A Histological Study on the Effect of Bisphenol A on the Testis of Prepubertal and Postpubertal Mice and the Protective Role of Nigella Sativa Oil

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List of Abbreviations

ABP	: Androgen binding protein
BPA	: Bisphenol A
BTB	: Blood testis barrier
CAT	: Catalase
EDC	: Endocrine disrupting chemicals
ER	: Estrogen receptor
FSH	: Follicle stimulating hormone
GnRH	: Gonadotropin-releasing hormone
GSH	: Glutathione
LH	: Luteinizing hormone
LPO	: Lipid peroxidation
MDA	: Malondialdehyde
NSO	: Nigella sativa oil
PCOS	: Polycystic ovarian syndrome
ROS	: Reactive oxygen species
SNTs	: Seminiferous tubules
SOD	: Superoxide dismutase
TQ	: Thymoquinone

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Abstract

Background: Bisphenol A (BPA) is a widely spread chemical that is incorporated in the production of food containers, water bottles, toys and sports equipment. Due to the daily human exposure to BPA, it has become a great concern to detect its effect on human health. BPA was proved to be an endocrine-disrupting chemical (EDC) which could affect the reproductive system **(Vandenberg et al., 2007).** Nigella sativa oil (NSO), one of the natural remedies, is known for its antioxidant properties and healing capabilities **(Darakhshan et al., 2015)**

Aim of the work: The current study was carried out to detect the effect of bisphenol A on the structure of the testis of prepubertal and postpubertal mice and the possible protective role of Nigella sativa oil.

Materials & methods: Fifty male mice were used and were divided into two groups: Group I (prepubertal group) (3-4 weeks) and Group II (postpubertal group) (9-10 weeks).

Each group was further subdivided into:

Control groups; (including 3 subgroups) receiving water and food *ad libitum* (IA1, IIA1), corn oil (IA2, IIA2), NSO (IA3, IIA3)

Experimental groups; (including 2 subgroups) BPA group (receiving orally daily 50mg/kg/day of bisphenol A) (IB1, IIB1), and BPA +NSO group (receiving bisphenol A orally daily (50mg/kg bw/day) and Nigella sativa oil (0.5ml/kg bw/day) orally concomitantly) (IB2, IIB2).

All mice were sacrificed after 3 weeks and the testes were processed for light microscopic examination (H&E, Masson's

trichrome, PAS, Caspase-3 immunohistochemistry) and tissue malondialdehyde (MDA). Serum testosterone hormonal assay, morphometric and statistical analysis were also performed.

Results: Microscopic examination of BPA groups (IB1, IIB1) revealed marked affection of the seminiferous tubules. The mean surface area and the mean thickness of the spermatogenic epithelium were significantly decreased. Most of the spermatogenic cells appeared distorted with darkly stained nuclei and vacuolated cytoplasm in the testes of both prepubertal and postpubertal mice. BPA groups (IB1, IIB1) also showed significant decrease in serum testosterone levels and significant increase in MDA levels as compared to their equivalent control groups. In BPA+NSO groups (IB2, IIB2), the testes appeared similar to those of the control groups with preservation of the testicular structure and function. These results were supported by the hormonal assay of serum testosterone and tissue malondialdehyde assessment.

Conclusion: BPA showed detrimental effects on the testis with reduced spermatogenesis in both prepubertal and postpubertal mice; the prepubertal group being more affected. Co-administration of Nigella sativa oil with bisphenol A resulted in a protective effect preserving the structure and function of the testis.

Keywords: Bisphenol A, Nigella sativa oil, testis, prepubertal, postpubertal, testosterone, ROS, apoptosis.

Introduction

Bisphenol A (BPA) is an environmental chemical contaminant that is widely used in the manufacture of polycarbonate plastics and epoxy resins **(Eid et al., 2015).** It is extensively used in the production of food containers, intravenous tubing, medical prosthetics, drinking water bottles, toys, sports equipment, in the inner lining of metal cans and water supply pipes, and as a dental sealant **(Michatowicz, 2014).**

The major human exposure source to BPA is diet, including ingestion of contaminated food and water **(Vandenberg et al., 2009)**. Bisphenol A is leached from the lining of food and beverage cans when they are cleaned with harsh detergents or when they contain acidic or high-temperature liquids **(Gao et al., 2015).**

BPA compound is not only widely spread in the environment, but also toxic even in low doses. Exposure to BPA has been associated with various diseases, including cardiac lesion **(Gear et al., 2017),** type II diabetes, altered insulin homeostasis, abnormal liver function **(Xia et al., 2014)** and even cancer **(Duan et al., 2013)**. Chronic BPA exposures demonstrate direct association of obesity in children, adolescents and even adults **(Trasande et al., 2012).** Additionally, it is suspected that exposure to BPA may result in disturbances in both male and female reproductive systems **(Erler and Novak, 2010).**

The observed effects of BPA on various metabolic changes in humans are related to hormonal regulation, therefore, it is considered as a xenoestrogen. Xenoestrogens include compounds

that may disturb the endogenous estrogens and, hence, may affect the growth, development and reproduction of organisms **(Tomza-Marciniak et al., 2017)**. Furthermore, the low doses of BPA generate ROS that decrease the activities of the antioxidant enzymes and increasing lipid peroxidation leading to oxidative stress **(Wahby et al., 2017).**

Nigella sativa, commonly known as the black seed, is an annual herbaceous plant. It grows in Eastern Europe, the Middle East, and Western Asia **(Gilani et al., 2004).** It is one of the most famous herbs known for its wide range of healing capabilities. N. sativa has been investigated for its biological effects and therapeutic potential and shown to have broad spectrum of activities including antidiabetic **(Razavi and Hosseinzadeh, 2014)**, hepato-protective **(Mollazadeh and Hosseinzadeh, 2014)**, renal protective **(Havakhah et al., 2014)**, anti-inflammatory, antimicrobial and antioxidant **(Hosseinzadeh et al., 2007)** properties.

Most of the experimental and clinical studies performed on N. sativa have proved that its pharmacological actions are due to its ability to eradicate free radicals and/or inhibit lipid peroxidation **(Salem, 2005).**

Aim of the Work

The current study was planned to assess the effect of bisphenol A on the structure of the testis of prepubertal and postpubertal mice and to evaluate the protective role of Nigella sativa oil.

Review of literature

The testis is a paired organ with endocrine as well as exocrine functions. The testes develop retroperitoneally in the dorsal wall of the embryonic abdominal cavity and move during fetal development to become suspended in the two halves of the scrotal sac, or scrotum, at the ends of the spermatic cords. During migration from the abdominal cavity, each testis carries with it a serous sac, the tunica vaginalis, derived from the peritoneum. This tunic consists of an outer parietal layer lining the scrotum and an inner visceral layer, covering the tunica albuginea on the anterior and lateral sides of the testis (**Mescher, 2010)**.

The tunica albuginea is a dense connective tissue capsule which thickens on the posterior side to form the mediastinum testis. From this fibrous region, septa penetrate the organ and divide it into about 250 pyramidal compartments or testicular lobules. Each lobule contains sparse connective tissue with endocrine interstitial cells (Leydig cells), and one to four highly convoluted seminiferous tubules in which sperm production occurs **(Singh, 2014).**

The seminiferous tubules:

Each seminiferous tubule is surrounded by a distinct basement membrane and a layer of flattened myoid cells. The seminiferous epithelium is an unusual, complex stratified epithelium with two cell populations: spermatogenic (or germ) cells and Sertoli cells. In a seminiferous tubule, germ cells are at various stages of spermatogenesis. The cells closest to the basement membrane with spherical nuclei are **spermatogonia**. Larger cells with

spherical nuclei but with distinctive spaghetti-like chromatin are **primary spermatocytes**. The haploid **secondary spermatocytes** are seldom seen; almost as soon as they form, they divide and produce spermatids. During a transformation period, **spermatids** attach to the relatively few Sertoli cells. Spermatids do not divide but mature into **spermatozoa**, which are released into the lumen and carried into efferent ducts (**Ovalle and Nahirney, 2013).**

The wave of the seminiferous epithelium describes the distribution of cellular stages along the length of the tubule. In rodents, each stage occupies a significant length of the seminiferous tubule, and the stages appear to occur sequentially along the length of the tubule. So, a transverse section through the tubule usually reveals only one pattern of cell stages. On the other hand, in humans each stage of the cycle has a patch-like distribution in the seminiferous tubule. Patches do not extend around the circumference of the tubule, nor are they in sequence **(Ross and Pawlina, 2015).**

Sertoli cells:

Sertoli cells were originally described by **Enrico Sertoli** in **1865** as branched cells surrounding different germ cell stages. Sertoli cells are located directly on the basement membrane of the seminiferous tubules and surround different germ cell stages with their cytoplasm. These cells are the only cells that reach from the basal membrane to the tubular lumen. **Immature Sertoli cells** exhibit a dark, round nucleus and less cytoplasmic branches (as there are only few gonocytes within the epithelium by this time),

whereas **adult Sertoli cells** can be recognized by their oval shaped to triangular nucleus, deep nuclear notches and a prominent nucleolus. Morphological changes start at the onset of puberty and have been described by **Sharpe et al. (2003)** as one prerequisite of Sertoli cell maturation.

Sertoli cells are derived from the coelomic epithelium and are the first differentiating cells in the fetal gonad, which enables seminiferous cord formation, colonization of the fetal gonad with gonocytes, differentiation, as well as function of Leydig cells **(Cupp and Skinner, 2005)**. Sertoli cells not only protect developing germ cells from endo- or exogenous substances and from the immune system but also take over nutritive and endocrine functions, by secreting the intratubular fluid. This shows a different composition from plasma as it contains more potassium and less sodium ions. In addition, they synthesize and release testicular transferrin, androgen binding protein (ABP), inhibin and fructose-rich medium. They also phagocytose cytoplasmic remnants shed during spermiogenesis (**Gartner and Hiatt, 2014**).

The Blood Testis Barrier:

The blood-testis barrier (BTB) forms between two adjacent Sertoli cells to maintain the immune privilege of the testis after onset of spermatogenesis. This barrier divides the seminiferous epithelium into **a basal compartment**, including only diploid spermatogonia and **an adluminal compartment**, containing the other germ cells types. **Dym and Fawcett (1970)** were the first to