Histological and Morphometric Changes of Aripiprazole Versus Quetiapine on The SeminiferousTubules of the Albino Rat

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Abstract

Population nowadays is suffering from some mental or behavioral disorders. Our community is complaining from increase in the appearance of mental disorders with a lot of treatment options. Increasing prevalence of psychiatric disorders is not limited to adults, but also children and adolescents are subjected to such deviations. Therefore, the uses of antipsychotics have increased. Two main drugs are used Quetiapine and Aripiprazole. These have been used with satisfactory results and fewer side effects. Both Aripiprazole and Quetiapine are known to-act on the dopaminergic receptor and this receptor has been detected in the germ cells. Hence it became the aim of the present work to detect the histological changes induced by Aripiprazole versus Quetiapine on the structure of the seminiferous tubules of albino rat, as their lining begin from a germ cell until reach the mature one.

Fifty two male albino rats were used in this study; twenty six rats aged two weeks (pre-pubertal rats) and twenty six rats aged three months (Post-pubertal rats).

The rats were divided into the following groups:

Group (I): Control group:

This group included twelve rats served as control untreated rats and was subdivided into: **I A**: six pre-pubertal rats and **I B**: six post-pubertal rats.

Group (II): Aripiprazole group:

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This group included twenty rats, subdivided into: **II A**: ten pre-pubertal rats and **II B**: ten post-pubertal rats.

Group (III): Quetiapine group:

This group included twenty rats, subdivided into: **III A**: ten pre-pubertal rats and **III B**: ten post-pubertal rats.

After ٤٥ days (immediately after stoppage of drug administration), half of the rats in each group were scarified.

The other half of each group were sacrificed after another ٤٥ days (a recovery period; they were left without taking any drugs).

Dissection of the testes was done. Samples from both testes of all groups were taken and processed for Light microscopic study.

The testes of all groups showed that the seminiferous tubules lost their normal architecture pattern. Multiple vacuolations replaced the cellular elements. Focal depletions or generalized cellular loss occurred. The lumen was empty in most of the tubules. Irregularity of the tubules was a common finding as well as congestion of the blood vessels and exudation in between the tubules. Morphometric measures were done for all the groups and revealed decrease in the epithelial height of the germinal epithelium and decrease tubules perimeter and surface area. The groups treated with aripiprazole were affected more than those treated with quetiapine.

The recovery groups of aripiprazole still showed damage in the seminiferous tubules, which is more evident in the group that started the treatment prior to puberty. The percentage of the damaged tubules was high in the group that started the experiment before puberty. The adult group included less damage.

The recovery groups of quetiapine showed improvement of the structure of the seminiferous tubules, approaching the control pattern. However, few sections still revealed residual damage. The percentage of damaged tubules decreased in both groups.

The results were discussed and it was concluded that the adverse effect of aripiprazole on the seminiferous tubules exceeded that of quetiapine. Almost no recovery could be detected after aripiprazole withdrawal, while marked improvement occurred after quetiapine withdrawal. According to the previous results it could be concluded that aripiprazole and quetiapine have adverse effect on the seminiferous tubules of the testis. Quetiapine is less injurious than aripiprazole. Moreover after withdrawal of the drugs, the quetiapine treated groups almost regain the normal seminiferous tubular pattern, a fact not observed with aripiprazole.

Key words:

Aripiprazole – Quetiapine – Seminiferous tubules – prepubertal - postpubertal

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Introduction

Many people nowadays suffer from mental or behavioral disorders ***(WHO International Consortium in Psychiatric Epidemiology,*** ٢٠٠٠***).*** In our community suffering is increasing from mental disorders with many treatment options.

Prevalence of psychiatric disorders is not limited to adults but recently is observed among children and adolescents. Most disorders in children are autistic disorders and attention deficit hyperactivity disorders ***(Amr et al.,*** ٢٠١٢***).***

In Egypt, schizophrenia is the most common chronic psychosis encountered. However, other problems have high incidence as well like: mood disorders ٦,٤٣٪, anxiety disorders ٢٢,٦٪, and depression١٩,٧٪ ***(Okasha,*** ٢٠٠٤***).*** Mental disorders are known to have a greater negative effect on health than many serious chronic physical illnesses ***(Kessler,*** ١٩٩٧***).***

In accordance to the previous statments, the use of antipsychotic drugs has increased nowadays. Two main antipsychotic drugs, Quetiapine and Aripiprazole, have been used in the psychiatric disorders with satisfactory results and relatively fewer side effects ***(Tayler,*** ٢٠٠٣***).***

Aripiprazole belongs to atypical antipsychotic family. It is primarily used in the treatment of schizophrenia and bipolar disorder ***(Tayler,*** ٢٠٠٣***).*** Other uses include treatment of major depressive disorder, and irritability associated with autism ***(Taylor,*** ٢٠٠٣***).***This drug exerts its effect by being a partial dopamine agonist ***(Burris et al.,*** ٢٠٠٢***).*** This means that Aripiprazole binds to the D٢ receptors with the same affinity as dopamine, but has a lower intrinsic efficacy, therefore the response it triggers is lower than dopamine but higher than the antagonist. Accordingly this drug is preferred more than other antipsychotics ***(Brunton et al.,*** ٢٠١٠***; Mailman and Murthy,*** ٢٠١٠***).***

The other drug is ***Quetiapine***. It is an atypical antipsychotic approved for the treatment of schizophrenia, bipolar disorder, and

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along with other antidepressants to treat major depressive disorder. It is also sometimes used as a sleep aid because of its sedating effect ***(James and Cassagnol,*** ٢٠٠٨***).*** This drug is apparently safe to be used in children less than ١٣ years old **(*Robert et al.,* ٢٠١١).** It exerts its effect through dopamine and serotonin antagonism but it has the advantage of having no effect on prolactin level ***(Atmaca et al.,*** ٢٠٠٢***).***

In spite of both Aripiprazole and Quetiapine are known to-act on the dopaminergic receptor and this receptor has been detected in germ cells ***(De Siqueira Bringle et al.,*** ٢٠١١***)***, only few studies reported their adverse histological effects on the structure of the seminiferous tubules of the testes.

Aim of work

The aim of the present work is to investigate the histological and morphometric changes induced by Aripiprazole versus Quetiapine on the structure of the seminiferous tubules of albino rats.

Male reproductive system

The male reproductive system consists of the scrotum, testes, spermatic ducts, sex glands, and penis. These organs work together to produce, maintain, and transport the sperm and protective fluid (semen) to the female genital tract during intercourse. These organs have a role in producing and secreting male sex hormones responsible for maintaining the male reproductive function ***(Widmaier et al.,*** ٢٠١٣***).***

Testes

The testis (from the Greek word Orchis) is the male gland responsible for both exocrine and endocrine reproductive functions ***(Stainberger,*** ١٩٧٥***).***

The testes are critical for normal development of internal and external genitalia in the fetus, for secondary sexual characteristics, sexual function, and initiation of spermatogenesis at the time of puberty. In adults, they maintain the male body features and function, sexual function, and fertility ***(Melmed et al.,*** 2015***).***

The testes are paired organs; each testis is located in the scrotum, separated from the other one by a scrotal septum, described as a large olive. The average volume of the adult testis is approximately ٢٥ ml.

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normally it measures ٣,٥ – ٥ cm in length, ٢,٥ – ٣ cm in width and ٣ cm in depth ***(Jezek,* ٢٠١٢*).*** The right testis is commonly slightly larger and weightier than the left ***(Stevens and Lowe,* ١٩٩٢*).***
The testis lies obliquely with its long axis vertical and a slight anterior and lateral slant to the superior pole. Superiorly it is suspended by the spermatic cord and anchored to the scrotum by the scrotal ligament and remnant of the gubernaculum ***(Swartz,* ٢٠٠٦*).***

In rats the testes are located in the abdominal cavity. No true scrotum was formed and the testes are laid in an inguinal pouch from which they might be withdrawn to the abdominal cavity when they are enlarged ***(Mark et al.,* ٢٠٠٥*).***

Each testis is surrounded by three coats from outside inwards: tunica vaginalis, tunica albuginea and tunica vasculosa. *The tunica vaginalis* (a remnant of processus vaginalis) surrounds the testis by a double layer separated by a potential space containing serous fluid and lined by a layer of flat mesothelial cells. The tunica vaginalis is absent at the superior and posterior borders of the testis, where the spermatic cord and the epididymis adhere directly to the testis. The visceral layer of tunica vaginalis is in close contact to the testis, epididymis and vas deferens. The parietal layer is adjacent to the internal spermatic fascia, it is more extensive and extends superiorly into the distal part of the spermatic cord. Deep to the tunica vaginalis is *the tunica albuginea*, a tough, fibrous outer covering of the testis. Posteriorly, it is redirected interiorly to form an incomplete vertical septum called the mediastinum testis. The *tunica vasculosa*, the innermost layer is a thin layer of connective tissue containing the superficial blood vessels, lymphatics, autonomic nerve fibers and genital ducts ***(Tintinalli et al.,*** ٢٠٠٤***)***.

The mediastinum testis extends from the superior to near the inferior portion of the gland. It decreases in width as it passes inferiorly. Anteriorly and laterally, numerous incomplete septa are given off, which radiate to the glands surface and are attached to the tunica albuginea. These divide the interior of the testis into many, cone-shaped spaces that have a wide base at the gland's surface, they narrow as they pass to the mediastinum. These septa extend internally to divide each testis into ٢٥٠ lobules of different sizes; the largest and longest is in the center. Each lobule contains one to four convoluted

seminiferous tubules. The mediastinum supports the ducts and vessels as they pass to and from the glandular substance ***(Standring et al.,*** ٢٠٠٥***).***

Microscopic Appearance

The testicular tissue is divided into two compartments: the tubular compartment and the interstitium. The seminiferous tubules comprise the tubular compartment and the interstitium is composed of Leydig cells, macrophages, fibroblasts, and blood vessels ***(Gnessi et al.,*** ١٩٩٧***).***

The rat testes possessed relatively less interstitial tissue and Leydig cells and more germ cells ***(Rizkalla,*** ١٩٨٦***).***

The Convoluted Seminiferous Tubules

These tubules are surrounded by a thick basal lamina and coated by ٣-٤ layers of smooth muscle cells (or myoid cells). Each tubule is about ١٥٠ µm in diameter and ٨٠ cm long. The seminiferous germinal epithelium formed of only one somatic cell type, the Sertoli cell ***(Hess and Franca,*** ٢٠٠٥***)*** and many different germinal cell types (**Russell et al.,** ١٩٩٠**).**

The spermatogenic cells

Spermatogonia

Are the first cells that begin spermatogenesis. They originate during the ٤th week of fetal development in the endodermal walls of the yolk sac and migrate to the primordium of the testis**.** Spermatogonia are diploid germ cells (٢n) that divide by mitosis and reside on the basement membrane ***(Coward and Wells,*** ٢٠١٣***).***

There are different types of spermatogonia as type-A, intermediate, and type-B. In well-studied laboratory rodents, such as rats and mice, four classes of spermatogonia are present: undifferentiated type A spermatogonia, differentiated type A spermatogonia, intermediate spermatogonia (In) and type B spermatogonia (B) ***(De Rooij and Russell,*** ٢٠٠٠***).***

In these species, the different spermatogonial classes can be described by light and transmission electron microscopy according to the presence and distribution of heterochromatin. It has also been suggested that undifferentiated spermatogonia are located in niches of the seminiferous epithelium, which are regulated by the Sertoli cell. Spermatogonia remain dormant until puberty ***(Phillips et al.,*** 2010***).***

Type A spermatogonia have an oval contour and a rounded nucleus with very fine chromatin grains and one or two nucleoli. They are stem cells which undergo division to form new generations of both type A and type B spermatogonia ***(Bergman et al.,*** ١٩٩٦***)***.

Intermediate spermatogonia divide by mitosis once to give type B spermatogonia. They have fine flakes of chromatin adjoining the nuclear membrane ***(Dym,*** ١٩٩٤***).***

Type B spermatogonia have rounded nuclei with chromatin granules of variable size, which often attach to the nuclear membrane, and one nucleolus. Although type B spermatogonia may divide many times, they do not function as stem cells and their final mitosis always results in the formation of primary spermatocytes ***(Bergman et al.,*** ١٩٩٦***)***.

The primary spermatocytes

They lie in the cell layer next to the spermatogonia, and appear larger than them. They immediately enter the prophase of the first meiotic division, which is prolonged (about ٢٢ days). Therefore spermatocytes are found in all stages, because meiosis is an elongated period of spermatogenesis. Meiosis extends over approximately ١٤ days in the mouse. A huge number of primary spermatocytes are always observable in cross-sections through the seminiferous tubules. Cell divisions, from the formation of primary spermatocytes and till the production of the spermatids, are incomplete. The cells remain connected by intercellular bridges of cytoplasm ***(Talwer et al.,*** ٢٠١٦***).***

The precursors of spermatocytes are small and situated next to the basement membrane near the spermatogonia, having filamentous chromatin of their nuclei and are known as leptotene spermatocytes. Other small spermatocytes possess the chromatin threads of their nuclei collected in a bouquet like configuration, and are known as zygotene spermatocytes. leptotene and zygotene are not recognizable in every section but only in some stages of the spermatogenic cycle. As the chromatin of the nucleus of the spermatocytes becomes less packed, they are known as pachytene spermatocytes. The completion of the first meiotic division results in the formation of secondary spermatocytes ***(Rizkalla,*** ١٩٨٦***).***

The secondary spermatocytes

They are smaller than the primary spermatocytes. They quickly enter and complete the second meiotic division and are therefore rarely seen in histological preparations. Their division results in the formation of spermatids ***(Khattab,*** ٢٠٠٧***).***

The spermatids

These are haploid gamete cells resulting from the meiosis of the secondary spermatocytes. The early (or rounded or immature) spermatids are rounded cells with large spherical nuclei ***(Khattab,*** ٢٠٠٧***).*** Later they undergo spermeogenisis and are transformed into late (or elongated or mature) spermatids ***(Cheng et al.,*** ٢٠١٢***).***

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They could be found in any layer of the tubule although they lied usually in groups near the lumen. They are small (about ١٠ µm in diameter) having at first very light (often eccentric) nucleus. The chromatin condenses during the maturation of the spermatids into spermatozoa, and the nucleus becomes smaller and stains darker ***(Rizkalla,*** ١٩٨٦***).***

The terminal phase of spermatogenesis is called spermiogenesis and consists of the differentiation of the newly formed spermatids into spermatozoa ***(Franca et al.,*** ٢٠٠٥***).***

The spermatozoa

The mature human spermatozoon is about ٦٠ µm long and actively motile. It is divided into head, neck and tail ***(kessel,*** ١٩٩٨***).***

The time required for spermatogenesis to be completed (the time required for type A spermatogonia to be transformed to spermatozoa) is variable between species. In human it is about ٧٢ days, while in rats it takes about ٥٤ days ***(Huang et al.,*** ٢٠٠٧***).***

In rats, each stage of the spermatogenesis cycle takes a significant length of the tubule, and the stages seem to occur sequentially along the length of the tubule creating waves of the seminiferous epithelium. In contrast, there are no classic waves in the human seminiferous tubules ***(Huang et al.,*** ٢٠٠٧***).***

Sertoli cells

A Sertoli cell (a kind of sustentacular cell) is the "nurse" cell of the testis because its main function is to nourish the newly formed

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sperm cells through the stages of spermatogenesis ***(Chung et al.,*** ٢٠١٠***)***. The Sertoli cell has numerous important roles in spermatogenesis, including: support and nutrition of the developing germ cells, compartmentalization of the germinal epithelium inside the seminiferous tubule by tight junctions, which offers a protected and specialized environment for the developing germ cells. Moreover, it controls the release of mature spermatids into the tubular lumen (spermiation), and the secretion of fluid, proteins and several growth factors. In addition, it allows phagocytosis of the degenerating germ cells and phagocytosis of the excess cytoplasm (residual body) that are remnants from the released sperm ***(Rato et al.,*** ٢٠١٢***)***. There is a significant relationship between sperm production rate and the number of Sertoli cells ***(Rizkalla,*** ١٩٨٦***).***

The Sertoli cell also facilitates the actions of follicle stimulating hormone and luteinizing hormone ***(Rato et al.,*** ٢٠١٢***)***.

Their shape is oval to pyramidal. Sertoli cells extend from the basement membrane to the luminal compartment of the seminiferous epithelium ***(Rato et al.,*** ٢٠١٢***)***.

Processes of the Sertoli cells spread out in between the spermatogenic cells (cell boundaries are therefore not obviously visible in the light microscope). The nucleus of Sertoli cells is ovoid or angular, large and lightly stained ***(Young and Heath,*** ٢٠٠٠***)***.

A prominent nucleolus is a constant feature with dense chromatin bodies. The cytoplasm contains a moderate number of mitochondria, lipid droplets and a small amount of rough endoplasmic reticulum (rER) ***(Young and Heath,*** ٢٠٠٠***)***.

Lateral processes of Sertoli cells are connected together by tight junctions, which are part of the blood-testis barrier ***(Bergman et al.,*** ١٩٩٦***).***

Spermatogonia and primary spermatocytes are located in the basal compartment; other cellular stages of spermatogenesis are located