"Modulatory effect of TNF-α inhibitor in experimentally-induced testicular toxicity in rats"

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Cadmium (Cd) is a serious environmental and occupational contaminant that represents a serious health hazard to humans and other animals. Reproductive health problems have been reported in men exposed to Cd. Testicular damage is one of the deleterious effects due to Cd exposure. Cd-induced testicular toxicity is mediated through oxidative stress, inflammation, testosterone inhibition and apoptosis. Thus, the present study was performed to assess the possible protective role of infliximab (IFX), anti-TNFα agent, against Cd-induced testicular damage and spermiotoxicity in rats. Rats were randomly allotted into six experimental groups: control, Cd sulphate treated, Cd sulphate treated with infliximab (5 mg/kg), Cd sulphate treated with infliximab (7 mg/kg), infliximab alone (5 mg/kg), and infliximab alone (7 mg/kg). To induce testicular damage, Cd sulphate (1.5 mg/100 gm body weight/day) was dissolved in normal saline and orally administrated for 3 consecutive weeks. The rats in infliximab-treated groups were given a weekly dose of 5 mg/kg/week or 7 mg/kg/week of infliximab intraperitoneally. Cd exposure reduced sperm count, markers of testicular function, sperm motility, as well as gene expression of testicular 3β-Hydroxysteroid dehydrogenase (3β-HSD) and 17β-Hydroxysteroid dehydrogenase (17β-HSD) and serum testosterone level. Additionally, it increased testicular oxidative stress, inflammatory and apoptotic markers. The histopathologic studies supported the biochemical findings. Treatment with infliximab significantly attenuated Cd-induced injury verified by the restoration of testicular architecture, enhancement of steroidogenesis, preservation of spermatogenesis, modulation of the inflammatory reaction along with suppression of oxidative stress and apoptosis. It was concluded that infliximab, through its antioxidant, anti-inflammatory and anti-apoptotic effects, represents a potential therapeutic option to protect the testicular tissue from the detrimental effects of Cd.

Keywords: Infliximab-Cadmium-Testicular damage-Spermiotoxicity-Steroidogenesis

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List of Abbreviations

ACP	Acid phosphatase
Ag	Antigen
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CAT	Catalase
Cd	Cadmium
cDNA	Complementary DNA
$CdSO_4$	Cadmium sulphate
COX-2	Cyclooxygenase-2
DHEA	Dehydroepiandrosterone
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTNB	5,5'-dithio-bis (2-nitrobenzoic acid)
ELISA	Enzyme linked immunosorbent assay
FSH	Follicle stimulating hormone
GnRH	Gonadotrophin releasing hormone
GSH	Reduced glutathione
H_2O_2	Hydrogen peroxide
HRP	Horseradish peroxidase
3β-HSD	3β-hydroxysteroid dehydrogenase
17β-HSD	17β-hydroxysteroid dehydrogenase

IFX	Infliximab
Ig G1	Immunoglobulin G1
IL	Interleukin
i-NOS	Inducible nitric oxide synthase
i.p.	Intraperitoneal
I/R	Ischemia/reperfusion
IV	Intravenous
LDL	Low density lipoprotein
LH	Luteinizing hormone
LPO	Lipid peroxidation
MDA	Malondialdehyde
mRNA	Messenger RNA
NaCl	Sodium chloride
NF-κB	Nuclear factor-Kappa B
Ni	Nickel
NO	Nitric oxide
O.D.	Optical density
PBS	Phosphate buffered saline
PGF-2	Prostaglandin F2
***p*-NPP**	*p*-nitrophenyl phosphate
***p*-NP**	*p*-nitrophenol
p.o.	Per os
PVC	Polyvinyl chloride
P450scc	Cholesterol side chain cleavage enzyme

qPCR	Quantitative polymerase chain reaction
RA	Rheumatoid arthritis
ROS	Reactive oxygen species
rpm	Revolution per minute
RQ	Relative quantity
RT-PCR	Real time polymerase chain reaction
SD	Standard deviation
StAR	Steroidogenic acute regulatory protein
TMB	3,3,5,5'-tetramethylbenzidine
TNF-α	Tumor necrosis factor-α
TNFR	Tumor necrosis factor receptor

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Human infertility

Infertility has been recognized as a worldwide public health issue by the World Health Organization (WHO) (**Ma *et al.*, 2019**). It is defined as the failure to conceive after 12 months of unprotected intercourse. It is a common disorder that can affect up to 15% of couples (**Lao *et al.*, 2018**). Over the past 25 years, a worldwide decline in human fertility rates and poorer reproductive outcomes have been described by multiple international literature (**de Angelis *et al.*, 2017**; **Durairajanayagam, 2018**; **Ma *et al.*, 2019**). According to WHO, the incidence of infertility ranges from 4% to 14% in different countries and regions (**Ma *et al.*, 2019**). Interestingly, nearly half of these cases can be attributed to a male factor (**Lao *et al.*, 2018**). A global decline in human semen quality was witnessed which is likely to be a contributing factor (**Hallak *et al.*, 2018**; **Sukhn *et al.*, 2018**). Now, there is a global concern with the increased male infertility and the possible underlying causes (**Ahmed *et al.*, 2018**). To tackle this problem, this requires an overview on the anatomy and the hormonal control of the male reproductive system as well as the possible contributing factors that can affect male reproduction **(Fig. 1)**.

Male reproductive system

The male reproductive system is specialized for the production of male gametes and their transportation to the female reproductive tract that is mediated by supporting fluids and production of testosterone (**Mohanty and Singh, 2017**).

It consists of:

1. **Penis**

   It is able to ejaculate semen (containing sperm) during sex and to relieve the body of urine (**Mohanty and Singh, 2017**).

2. **Urethra**

   It acts as a way for urine from the bladder, out of the male body and for the ejaculation of semen (**Mohanty and Singh, 2017**).

3. Epididymis

It is divided into the head, body, and tail (**Ruberte *et al.*, 2017**). There are three primary functions of the epididymis: (1) it is responsible for the maturation of infertile testicular sperm to fertile mature sperm, (2) it acts as a peristaltic conduit for the active transport of sperm from the testis to the vas deferens, and (3) it serves as a storage site for mature sperm (**Roberts and Pyor, 1997**).

4. Vas deferens

It is a thick, muscular tube that carries sperm from the cauda epididymis to the ejaculatory ducts in the prostate (**Roberts and Pyor, 1997**).

5. Accessory glands

The mass of semen is produced with the help of three accessory glands of the male reproductive system: the seminal vesicle, the prostate, and the bulbourethral glands (**Mohanty and Singh, 2017**).

5.1. Seminal vesicles

The seminal vesicles are basically glands that add approximately 60% of the semen volume. The fluid contains maximum amount of sugars, which are used by sperm to generate ATP to permit movement through the female reproductive tract (**Mohanty and Singh, 2017**).

5.2. Prostate gland

The prostate gland produces a variety of proteases, which help in semen liquefaction to facilitate the release of sperm (**Mohanty and Singh, 2017**).

5.3. Bulbourethral glands

They release a thick, salty fluid that lubricates the end of the urethra and the vagina and helps in cleaning the remaining urine from the penile urethra and helps neutralize the acidic vaginal pH and turns the environment favorable to permit sperm mobility (**Mohanty and Singh, 2017**).