بسم الله الرحمٰن الرحيم

قرأ باسم ربك ا لذي خلق ١ خلق الإنـسان مـن
علق ٢ قرأ وربك الأكرم ٣ ا لذي علم بـالقلم
٤ علم الإنسان ما لم يعلم ٥

صدق الله العظيم

سورة العلق

الآيات (١-٥)

Histological study on the effect of Nitrofurazone on the testis of albino rat

Thesis
Submitted for fulfillment of
M.Sc. degree in Histology

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2010

Abstract

Nitrofurazone{NF;2-[(5-nitro-2-furanyl)-methylene] hydrazide carboxamide}, is a synthetic furan derivative, active against a broad spectrum of bacteria, which has been used both therapeutically and prophylactically against gastro-enteritis in a number of food-producing species, including sheep, goats, cattle, chickens, pigs, fish and turkeys. In non food-producing animals, pet animals, it is used in the treatment of wound and urinary tract infections. In human, it is used topically in the treatment of skin infections and burns and also as NF-impregnated urinary catheters to reduce the urinary tract infections in catheterized patients.

This work was designed to study the histological and immunohistochemical changes that might occur in the testis of albino rat in response to a single oral administration of Nitrofurazone.

This study was conducted on twenty-four male albino rats divided into two main groups; the experimental group (eighteen rats) which was given a single oral dose of 300 mg/kg Nitrofurazone, then subdivided into three subgroups A, B and C (six rats each), and the control group (6 rats).

These three subgroups (A, B, and C) were sacrificed together with the rats of the control group (two rats with each subgroup), respectively after, two days, four days and four weeks from Nitrofurazone administration and the testes were excised.

Haematoxylin (H.) and Eosin (E.) stained sections, immunohistochemical stained sections for FADD (Fas Associated Death Domain, which is an apoptosis marker) and Semi-thin sections were prepared, followed by morphometric studies to assess the area percent of FADD positive nuclei and to count the number of Leydig cells in the interstitial tissue.

Abstract

Two and four days following Nitrofurazone administration, the spermatocytes and the spermatids in the H. and E. stained sections and in semi-thin sections showed features of apoptosis. They also showed positive immunoreactivity for FADD in immunohistochemical stained sections. Sertoli cells were affected in the form of vacuolation of the cytoplasm and enlargement of their nuclei. While, Leydig cells were histologically normal with increase in their count and spermatogonia were preserved.

By four weeks following Nitrofurazone administration recovery was evident in the cells lining the seminiferous tubules.

It could be concluded that single oral dose of Nitrofurazone induces histological and pathological changes in the testes of the albino rats that might cause male infertility.

Keywords: Nitrofurazone, Albino rat, Testis, Apoptosis, Leydig cells, Fas, FADD.

Acknowledgement

Firstly and foremost, I am thankful to "**ALLAH**" the most gracious and the most merciful.

I am greatly honored to express my deep respect and gratitude to **Prof. Dr. Hanaa Abdel Kader**, Chairman of Histology Department, Faculty of Medicine, Cairo University, for her efforts and great help in providing the materials needed for this work.

I wish to express my thanks and sincere gratitude to **Prof. Dr. Eman Mostafa Sadek**, professor of Histology, Faculty of Medicine, Cairo University as she offered me much of her time. I also deeply appreciate for patience, valuable suggestions, meticulous supervision and faithful support.

I wish also to express my sincere gratitude and deep appreciation to **Prof. Dr. Manal Mohammad Hatem**, professor of Histology, Faculty of Medicine, Cairo University. She helped me in choosing the idea of this work and also gave me a great deal of her time and effort in supervising this work.

I am greatly indebted to **Dr. Safinaz Salah El-Din Sayed**, Assistant professor of Histology, Faculty of Medicine, Cairo University, for her kind, close and meticulous supervision and her valuable comments in photographing the slides, writing the results and the discussion and finally, in the revision of this study.

Finally, I am thankful to my professors and my colleagues for their continuous encouragement.

"To My Dear Family for Their Kind And Unlimited Support Thank You with All the Love and Gratitude."

List of Abbreviations

AMDUCA: Animal Medicinal Drug Use Clarification Act.
AP-1: Activating Protein-1.
Apaf-1: Apoptosis aggregation Factor.
bax, bcl-xs, bak, bad, bid: Pro apoptotic members of bcl-2 family.
bcl-2, bcl-x_1: Anti-apoptotic members of bcl-2 family.
BID: Bcl-2 Interacting Domain.
DAB: Di-Amino-Benzedine.
DDSA: Dodeceyl Succinic Anhydride.
DNA: Deoxy-ribo Nucleic Acid.
DRs: Death Receptors.
FADD: Fas Associated Death Domain.
FDA: Food and Drug Administration.
FSH: Follicle Stimulating Hormone.
G1: First Gap stage of the cell cycle.
G2: Second Gap stage of the cell cycle.
H. and E.: Haematoxylin and Eosin.
IGF: Insulin like Growth Factor.
IGF-Bp3: Insulin like Growth Factor Binding protein 3.
ISEL: In Situ End Labeling.
LC: Liquid Chromatography.
MAO: Mono Amino Oxidase.
MS: Mass Spectrometry.
NF: Nitrofurazone.
NF-KB: Nuclear Factor-KB.
NK-cells: Natural Killer cells.
NTP: National Toxicology Program.
PBS: Phosphate Buffer Saline.
p-FADD: phosphorylated FADD.
RIP: Receptor Interacting Protein.
RNA: Ribo Nucleic Acid.
ROS: Reactive Oxygen Species.
SEM: Semicarbazide.
TNF-Rs: Tumor Necrosis Factor Receptors.
TUNEL: TdT-mediated Nick End Labeling.

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Introduction and Aim of work

Nitrofurazone{NF;2-[(5-nitro-2-furanyl)-methylene] hydrazide carboxamide}, a synthetic furan derivative, is a broad spectrum antibacterial and anti-parasitic. Because of its high efficiency in preventing gastroenteritis, it was used both prophylactically and therapeutically in gastro-intestinal infections, in addition to its use topically in cases of pink eye and wounds in food and meat producing animals like cattle, goats, sheep, rabbits, chickens and turkeys **(Chamberlain, 1976)**. In human, NF (Becafurazone or Demafural) was used locally in cases of skin infections and burns, but there were reports of contact dermatitis following exposure to it **(Bajaj and Gupta, 1986 and Lo et al., 1990)**. Nitrofurazone-impregnated urinary catheters are used to reduce urinary tract infections associated with catheterization.

Studies in which NF was administered in the basal diet of rats revealed that, it elevated the incidence of mammary fibroadenomas in female rats **(Morris et al., 1969)**. On the other hand, **Maronpot (1987)** reported that NF increased the incidence of ovarian granulosa cell and benign mixed tumors in mice and rats. Both findings were detected by other authors **(National Toxicology Program "NTP", 1988; Kari et al., 1989 and Huff et al., 1989)**.These benign neoplasms are known to progress to malignancy so; NF was considered as a potential carcinogenic compound in rodents **(NTP, 1988 and Huff et al., 1989)**.

Non-neoplastic effects for NF were reported; they included genotoxicity in vitro but not in vivo **(Skeggs et al., 1984 and NTP, 1988)**, joint articular cartilage degeneration in male and female rats in several areas like vertebral and knee joints **(NTP, 1988 and Huff et al., 1989)** and cytotoxicity on hepatocytes in vitro **(Hoogenboom et al., 1991)**.

Introduction and Aim of work

Several studies have been performed on the effect of oral Nitrofurazone, in basal diet, on the male genital system in various experimental animals, including rats and mice. Reversible testicular atrophy was reported **(Uematsu, 1966)**.

This work was designed to study the histological and immunohistochemical changes that might occur in the testis of albino rat in response to a single oral administration of Nitrofurazone.

Normal histology of the testis

The testis is an avoid organ, 4.5 cm length, 2.5 cm in width and 3cm in antero-posterior diameter. Its temperature is kept at a level lower than that of the body, 35°c, by its presence in the scrotum, which is a highly specialized skin pouch outside the abdomen characterized by absence of fat, numerous merocrine sweat glands and smooth muscle fibers arranged haphazardly (Dartos muscle) whose contraction results in wrinkling of the scrotal skin.

Inside the scrotum, testis is surrounded by three coats **(Trainer, 1987)**:

1. The outermost mesothelial one (tunica vaginalis), formed of two layers (inner visceral and outer parietal) by evagination of the peritoneum, pushed during descent of the testis from the posterior abdominal wall to the scrotum during embryological development.

2. Underlying tunica vaginalis, there is a thick fibrous capsule formed of collagenous connective tissue fibers and because of its whitish appearance, it is called tunica albuginea. It is thickened posteriorly forming mediastinum testis through which blood vessels enter and ducts leave the testis.

3. The innermost layer is formed of thin loose areolar connective tissue rich in blood vessels called tunica vasculosa.

The testis is divided by thin, fibrous, incomplete septa radiating from mediastinum testis to the tunica albuginea into 200-250 pyramidal compartments called testicular lobules **(Trainer,**

1987). The lobules are partially intercommunicating with each other due to the incomplete septa and each lobule contains 1-4 seminiferous tubules surrounded by network of loose connective tissue called interstitial tissue. The seminiferous tubules produce male germ cells while the interstitial cells of Leydig, present in the interstitial tissue, secrete testicular androgens **(Oliver, 1995)**.

Seminiferous tubules:

They are coiled tubules forming about 70-80% of the testicular volume. In man, they may reach 70 cm long and 150-250 micrometers in diameter. Both ends of each tubule empty into the rete testis. Each tubule is surrounded by dense capillary network **(skinner and Fritz, 1985 and Johnson and Everitt, 2000).**

The seminiferous tubules are lined by seminiferous epithelium, also called germinal epithelium, and bounded by the tunica propria which is a multi-layer capsule, its inner layer represented by the basal lamina of the seminiferous epithelium. The other layers vary according to the species**(Tung and Fritz, 1980)**.

In rodents the basal lamina is surrounded by a layer of flat cells called myoid cells that have the ultra-structural characteristics of smooth muscles and responsible for the rhythmic contractions of the seminiferous tubules**.** In other bigger species, the layer of myoid cells is surrounded by multiple layers of fusiform fibroblast cells while in human; the tubules are only surrounded by layers of fibroblasts without myoid cells **(Skinner and Fritz, 1985).**

The seminiferous epithelium is a complex stratified epithelium made up of supporting, non proliferating cells (Sertoli cells) and the germ cells which include: stem cells (spermatogonia)