The effect of melatonin on the testes of rats treated with cyclophosphamide (Histological & Immunohistochemical studies)

Thesis submitted for fulfillment of
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بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا ۖ
إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

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All Love

For my dear mother ,father, husband and beautiful daughters

For unlimited patience & continous support

Abstract

Cyclophosphamide (CP), is cytotoxic alkylating agent, used extensively as an antineoplastic agent for the treatment of specific types of cancers, as well as an immunosuppressive agent during organ transplantation . However, its clinical utility is limited because of its several adverse actions especially on the human reproductive system. Cyclophosphamide treatment in patients is associated with oligozoospermia and azoospermia, as well as biochemical and histologic alterations in the testes and epididymis of rats and humans. Melatonin hormone is natural compound found in humans and animals ,it was detected in the reproductive system of human and animals ,therefore it seems reasonable to assume that melatonin may play a useful function to the cells of the reproductive system . **Aim of this work** was to investigate the effect of melatonin on the histological and immunohistochemical changes which may appear in the testicular cells of the testes of albino rats previously treated with cyclophosphamide alone or in combination with melatonin . **Material& Methods :**forty two adult male albino rats weighing about 150 -200 gm were enrolled in this study .The animals were classified into three groups .At the end of experiment ,the rats were sacrificed ,weighted ,right testes were processed for paraffin section. Prepared histological slides were stained with H & E, Masson's trichrome ,Immune histochemical studies with BCL2 onchoprotein and Morphometric study .**Results** showed certain histological and immunohistochemical changes concerning that melatonin could exhibit partial protection against cyclophosphamide induced testicular atrophy.

key words : Cyclophosphamide, melatonin , testis.

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Content

List ofAbbreviations

ABP: androgen-binding protein.

AMH : anti-müllerian hormone. .
AFMK :N(1)-acetyl-N(2)-formyl-5-methoxykynuramine .
AANAT or NAT :aryl alkylamine *N*-acetyl transferase.

Bid: Bcl-2 interacting Domain **.**

Bcl_2: bcl_2 onchoprotein.

COX-2 :cyclooxygenase 2.

CP: Cyclophosphamide

CYP: cytochrome p450 **.**

DNA: deoxyribonucleic acid.

ETC: electron transport chain.

FSH: follicle-stimulating hormone

G-Px: glutathion peroxidase .

HCG: human chorionic gonadotropin.

HIOMT: hydroxyindole-*O*-methyltransferase.

HIV: Human Immunodiffiency Virus.

I/R : Ischemia –Reperfusion.

INSL3: insulin-like hormone 3.

IL-2: interleukin-2 **.**

iNOS : inducible NO synthase.

MTP: mitochondrial transition pore.

MIS :müllerian inhibiting substance.

PGC: Primordial germ cells.

ROS/RNS :reactive oxygen/ nitrogen species.

rER:rough endoplsmic reticulum**.**

SCN: suprachiasmatic nucleus.

SLE:systemic lupus erythematosus**.**

SOD: superoxide dismutase.

StAR :steroidogenic acute regulation protein

SRY(Sex determinding Region on Y gene)**.**

T: testosterone.

Introduction And Aim of Work

Most of the chemotherapeutic drugs used in the treatment of neoplastic cells cause various sorts of damage to normal cells. Cyclophosphamide (CP) is used in the treatment of certain malignant tumors. It has also an immunosuppressive effects during organ transplantation and in the treatment of some autoimmune diseases as glomerulonephritis .Previous studies have shown that CP alters the process of sperm chromatin structure and the composition of sperm head basic proteins **(Cerbasi et al .,2010)**.

Melatonin acts as a powerful antioxidant and as a free radical scavenger of hydroxyl and peroxyl radicals. Indeed, melatonin was shown to be twice as potent as vitamin E in removing peroxyl radicals and it is more effective in scavenging hydroxyl radicals than glutathione and mannitol . Since melatonin binding sites were detected in the reproductive system of different species ,it seems reasonable to assume that melatonin exerts its actions via direct interaction with the steroidogenic cells of the reproductive organs **(Ilbey et al. ,2009).**

Aim of Work:-

The aim of this work was to investigate the histological and immunohistochemical changes which may appear in testicular cells of albino rats previously treated with cyclophosphamide alone or in combination with melatonin.

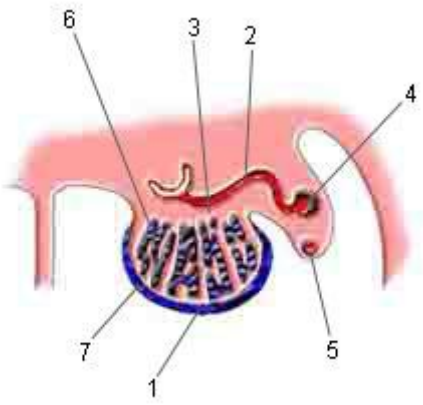
DEVELOPMENT, ANATOMY AND HISTOLOGY OF TESTIS

The testis is an exocrine and endocrine organ . The exocrine portion consists of a series of highly coiled seminiferous tubules that produce sperms (spermatogenesis);the endocrine portion consists of specialized cells called interstitial cells of Leydig that secret testosterone(**Nieschlag et al.,2010).**

A-Embryogenesis:

The early gonads are capable of becoming either ovaries or testes . In males , the sex-specific gene SRY(Sex determinding Region on Y gene) that is found on the Y-chromosome initiates sex determination by downstream regulation of sex-determining factors such as AMH(anti-müllerian hormone), which leads to development of the male gonads **(Sadeler , 2010)**.

In humans, starting at about week 4, the gonadal rudiments are present within the intermediate mesoderm adjacent to the developing kidneys. At about the 6^{th} week, The primitive sex cords develop and continue to proliferate and penetrate deep into the medulla to form the testis (medullary) cords and rete testis. Later these testicular cords lose contact with the surface epithelium because of the formation of a dense connective tissue capsule around the testis, the tunica albuginea. The sex cords are composed of supporting Sertoli cells, derived from the mesothelium (coelomic epithelium) and spermatogonia, derived from the primordial germ cells. The interstitial Leydig cells are derived from the genital ridge mesenchyme. The primitive sex cord gives rise to the seminiferous tubules, rete testis (an anastomosing network of channels in the mesorchium) and tubuli recti. The efferent ductules derived from the remnants of the mesonephric tubules connect the rete testis to the derivatives of the mesonephric duct (epididimis, ductus defferens, seminal vesicle and ejaculatory duct) fig (1) **(Drake et al.,2010).**



Development of the testis

Fig(1) : 1. Tunica albuginea 2. Degenerating mesonephric tubule3. Rete testis 4. Mesonephric duct 5. Paramesonephric duct 6.Testis cords7. Mesothelium cords **(Drake et al.,2010)** .

Testis contain three principal cell types**(James and Jean,2003).**

1-Primordial germ cells(PGC) , derived from endodermal cells of the inner cell mass(initially identifiable in the yolk sac).

2- Supporting cells derived from the coelomic , epithelium of the gonad ridge that differentiate into the Sertoli cells in the testis .

3-Stromal (interstitial) cells, derived from mesenchyme of the gonadal ridge that differentiate into leydig cells and peritubular myoid cells.

The Primordial germ cells express specific cell markers and are recognizable in the 4^{th} to 5^{th} day in human blastocyst. Before day 23 of human gestation ,these cells are located in the dorsal and caudal portions of the yolk sac endoderm , and then migrate by amoeboid movement from the gut endoderm through the mesentery to reach genital ridge **(James and Jean,2003).**

On reaching the genital ridge the germ cells, together with adhering epithelial cells , infiltrate the underlying mesenchyme .This process is identical in male and female embryos and culminates by 5-6 weeks of gestation in the formation of the genital blastema containing the three cell types**(Castrillon et al.,2000).**

The germ cells are named gonocytes once they reach the gonad, in which they proliferate .The gonocytes then enter quiescent period during which their number does not change .This period extend to birth in the mouse and postnatal day 3 in the rat ,when mitosis resumes and gonocytes differentiate into spermatogonia **(Olaso and Habert,2000)** .

Primordial germ cells that fail to reach the genital ridge degenerate or differentiate into other cell types and may serve as progenitors of extra-gonadal cell tumors in later life. The somatic cells of the gonad can undergo partial organization into ovary or testis as specified by the sex chromosomes even if the germ cells are prevented from migration to the genital ridge ,suggesting that some determinants for gonadal development are inherent in the cells of the genital ridge **(James and Jean,2003).**

The Sertoli cells are the first to differentiate ,they surround the germ cells to form the seminiferous cords. Sertoli cells divide actively until puberty and remaining quiescent thereafter**(Olaso and Habert,2000).**

The seminiferous tubules are separated by mesenchyme that gives rise to the interstitial cells of Leydig .By the eights week ,these cells begin to secret androgenic hormones (testosterone- androstenedione) , which induce masculine differentiation of the mesonephric ducts and the external genitalia .Testosterone production is stimulated by human chorionic gonadotropin (HCG) which reaches peak amount during the 8- to12-week,In addition to testosterone the fetal testes produces a glycoprotein known as AMH or