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Original Article

The Protective Effect of Aromatase Inhibitor Anastrozole on Varicocele-Induced Testicular Dysfunction in Rats

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A B S T R A C T

Aim: This study aimed to investigate the protective effect of anastrozole on experimental varicocele - induced testicular dysfunction in rats.

Material and method: adult male albino rats were divided randomly into three groups; group 1 act as control. In group 2 (varicocele rats) varicocele was induced by partial ligation of the left renal vein. Group 3 (anastrozole-treated group) varicocele rats received anastrozole (400mg/l) in the drinking water for a period of 9 weeks immediately after ligation of the left renal vein. Serum FSH, LH, testosterone and intratesticular testosterone were measured. Seminal parameters (progressive motility, sperm cell concentration, epididymal sperm abnormal forms) were estimated. Testicular tissue specimens were histopathologically examined by hematoxylin & eosin staining.

Results :after 9 weeks of operation, the varicocele induced significant decreased of bilateral intratesticular testosterone levels and marked impairment of seminal parameters and histopathological changes in the rat tests compared with control group ($p<0.05$). While, serum FSH, LH and serum testosterone levels insignificantly decreased. All these factors were significantly improved by anastrozole. These results clearly pointed the pivotal role of aromatase inhibitors in varicocele induced testicular dysfunction.

Key Words: Aromatase inhibitors, varicocele, testicular dysfunction, anastrozole.

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1. INTRODUCTION

Varicocele is observed in 10-20% of the general male population, in 35-40% of men with primary infertility and in up to 80% of men with secondary infertility **(Bechara et al., 2009; Razi et al., 2011).** Varicoceles are being reported in up to 91% of sub-fertile cases, most of who were previously regarded as having idiopathic etiology **(Resim et al., 1999).** Recently, the pathogenesis mechanisms by which varicocele induce testicular degeneration, spermatogenesis arrest and finally infertility are not completely understood. The suggested mechanisms include reflux of toxic metabolites from adrenal and renal origin, impairment of the hypothalamic-gonadal axis, venous stasis leading to testicular hypoxia and elevation of temperature in testicles (Comhaire**, 1991; Benoff and Gilbert 2001).** The previous studies indicated that the intra-testicular testosterone decreases in varicocele patients **(Naughton et al., 2001).**

Recent studies showed that aromatase inhibitors can increase endogenous testosterone production and serum testosterone levels. Treatment of infertile males with the aromatase inhibitors testolactone, anastrazole, and letrozole has been associated with increased sperm production and return of sperm to the ejaculate in men with non-obstructive azoospermia **(Tadros and Sabanegh, 2017; ShoshanySchlegel, 2017)**.Aromatase is a cytochrome p-450 enzyme that converts testosterone to estradiol and androstenedione to estrone. Aromatase found in the testis, liver and brain in addition to female reproductive tract and adipose tissue. In the testes, aromatase are localized to Leydig and Sertoli cells and are found in germ cell tumors **(Inkster et al., 1995).**

Thus, the aim of the present study was to evaluate the possible protective effects of anastrozole-aromatase inhibitor- on the testicular dysfunction through measurement of the serum level of testosterone, LH and FSH, evaluation of seminal parameters and

histopathological changes in experimentally-induced varicocele in rats.

2. MATERIALS AND METHODS

2.1. Experimental protocol

2.1.1 Drugs and chemicals:

- Anastrozole: AstraZeneca UK Ltd.
- Urethane crystal: (Ethyl carbamate). (Prolabo, Paris).

2.1.2 Animals used

Fifty adult male albino rats, weighting (120-150gm). They were brought from Experimental Animal Breeding Farm, Helwan - Cairo .All animals were housed in controlled laboratory condition at 20 -25C in a 12h light/dark cycle and had free access to standard laboratory chow (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and water. They have acclimatized for one week and were caged (10/cage) in fully ventilated room at room temperature. All experimental protocols were approved by the ethics committee.

2.1.3 Experimental design:

Sixty rats were randomly divided into three groups:

Group (1): Control group, a suture was placed around the renal artery but not tied. This group did not receive any drug.

Group (2): varicocele non- treated rats. Rats were anaesthetized with urethane (white crystals 0.6ml/100g 25% freshly prepared solution). Varicocele was done by a clamp passed behind the left renal vein distally to the spermatic vein insertion. A silk ligature was placed around the left renal vein at this site and was tied over the top of a probe. The probe was then withdrawn and the vein was allowed to expand **(Turner, 2001)**.

Group (3): varicocele treated: rats were treated with anastrozole (400mg/L) in the drinking water for 9 weeks **(Turner et al, 2000).**

2.2.1 Parameters used:

2.2.1 Hormonal assay parameters:

Serum levels of FSH, LH, and testosterone:

At the end of the 9th week rats were anaesthetized with urethane. Blood samples were collected from the abdominal aorta to determine the serum levels of FSH, LH and testosterone hormone. The blood samples (2mleach) were allowed to clot at room temperature, centrifuged at 3000 rotation/minute and the sera were separated. Samples were stored at -20 Ć in dark containers.

Serum level of FSH:

The serum FSH, was measured with the commercially available kits, follicle stimulating hormone (Amer-sham pharmacia, Biotech Ltd., Buckinghamshire, U.K.).

Serum levels of LH: were measured by using lutenizing hormone assay system (Amersham Pharmacia, Biotech Ltd., Buckinghamshirc, U.K.).

Serum levels of testosterone: Diagnostic Products Corporation. Los Angeles. U.S.A).

2.2.2 Intratesticular testosterone:

At the end of the experiment the rats were sacrificed and testes were cut and weighed. Right testes homogenized in 10 ml distilled water and undergo centrifugation at 3000 rpm for 10 min. at 4 ºC the supernatal fraction was used as a sample to determine testicular testosterone.

2.2.3 Seminal parameters:

The epididymal content of each rat was obtained after cutting the tail of epididymis and squeezing it gently in sterile clean watch glass and examined using the following parameters:

Progressive motility:

The progressive motility of sperms was examined according to the method reported by **Bearden and Fluquary (1980),** where a small droplet of semen was added to one drop of sodium citrate solution 2.9% on a warm slide. Several fields were examined and the incidences of progressively motile sperms were estimated and recorded.

Sperm cell concentration:

This was performed according to the technique adopted by **Bearden and Fluquary (1980),** using pipette of haemocytometer. The undiluted semen was withdrawn up to the mark 0.1 and the pipette was then filled up to the mark 101 by normal saline stained with eosin. The contents of the pipette were mixed by holding the end of the pipette with thumb and index fingers and shaking it vigorously. The cover slide was placed over the counting chamber and a drop of diluted semen was spread between the haemocytometer slide and its cover. The sperms in 5 large squares contains (contain 80 small squares) were counted using the high power objective lens. The sperm cell concentration in cubic milliliters was estimated by multiplying the counted number of sperms by 10 (depth) and 1000 (dilution).

Epididymal sperm abnormal forms:

A drop from epididymal content of each rat was mixed with an equal drop of eosin-nigrosin stain. The semen was carefully mixed with the stain. Then films were spread on clean slides. A hundred sperms were

examined at random per slide under high power objective lens and percentage of abnormal sperms was recorded.

2.3. 1 Histopathological techniques:

The testes were removed and half of each testis was fixed in Bouin's solution **(Bouin, 1897),** and embedded in paraffin wax sectioned at 5 microns **(Harris, 1900)** and stained with haematoxillin and eosin stain. Histological changes were observed by light microscopy.

Statistical Analysis: was performed using one-way analysis of variance (ANOVA) to detect significant differences between the group means. Probability (P) values of< 0.05 were considered as statistically significant. *Statistical Analysis* results are presented as mean ± standard error (mean ± SEM).

3. RESULTS

3.1 Effects of varicocele-induced changes in rats:

Varicocele induced insignificant decrease of serum levels of FSH, LH and testosterone (table 1). While, intratesticular testosterone level was significantly decreased (figure 1).Also, Sperm count, sperm motility and sperm viability were significantly decreased. On the other hand, varicocele significantly increased abnormal sperm forms in compared with control group (figure 2). Histopathological changes showed moderate degenerative changes in some spermatogenic cells and necrosis of seminiferous tubules. Also, sertoli cells showed degeneration with significant decrease in the number of spermatids and sperms inside the seminiferous tubules (figure 3).

3.2 Effects of anastrozole administration on varicocele -induced changes:

3.2.1 Serum levels of FSH, LH, and testosterone:

Administration of anastrozole (400mg/L) had no significant effect on serum levels of FSH, LH and testosterone figure (1). On the other hand, anastrozole significantly increase intratesticular testosterone levels when compared with the control group (figure 2).

3.3 Seminal parameters:

3.3.1 Sperm count changes:

In varicocele non- treated rats, there was significant decrease in sperm count $p<0.05$ compared to control group. Administration of anastrozole (400mg/L) significantly increase sperm count, compared with varicocele non- treated group but it was at non-significant lower level ($p<0.05$) if compared to control group.

3.3.2 Sperm viability changes:

In varicocele non -treated rats, there was significant decrease of viability $p<0.05$ compared with control group. Administration of anastrozole (400mg/L) significantly increase sperm viability when compared with varicocele group and it was at non-significant lower level ($p<0.05$) if compared to control group.

3.3.3 Sperm motility changes:

In varicocele non- treated rats, there was significant decrease of motility $p<0.05$ compared with control group. Administration of anastrozole (400mg/L) significantly increase sperm motility when compared with varicocele group and it was at non-significant lower level ($p<0.05$) if compared to control group.

3.3.4 Sperm abnormal forms changes:

In varicocele non - treated rats, there was significant increase of abnormal forms $p<0.05$ compared with control group. Administration of anastrozole (400mg/L) significantly decrease sperm abnormal forms when compared with varicocele group and it was at non-significant higher level ($p<0.05$) if compared to control group. (Table 1, figure 3).

3.4.1 Histopathological changes:

Anastrozole group showed improvement of testicular histology compared to varicocele group and nearly normal germinal epithelial layers with preservation of sertoli cells. Also, there was increased growing of spermatogenic cells with many spermatozoa in the lumen of seminiferous tubules (figure 4).

Table (1): Effect of administration of anastrozole (400mg/L) in drinking water for 9 weeks on sperm parameters in experimentally varicocele induced male infertility for 9 weeks:

Groups	Sperm count Millions/mm^3	Sperm viability %	Sperm motility %	Sperm abnormal forms %
Control	77.34± 4.2	90.31±2.4	80.61±3.1	16.31±0.12
Varicocele non-treated	50.71±3.8[a]	66.4±3.1[a]	55.32±2.7[a]	25.1±1.45[a]
Anastrozole treated	70.22±1.5[b]	84.27±1.1[b]	71.43±2.5[b]	20.91±0.81[b]

a: significant difference versus control at $p<0.05$.

b: significant difference versus varicocele infertile group.

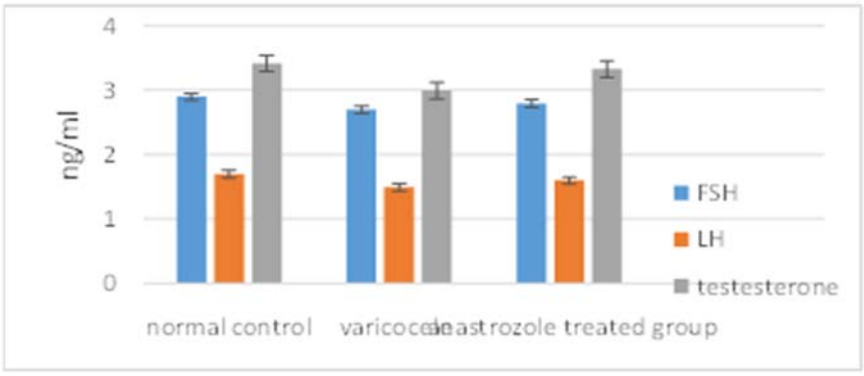


Figure (1): Effect of treatment with anastrozole (400mg/l) in drinking water for 9 weeks on serum levels of FSH, LH and testosterone in varicocele model induced experimentally in rats. There were insignificant decrease of serum levels of FSH, LH and testosterone level after 9 weeks of varicocele induction. Anastrazole treatment increase FSH, LH and testosterone insignificantly after 9 weeks. Data are presented as mean ±SEM.

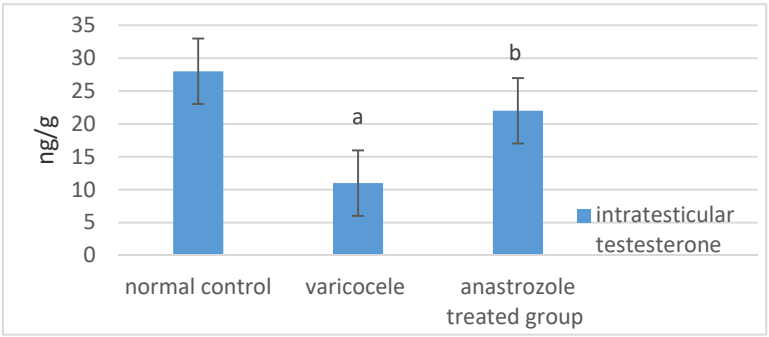


Figure (2): Effect of treatment with anastrozole (400mg/l) in drinking water for 9 weeks on intratesticular levels of testosterone in varicocele model induced experimentally in rats. There were significant decrease of intratesticular levels of testosterone after 9 weeks of varicocele induction. Anastrazole treatment significantly increase intratesticular testosterone after 9 weeks.
Data are presented as mean ±SEM.
a:significant difference versus control group at $p<0.05$.
b:significant difference versus varicocele infertile group at $p<0.05$.

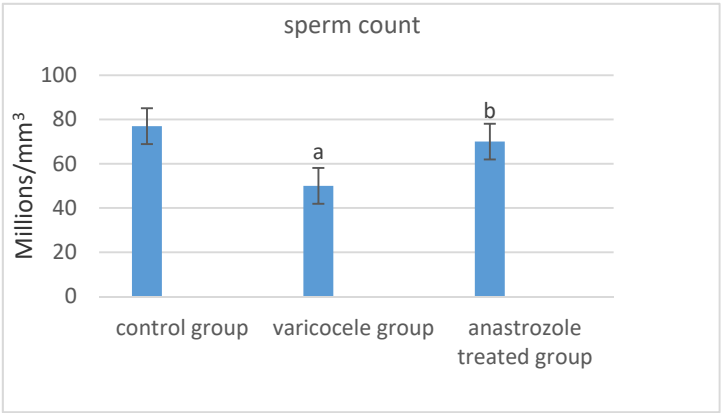


Figure (3): Effect of treatment with anastrazole (400mg/L) in drinking water for 9 weeks on sperm count in varicocele induced experimentally in rats.
Data represented as mean ±SEM
a: significant difference versus control group at $p<0.05$.
b: significant difference versus varicocele infertile group at $p<0.05$.

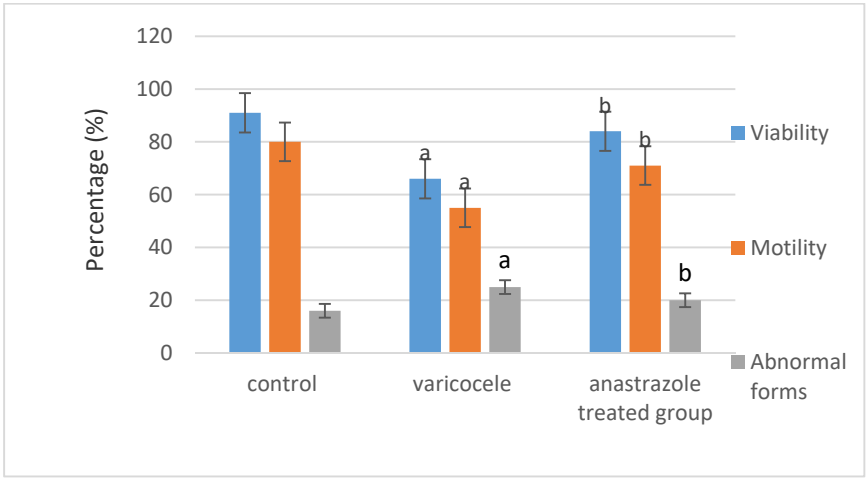
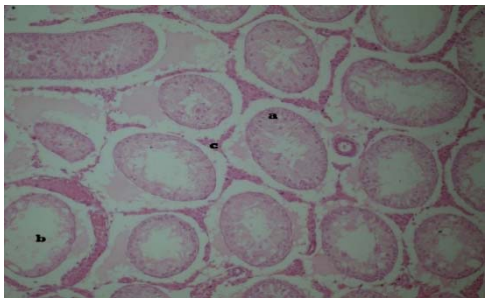


Figure (4): Effect of treatment with anastrozole (400mg/L) in drinking water for 9 weeks on sperm viability, motility and abnormal forms in varicocele induced experimentally in rats.

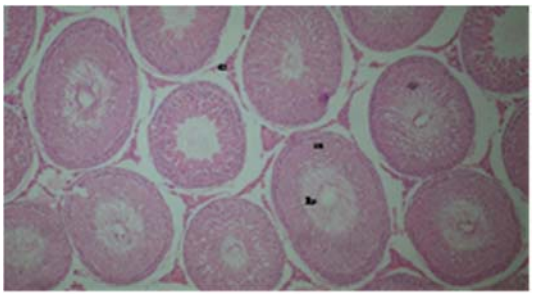
Data are represented as mean ±SEM

a: significant difference versus control group at $p<0.05$.

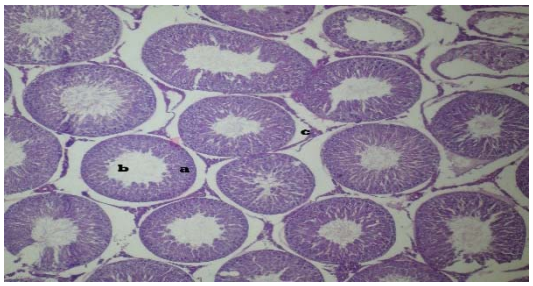
b: significant difference versus varicocele group at $p<0.05$.



A)



B)



C)

Figure (5): Effect of treatment with anastrozole (400mg/L) in drinking water for 9 weeks on histological changes in rat testes with varicocele.

A) A photomicrograph of a cut section in the testis of a control rat (group 1) showing normal histological structure of seminiferous tubules :(a) lining with normal layers of germinal cells (b) lumen filled with sperms and spermatids (c)normal spaces between seminiferous tubules with normal stroma. (H&Ex10).

B) A photomicrograph of a cut section in the testis of a rat with varicocele (group 2) showing :(a) mild decrease in Sertoli cells (b) decreased in number of sperms and spermatids in seminiferous tubules (c) normal spaces between seminiferous tubules with normal stroma. (H&Ex20).

C) A photomicrograph of a cut section in the testis of anastrozole treated rat (group 3)showing certain improvement of histological findings :(a) increased growing of spermatogenic and Sertoli cells (b) many spermatozoa in seminiferous tubules (c) normal spaces between seminiferous tubules with normal stroma. (H&Ex20).

4. DISCUSSION

Varicocele is the main cause of primary and secondary male infertility. The high incidence of varicocele in men with secondary infertility at puberty suggest that the varicocele can cause a progressive decline in fertility. Although varicocelectomy is effective treatment of infertility, a significant number of men remain infertile after varicocelectomy**(Silva et al., 2002; Singh et al., 1995; Allan et al., 2004).** Thus, there is increasing need to identify the exact pathophysiology of varicocele and the possible conservative treatment.

In the present study, varicocele resulted in insignificant decrease in serum levels of FSH, LH and testosterone. On the other hand, intratesticular testosterone was significantly decreased. There were significant decrease in sperm count, motility, viability and increase in sperm abnormal forms. Also, there were significant histopathological changes in the testicular tissue in the studied rat groups. These results were in line with previous studies on the effect of varicocele on testosterone level in serum and testes of rats **(Liu et al., 2007; Luo et al., 2011).**These effects were also in agreement with **Taghizadeh et al., (2017)** who found that varicocelized rats showed decrease in sperm count, motility and damage in testicular architecture.

Administration of anastrozoleto varicocele rats caused significant increase in serum levels of FSH, LH and testosterone. At the same time it caused significant increase in intratesticular testosterone compared to varicocele none treated rat group. These results agreed with **Gulino et al., (2014); Xu et al., (2017**)who reported that the aromatase inhibitors decrease the conversion of androgen to estrogens, increasing serum levels of androgens, resulting in an increased release of gonadotropins in male infertile patients. In this way, the local production of testosterone by leyding cells and androgen binding protein by sertoli cells, both known to be involved in the maintenance of normal spermatogenesis, might be increased.

Oral administration of anastrozole caused significant increase of sperm count, sperm viability and motility. Also, it caused non- significant decrease in abnormal forms compared to varicocele non-treated group. These results in consistent with **Saylam et al., (2011**)who reported that aromatase inhibitor has positive effects on spermatogenesis and semen quality in infertile men with a low serum testosterone-estrogen ratio. A positive effect of anastrozole on sperm kinetics was proven in a systematic review of randomized controlled trials in treatment of oligozoospermic or azoospermic men by (**Ribeiro et al., 2016).**

Spermatogenesis is highly dependent on intratesticular testosterone in rodents **(singh et al., 1995).** Additionally, high intratesticular testosterone is necessary for transition from type A to type B spermatogonia, an early step during spermatogenesis **(McLachlan et al., 2002). Ruwanpura et al., (2010)** reported that testosterone is involved in the survival of spermatocytes and spermatids through anti-apoptotic mechanisms. These finding are very important in the clinical setting because we can increase intratesticular testosterone by using aromatase inhibitors.Many studies have reported aromatase receptors localization in germ cells, mainly in spermatogonia and spermatocytes **(Kimura et al., 1993; Guillaume et al., 2001).**

The increase in sperm count reported in our study with anastrozole administration was consistent with results obtained by **Tadros and Sabanegh, (2017)** who reported the positive effect of anastrozole on sperm concentration. The authors showed that anastrazole administration in male caused elevation in serum FSH, LH and testosterone levels andsupposed that this may be the cause of improvement in sperm parameters by aromatase inhibitors administration. Moreover, the conversion of spermatogonia to spermatocytes and the conversion of spermatocytes to round spermatids depend on the synergistic action of both FSH and testosterone. But, the effect of FSH is greatest on the conversion of spermatocytes to spermatids **(Sun et al., 1990).**

The increase in sperm count reported in our study with anastrozole administration was consistence with results obtained by **Zhao et al., (2014)** who reported the activation of spermatogenesis in man who was non-obstructive azoospermia with elevated FSH level resulting from the use of aromatase inhibitors.

In the present study, histological examination revealed moderate changes in the form of loosely packed connective stroma around seminiferous tubules, reduction in number of spermatogenic cells with sloughing of many spermatocytes within the lumen of some tubules in varicocele none treated rats. This results was in line with **Liu et al., (2007**) who found that experimental varicocele could reduce the level of bilateral intratesticular testosterone but not that of serum testosterone by damaging leyding cells in rats. In addition, the deleterious effects of varicocele on both tests of rats were observed by **Ozturk et al., (2013)** and there was no statistically significant difference as for histologic recovery following varicocelectomy.

The administration of anastrozole may exert a positive influence on spermiogenic epithelium. These results were in line with **Turner et al., (2000)** who found that the majority of anastrozole-treated animals had testes with normal spermatogenesis but, occasionally, seminiferous tubules showed abnormal loss of germ cells or contained only Sertoli cells. Ten percent of anastrozole-treated animals had testes that appeared to contain only Sertoli cells, and one rat had 'giant' testes in which the tubule lumens were severely dilated. Morphometric analysis of the normal testes at 19 weeks

showed no difference in the number of Sertoli cells or germ cells, or the percentage volumes of the seminiferous epithelium, tubule lumens and interstitium between control and anastrozole-treated rats. The drug induced inhibition of aromatase in leyding and sertoli cells might increase their responsiveness to gonadotropin stimulation. In this way, the local production of testosterone by leyding cells and androgen binding protein by sertoli cells, both known to be involved in the maintenance of normal spermatogenesis, might be increased.

From previous data one may assume that anastrozole has positive effects on spermatogenesis and semen production and quality, and it increases fertility in varicocele. These effects of anastrozole are related to its aromatase inhibition which leads to increase intratesticular testosterone. This work also confirmed the positive effects of anastrozole on histopathologic changes following varicocele in rats.

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