt in normal saline and quaff to the rats by oral intubations using a stomach tube for 90 days.

HISTOPATHOLOGICAL STUDY

Testes removed from the animals after sacrificing by using chloroform and fixed immediately in 10% buffered formalin solution before being histologically examined and embedded in paraffin. The slides were then routinely stained using Hematoxylin and Eosin stain after the histological sections were cut using a rotary microtome (Bancroft and Gamble, 2008).

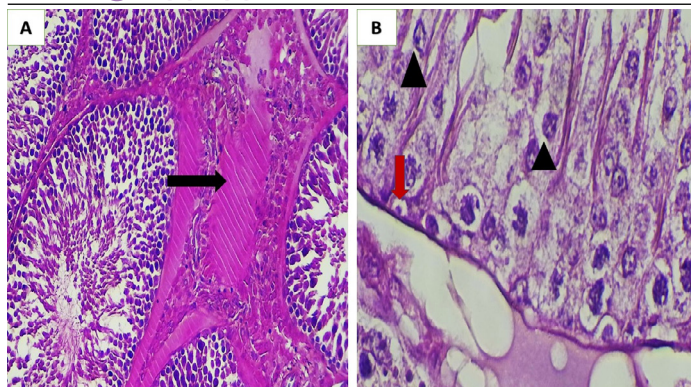


Figure 2: Histopathological section of rat testis for GII group; **A:** showing interstitial thickening with hyalinization (black arrow) (H and E, stains, 200x); **B:** severe necrosis (nuclear changes) in the germinal cells (head arrow) and apoptosis also seen (red arrow) (H and E, stains, 400x).

IMMUNOHISTOCHEMICAL STUDY

The result of estimation of apoptotic activity in the testes showed increased in the numbers of apoptosis in the spermatogonia that appeared as brown in color indicate positive reaction as in (Figure 3).

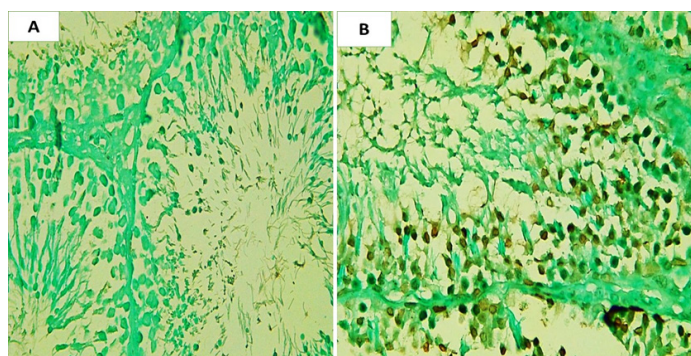


Figure 3: Immunohistochemical section of rat testes; **A:** for GI group; **B:** for GII group showing positive reaction for apoptotic activity appear as dark brown color of spermatogonia (DAB chromogen stain, 200x).

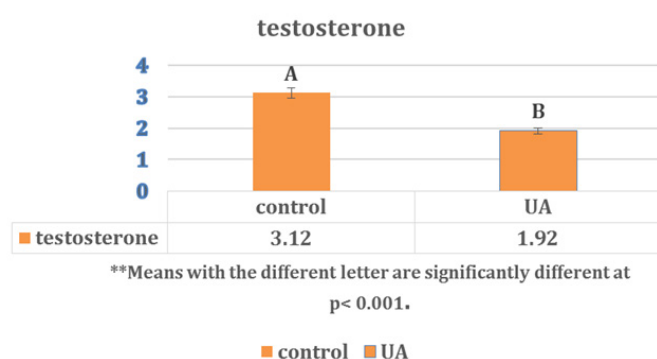


Figure 4: levels (mean $\pm$ SE) of testosterone in serum (nmol/ml) of rats treated with Uranyl acetate for 90 day.

ESTIMATION OF TESTOSTERONE LEVEL

The results for estimation of testosterone level in the serum of rat showed in Figure 4. The level of testosterone was

significantly ($p < 0.001$) decline to (1.92 ± 0.03) compared with control group (3.12 ± 0.016).

DISCUSSION

Recent decades have seen an increase in the amount of evidence supporting a possible link between exposure to toxicants of the environmental and unfavorable consequences for reproductive health (Campion *et al.*, 2012). Our histopathological results showed many of degenerative changes such as vacuolation of spermatogonia, and severe necrosis and apoptosis in seminiferous tubules. This result might be attributed to uranium's capacity to damage reproduction because of its chemical and radioactive characteristics (Ma *et al.*, 2020). It has also been shown in previous investigations that uranium accumulates in the reproductive organs. Moreover, our findings imply that DU may originate from testicular fluid, which would allow it to avoid the blood-epididymal barrier and/or the BTB. Male reproductive organs are well-protected by both barriers (Mital *et al.*, 2011). According to the Sertoli cells' ability to sustain testicular homeostasis, germ cell death through apoptosis plays a role in maintaining it. This process may also be connected to vacuolization (Lagos-Cabre and Moreno, 2012). The two distinct pathways of testicular apoptosis—the mitochondrial-mediated pathway and the receptor-mediated Fas/FasL pathway—play a crucial role in preserving the germ cell population. The immunohistochemical and histopathological sections of the current study demonstrated that exposure to low doses of UA resulted in increased apoptotic activity. The mitochondrial apoptosis route and the Fas/FasL-mediated apoptotic signal transduction pathway can both be induced by reactive oxygen species (Chandra *et al.*, 2000). According to a research by Monleau *et al.* (2006), these findings reveal that the generation of ROS and inflammatory processes contributed to some of the DNA damage, and that recurrent exposure to insoluble DU particles may have a potentiation impact. Gonadotropins and steroid hormones precisely control the hormonal balance essential for male reproductive function, including spermatogenesis and BTB integrity. Nonetheless, our data showed a decrease in testosterone levels following a 90-day exposure to UA; this finding was consistent with earlier research (Grignard *et al.*, 2008; Angenard *et al.*, 2011), which also noted changes in sexual hormones following DU exposure. While both DU groups had lower intratesticular levels of estradiol, there was no significant change in intratesticular or plasma testosterone levels. Furthermore, a toxin may potentially impact rodent reproduction and fetal development through a number of different routes. Mutagenicity and genotoxicity, disruptions in cell division, modifications or inhibition of protein or steroid synthesis, disruption or inhibition of enzyme systems, and distortion of behavior patterns associated in normal reproduction are among the harmful effects of DU alloy. These mechanisms

can result in altered morphogenetic pattern formation, decreased or increased cell death, disrupted cell-to-cell contact, decreased biosynthesis, or tissue disruption that can cause aberrant pathogenesis in the developing fetus or reproductive system (Peters and Garbls-Berkvens, 1996; Schardein, 2000).

CONCLUSIONS AND RECOMMENDATIONS

In Conclusion of this study showed that the chronic exposure to UN can induce reproductive toxicity in male rats, mainly in the spermatogonia by increased the apoptotic activity, also decreased the testosterone level.

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NOVELTY STATEMENT

This study investigation the exposure to uranium acetate and Immunohistochemical Estimation of Apoptotic Activity and Testosterone Level In The Testes of Rats.

AUTHOR'S CONTRIBUTIONS

Ghusoon Abdul Kareem Neamah, created and designed the study. Muna Abdul Sahib Alkhfaji, sorted and compiled the references examined and evaluated the information. Heba Saleh Shaheed prepared the first draft of the text, and gave insightful feedback that helped to mold the work into its present state. The final manuscript was critically examined and approved by all writers. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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