



The efficiency of *Zephranthes candida* in inhibited action of uranyl acetate toxicity on test of male rats

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Abstract

Thirty male mature sprague-Dawley rats were divided into three equal groups, control group administered distilled water, treated group were administered 75mg/kg/b.w of uranyl acetate and treated group administered 75mg/kg/b.w of uranyl acetate with aquatic extract of *zephranthes candida* (5%). The route of administration was oral intubation for every other day for 53 days. Animals were administered 75mg/kg/b.w include: degeneration changes were appeared as spermatogenic cells depilation and showed number of atrophied seminiferous tubules, necrosis compared with animal treated with *Z.C.* aquatic extract. Also recorded a significant decrease ($P < 0.05$) in the number of spermatogonia and primary spermatocyte in two treated groups, and it can be concluded that *zephranthes candida* aquatic extract contains material that ability to inhibit side effect of uranyl acetate. It is hoped that the plant will be studying the importance of addressing an alternative to chemical treatments.

Keywords: Uranyl acetate, *Zephranthes candida*, Testis, Seminiferous tubules.

Introduction

Depleted uranium (DU) is a man-made, radioactive heavy metal derived from uranium ore. It is chemically identical to natural and enriched uranium, although it is approximately 40% less radioactive than the naturally occurring metal (ECRR, 2010). Uranyl acetate is one of the U salts, which is the most commonly associated with oxygen as the uranyl ion to form uranyl oxide (UO_2) (Lide, 1992). The effects of exposure to uranium are not, of course, restricted to DU and passive weapons fallout. Uranium is increasingly contaminating the environment, near nuclear sites, near isotope separation plants, near fuel manufacturing, near uranium mines and in atomic and thermonuclear weapons fission fallout, near and remote from the test sites. Uranium is increasing found in food and drinking water as it is significant component of agricultural fertilizer. It is therefore also found near fertilizer factories and phosphate mines and in the transportation of phosphate ore and its agricultural products (ATSDR, 1990; CRAFT 2004). Several previous investigations have been published concerning the effects of uranium salts on numerous organs such as bone, liver, lungs, kidney and blood (Whitnall *et al.*, 2009; Alkaisy *et al.*, 2002; Alkaisy *et al.*, 2005).

Several investigations have shown that uranium salts, such as uranyl nitrate and uranyl acetate, have the ability to induce several renal dysfunction and tubular necrosis (Domingo *et al.*, 2009). In addition, several published results have shown that U or DU causes decreased fertility, embryo/fetal toxicity including teratogenicity, and reduced growth of the offspring have been observed following uranium exposure at different gestation periods (Domingo *et al.*, 2009; Radulesen *et al.*, 2009).

Zephranthes candida (Lindl.) Herb (Family: Amaryllidaceae) is a perennial herb. The leaf is glossy deep green, linear and about 3mm wide. The flower is erect in perianth, the scape is slender and hollow and the flower is single borne on top of scape. The bottom of the spathe-shaped involucre is tubular. The capsule is nearly spherical and the seed is black and flat. The plant is widely cultivated as an ornamental flower and used as a medicinal plant in China. The whole plants of *Z. candida* are used to treat infantile convulsions, epilepsy, and tetanus. Phytochemical studies on *Z. c.* have focused on the bulbs, leading to reports of four ceramides and nine alkaloids (Chowdhury and Hubsten, 2006; Pettit, 1994). Luo *et al.* (2012) concluded that *Z. c.* extract contains materials which have cytotoxic activities against five human cancer cell lines and human bronchial epithelial cell line (Wu *et al.*, 2009).

Material and Methods

Thirty sexually mature laboratory males and Sprague-Dawley Albino rats of an average body weight of 230 ± 3.565 gm and 12-15 weeks old were obtained from animals house of the national center for censorship and curative researches in Baghdad. Animals divided into three equal groups, control group administrated distilled water, second group administrated 75mg/kg/b.w of uranyl acetate and third group administrated 75mg/kg/b.w with concentration 5% w/v of *Zephyranthes* aqueous extract the route of administration was oral intubation for every other day for 53 days.

Testes were taken for study of histopathological changes. the histological section were made according to Humson (1972), the organ was fixed by 10% formalin (10ml formalin + 90 ml 0.9% NaCl), then washed by tap water for several min. passing through a serial concentration of alcohol (50%, 70%, 80%, 90% and 100%) for 2hrs. in each concentration, then cleared by xylol, saturated with paraffin at 60° for 3hrs. embedded in pure paraffin; the blocks were cut into section with 5 μ m in thickness by using microtome. these section were held on glass slides using Myer's albumin; they were left for drying at 37° . Haematoxylin stain was used for 5-10min. washed by tap water then with acidic alcohol then washed by tap water. After that Eosin stain 15-30sec. and then washed by D.W. serial concentration of alcohol were then used (70%, 90% and 100%) for 2min. in each concentration, cleared by xylol for 10min; then Canada balsam was used, covered by cover slide and examined by light microscope.

Results and Discussion

Several changes were observed in the testis of animals received UA and UA with Z compared with control animals. These changes include atrophied seminiferous tubules surrounded by normal ones presented even within the same microscopic field (figure1).

The degenerative changes in seminiferous tubules were varied from one damaged tubule to other, the degenerative changes were appeared as spermatogenic cell depletion, degenerate spermatocytes characterized (figures 2 and 3). Some degenerated tubules showing shrinkage, others were filled with necrotic cells (Figure 4), while the animals treated U.A with Z. c. observed some alteration as a compare with animals that treated UA alone, these alteration include blood congestion and giant cell were present of some degraded tubule (Figures 5 and 6).

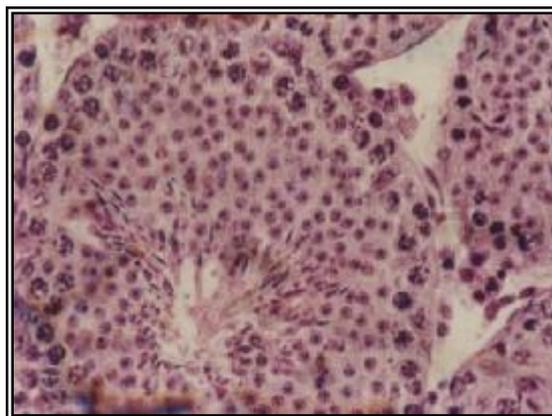


Figure (1): Section of rat testes(control group) showing various stages of maturation (H&E 400 X).

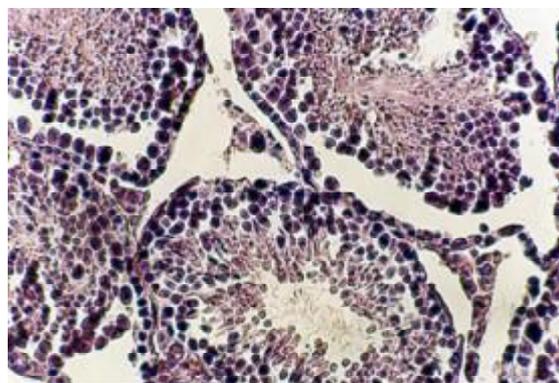


Figure (2): Section of rat testes treated with 75mg/kg uranyl acetate showing complete sloughing and atrophied seminiferous tubule and large interstitial space (H & E 200X).

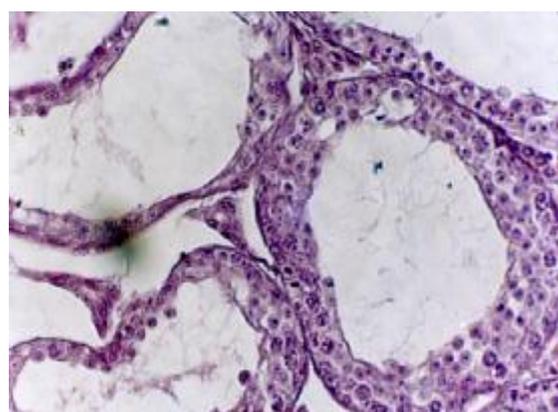


Figure (3): Section of rat testes treated with 75mg/kg uranyl acetate showing the degeneration of spermatocytes (H&E 200X).

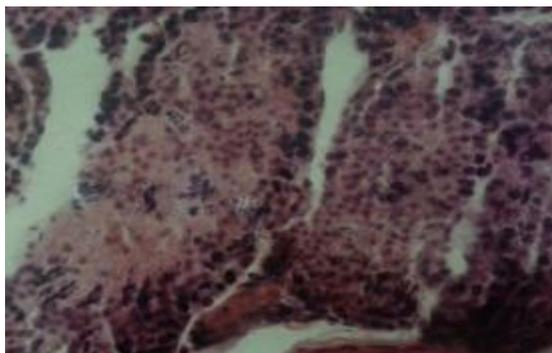


Figure (4): Section of rat testes treated with 75mg/kg uranyl acetate showing the degeneration & necrosis in seminiferous tubules (H&E 400X).

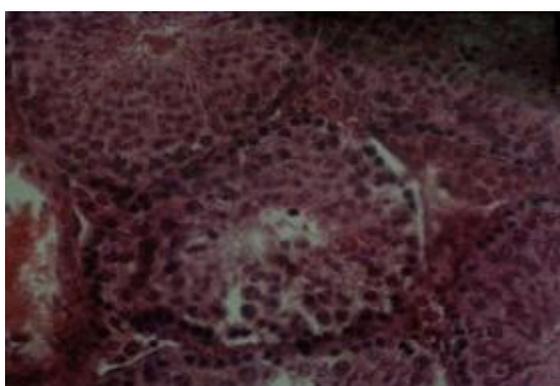


Figure (5): Section of rat testes treated with 75mg/kg uranyl acetate with *Z. c.* showing blood congestion & giant cells were present in some degraded tubules (H&E 400X).



Figure (6): Section of rat testes treated with 75 mg/kg uranyl acetate with *Z. c.* showing normal structure in many tubules & blood congestion (H&E 100X).

The count of spermatogenic cells showed a significant number of spermatogonia, primary spermatocytes and spermatids at $P < 0.05$, while the decreasing number of Sertoli cells was not significant in the two groups of experiment, as compared with the number of the same cells in the control group (Table 1).

In the last few years, medicinal plants used for the treatment of many diseases. Plants of the family Amaryllidaceae comprise ca. 85 genera and 1100 species that are distributed widely in the tropical region of the world. More than 500 Amaryllidaceae alkaloids representing 18 skeletal types have been isolated and reported to have acetylcholinesterase (AChE) inhibitory, antibacterial, antifungal, antimalarial, antitumor, antiviral and cytotoxic activities (Chowdhury and Hubsten, 2006; Pettit, 1994).

Table (1): The number of spermatogenic cells and Sertoli cells in the two administered groups as compared with the control group.

Number of cell	Control group	UA 75 mg/kg/b.w	UA 75 mg/kg/b.w+Z
No. of spermatogonia	35 ± 0.23*	29 ± 0.26*	31 ± 0.34*
No. of primary spermatoids	76 ± 0.56*	66 ± 0.29*	129 ± 0.50*
No. of spermatids	140 ± 0.63*	107 ± 0.38*	129 ± 0.58*
No. of Sertoli cell	24 ± 0.28*	22 ± 0.20*	23 ± 0.22*

No: 30, *Significant at $P < 0.05$, Means ± SE

After administration of other chemical compounds. Also, these results are in agreement with (Rchard *et al.*, 1997) who reported that paracetamol may cause depletion and atrophy in several seminiferous tubules. The results are similar to other studies where uranium was administered orally or subcutaneously to mice. Effects such as, decreased number of fetuses, embryonic/fetal toxicity, increased rate of abortion

(Paternain *et al.*, 1989). The histological section appears in the testicular tissue while the histological section in the testicular tissue of the treated group with aquatic extract of *Z. c.* appeared to be normal structure in many tubules although degenerative in some regions, maybe due to the ability of saponin and tannins in habituating the activity of uranyl acetate faster than the cellular reversible (Maasoura *et al.*, 1999).

The count of spermatogenic cells showed significantly decreasing in number of round spermatid and primary spermatocyte in both experimental animals, on the other hand the histological examination revealed that the degenerative changes were presented among all stage of spermatogenic cell and these change were noticed as spermatogenic cell depletion and sloughing of some degenerated spermatocytes.

These result arrangement with (Creasy, 2001) who said that different germ cell population of spermatogonia, spermatid were displayed their own sensitivity to different toxicants.

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