Research Article

Ameliorating Effect of Linseed Oil Against Fluconazole Induced Adverse Effect on Male Fertility of Cocks

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Abstract | This study was designed to investigate the modulating effects of linseed (LIN) oil on some fertility alterations induced by fluconazole (FCZ) in cocks. Thirty White Leghorn cocks were used and divided into three groups (ten in each). The 1st group was served as control, the 2nd group received FCZ (5 mg/kg body weight (BW)/day for 7 days) in drinking water, while the 3rd group received FCZ in drinking water for a week, then given LIN oil (60 ml/kg basal diet) for two weeks. FCZ administrated cocks showed elevation in abnormal sperm % and significant decrease in serum testosterone hormonal levels and spermatozoa (viability and motility) which accompanied by some histopathological damage in testes and epididymis. On a molecular basis, mRNA expression of CYP17A1 and LHR genes were significantly reduced in testicular tissues of FCZ group while aromatase gene was elevated. The administration of LIN oil after FCZ treatment markedly improved the aforementioned alterations caused by FCZ. So linseed oil is capable to ameliorate the fluconazole induced fertility disorders.

Keywords | Male fertility, Antifungal, Medicinal plant, Testosterone hormone, Aromatase

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INTRODUCTION

Fertility is considered an essential part of poultry industry and a lot of money are paid annually to improve the fertility parameters (Hamzehnezhad et al., 2019). Cocks play a vital role in flock fertility because in poultry breeding the ratio of male to female is low (Ommati et al., 2013). Therefore, promoting fertility indices in cocks is crucial to achieve the highest productivity (Hamzehnezhad et al., 2019).

Fluconazole (FCZ) is a triazole antifungal agent which can be administered both orally and intravenously. It has inhibiting action on the synthesis of ergosterol and the essential sterol in fungal cell membranes via suppressing fungal cytochrome P450. It is active against *Aspergillus species, Candida species, Blastomyces dermatitidis, Histoplasma capsulatum, Coccidioides immitis and Cryptococcus neoformans* in diseased animals (El-Medany and Hagar, 2002). FCZ has shown functional alterations in male fertility of treated animals (El-Medany and Hagar, 2002). Moreover, FCZ has been proved to decrease cock's fertility as it reduced serum testosterone level and caused direct damage at the level of seminiferous tubules (Hammad et al., 2018).

Herbs and herbal extracts are one of the pillars of alternative and complementary medicines (Dhama et al., 2018), which have exhibited mitigative actions against deleterious effects caused by several xenobiotic (Waman et al., 2018). Medicinal plants have been used in many nations to treat male infertility problems (Khojasteh et al., 2016).

Linseed (LIN) or flaxseed oil (*Linum usitatissimum*) is a member of the Linaceae family, which contains docosahexaenoic acid and omega-3 fatty acid. Omega -3 fatty acids has a role in maintaining cell membrane

integrity which in turns increase production of healthy sperms. Moreover, it has been proved that docosahexaenoic acid increased serum testosterone level, sperm quality and sperm motility in man, boar, sheep and goat (Abu-Heakal et al., 2016).

To the best of our knowledge, the effect of LIN oil on the changes induced by FCZ on cocks' fertility hasn't yet been investigated. Therefore, the aim of this work was to explore the possible ameliorative action of LIN oil on the adverse effects induced by FCZ on cocks' fertility.

MATERIALS AND METHODS

Experimental animals and diet

A total of thirty healthy adult White Leghorn cocks, 6 months old, weighing 1.8-2.1kg were obtained from local poultry farm (Abo-Keber city, El-Sharkia, Egypt). The health status of cocks was evaluated through physical examination. Cocks were kept in clean floor pens (10 cocks/pen) under hygienic conditions at 25±1 and 60-70% relative humidity. They were allowed to acclimate 2 weeks before the start of the experiment. Each pen was provided with wood shaving as litter material at 10 cm depth. The basal growing diet was formulated according the permissible measures of NRC (1994) for layers. The cocks were supplied with ad libitum access to water. The planned protocol was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt (Approval No. 6).

Oil extracts and drug

Linseed oil (LIN) (0.93 g/ml, Product No: 430021) was procured from Sigma Aldrich Co. (St. Louis, MO. USA). Fluconazole (flucoral®, 150 mg) was bought from Sedico Co. (6th of October City, Egypt) (Reddy, 2012).

Animal and experimental design

Thirty cocks were randomly divided in to 3 groups (10 cocks/ group). The 1st group was served as control fed basal di*et al*l over the period of the experiment. The 2nd group fed basal diet and received FCZ (5 mg/kg body weight (BW)/ day for 7 days) (Steneroden, 2014) in drinking water. The 3rd group fed on basal diet and received FCZ in drinking water for a week, then supplemented with LIN oil (60 ml/ kg basal diet) (Castrovilli et al., 2003) and clear water for the next two weeks.

Samples collection

At the end of the 3rd week post treatment, 5 cocks in each group were selected haphazardly. Blood samples were withdrawn from the wing vein of each chosen cock in plane tubes and allowed to clot, then centrifuged at 1500 x g for 15 min to separate the serum. The obtained sera were preserved at –20 °C for further analysis of serum total testosterone (Stoffregen et al., 1997). Then, all selected cocks were euthanized, and the reproductive organs (testis and epididymis) were taken for sperm quality analysis. Parts of the testis and epididymis were fixed in 10% formalin for histopathological investigation (Bancroft et al., 1996). Another part of the testis was kept at -80 °C for conducting quantitative real time polymerase chain reaction (real-time PCR) for gene expression investigation (Pfaffl, 2001).

Sperm quality analysis

Sperm quality analysis (sperm motility, morphology, and viability) was determined as reported by Zemjanis et al. (1970); Barth and Oko (1989) and Evans (1987), respectively.

Assessment of total serum testosterone

The level of total testosterone in serum was measured by Enzyme Linked Immunosorbent Assay (ELISA) method according to Tietz et al. (1995).

Transcription of target genes (Lutenizing hormone receptor, Aromatase, and Cytochrome P450 17A1 using real-time PCR

RNA extraction and reverse transcription

Total RNA was extracted from 50 mg of cock testis using Trizol reagent according to the manufacturer's instructions (Direct-zolTM RNA MiniPrep, catalog No. R2050). The cDNA of each sample was synthesized following the manufacture protocol (Sensi Fast TM cDNA synthesis kit, Bioline, catalog No. Bio- 65053).

Quantitative real time PCR analysis

Relative quantification of mRNA levels of luteinizing hormone receptor (LHR), Aromatose and Cytochrome P450 17A1 (CYP17a1) in cock testis was performed using SYBR Green PCR Master Mix (2x SensiFastTM SYBR, Bioline, catlog No. Bio-98002). Primer sequences of target genes are shown in Table 1. The housekeeping gene GAPDH was used as an internal control. The relative expression of the gene was calculated as reported by Pfaffl (2001) using the comparative $2^{-\Delta\Delta Ct}$ method (Ct: cycle threshold).

Histopathological studies

The testis and epididymis were fixed in 10% formalin. After that, the samples were treated until being blocked in hard paraffin which then were cut into sections of 5µm thickness and was applied for staining by Hematoxylin and Eosin (H and E), according the method published by Bancroft et al. (1996).

Table 1: Showed the primers used for real-time PCR amplifications.

Gene	Oligonucleotide sequence	Annealing temperature (C^0)
LHR	F5: GTATGGCTGTGACCACCACGG-3, R5: CTGCACGGTGCAGTCGGAG-3,	64
Aromatase	F5: CGACAAAAGCTTTCCACTGT-3, R5: AGCAGCAATCATCATCTCCA--3,	62
CYP17a1	F5: GCCAGCGTCACCGAGATGAT-3, R5: GCAGCCTGAGAGTCTCCTTGATG-3,	60
GAPDH	F5: CACTATAAAGGC GAGATG-3, R5: GGCTGTGTGCTT GGCTCA-3,	64

CYP17a1: Cytochrome P450 17A1, LHR: Luteinizing hormone receptor. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

Statistical analysis

All data have been analyzed statistically by computerized SPSS program (Version 19) using one-way ANOVA to compare different groups. The significant difference between groups at different times were determined by using Tukey's test post HOC multiple comparison. The results considered significant when $P \leq 0.05$ according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect of linseed oil and fluconazole on total serum testosterone hormone levels (ng/ml)

Fluconazole medicated cocks (G2), exhibited a significant decrease ($p<0.05$) in serum testosterone levels relative to the control negative group (G1). While testosterone hormonal levels of LIN oil supplemented group after FCZ (G3) were significantly elevated ($p<0.05$) relative to the FCZ treated group (G2). Whereas, there was no significant difference in testosterone levels among LIN oil and control groups.

Effect of linseed oil and fluconazole on semen analysis (mass motility %, abnormal sperm % and live/dead sperms %) of clinically healthy adult cocks

Notably, FCZ medication induced alterations in semen picture of treated cocks which represented in a significant decrease ($p<0.05$) in mass motility and live/dead % with a marked rise ($p<0.05$) in sperm abnormalities relative to the control group. LIN oil supplementation after FCZ induced a non-significant raise in mass motility% and non- significant reduction in sperm abnormality %, while live/dead ratio was not significantly changed relative to cocks treated with FCZ only.

Histopathological results

Testicular lesions

The obtained results regarding the effect of the tested oils on histopathological finding of testis in all treated groups were illustrated in Figure 2, compared with the control. The pathological finding showed that, testicular sections from G2 that was given therapeutic dose of fluconazole at 3rd week post treatment appearing wider lumen of seminiferous tubules when compared with control one (G1) that contained a large amount of exfoliated germ cells and vacuolated spermatocytes (Figure 2). Moreover, in group (G3) supplemented with LIN oil after FCZ, the lumen of seminiferous tubules still wide and mildly vacuolated spermatocytes were observed.

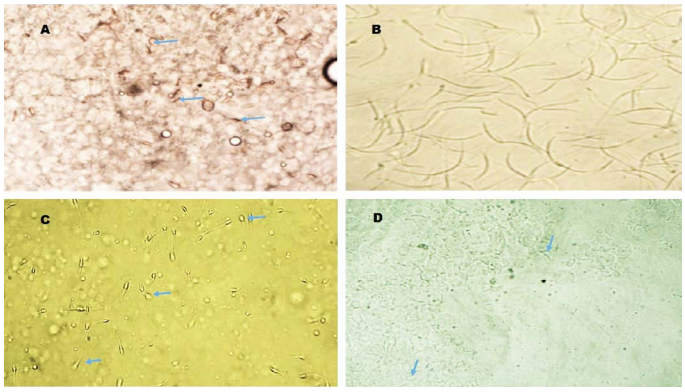

Figure 1: showing sperm morphology of clinically healthy adult cocks. A and B: normal sperm (arrow) in control group G1, C: large head sperm (arrow) in G2, D: bend middle piece sperm (arrow) in G3 (X: 400-1000). G1: Control (non-treated); G2: Fluconazole (5mg/kg b.wt); G3: Fluconazole (5mg/kg b.wt) then Linseed oil (60ml/kg).

Epididymal lesions

Histopathological sections of epididymis of fluconazole treated group (G2) at 3rd week post dosing has showed marked decreased in diameter of epididymal duct and decreased amount of spermatozoids in lumen with presence of large amount of exfoliated germ cells and round bodies in the lumen (Figure 3). Moreover, hyperplastic alteration in the lining epithelium forming infolding or cribriform change and pseudo-glandular structures besides hyperplasia of clear cells (Figure 3) were observed in epididymial. While in group 3 that supplemented with LIN oil after FCZ showed a slight increase in amount of spermatozoids in epididymal lumen with slightly decreased hyperplastic alteration in the lining epithelium, besides presence of few

exfoliated germ cells and round bodies in the epididymal lumen (Figure 3).

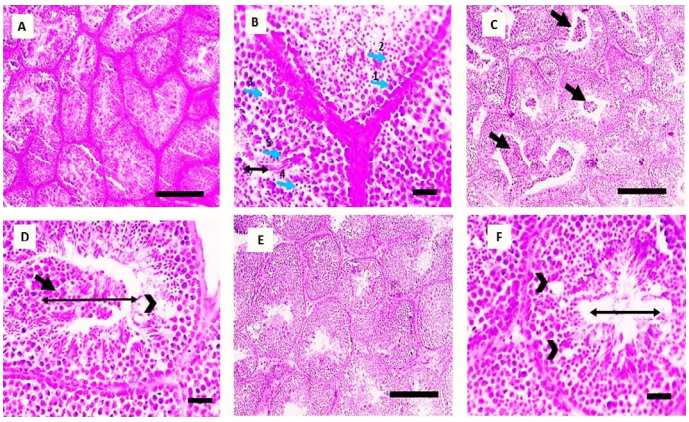

Figure 2: (A and B) Testis, Gp1 control showing compact seminiferous tubules with narrow lumen (double headed arrow) and lined with several layers consisting of spermatogonea cells (1), spermatocytes (2), Sertoli cell (3), spermatid (4) and spermatozoids (5). (C and D), Gp2 showing wider lumen (double headed arrow) containing large amount of exfoliated germ cells (black arrow) and vacuolated spermatocytes (arrowhead). (E and F), Gp3 showing wide lumen (double headed arrow) and mildly vacuolated spermatocytes (arrowhead). H and E, Low magnification X: A, C and E:100 bar 100 and high magnification X: B, D and F:400 bar 50.

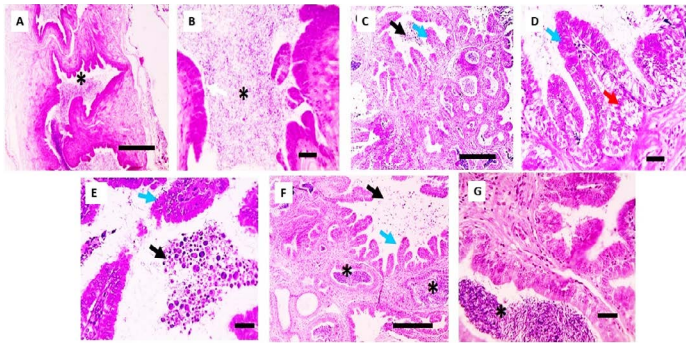

Figure 3: (A and B) Epididymis, Gp1 control showing epididymal duct with great quantity of spermatozoids (asterisk) in lumen and normal epithelial lining (white arrow), (C, D and E), Gp2 showing marked decrease in diameter of epididymal duct and amount of spermatozoids in lumen with presence of large amount of exfoliated germ cells and round bodies in the lumen (black arrows), hyperplastic alteration in the lining epithelium forming infolding or cribriform change and pseudoglandular structures (blue arrows) besides hyperplasia of clear cells (red arrow). (F and G), Gp3 showing slight increase in amount of spermatozoids in lumen (asterisk) with slightly decreased hyperplastic alteration in the lining epithelium (blue arrows) besides presence of few exfoliated germ cells and round bodies in the lumen (black arrows). H and E, Low magnification X: A, C and F: 100 bar 100 and high magnification X: B, D, E and G:400 bar 50.

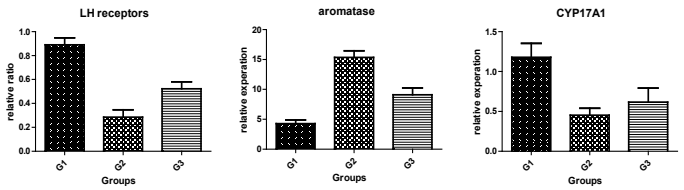

Figure 4: Effect of linseed oil (60ml/kg) and fluconazole (5mg/kg b.wt) in 3rd week post fluconazole administration on fertility related genes of clinically healthy adult cocks. G1: Control negative (non-treated); G2: Control positive (treated with Fluconazole 5mg/kg b.wt); G3: Fluconazole (5mg/kg b.wt) then Linseed oil (60ml/kg).

Expression of fertility related genes

Effect of linseed oil and fluconazole on LH receptor (LHR), aromatase (CYP19A1) and CYP17A1 genes

The mRNA expression of certain testicular steroidogenic genes was altered in testicular tissues of FCZ medicated cocks in comparison with control group, LHR and CYP17A1 genes were significantly decreased ($p<0.05$) in FCZ treated group. While, aromatase gene was significantly elevated. However, the ameliorative effect of LIN oil was notable in G3 in which LHR and CYP17A1 genes were up-regulated while aromatase gene was down-regulated when compared with the respective expression of those genes in cocks treated with FCZ only.

Testosterone produced entirely by Leydig cells in the testicular tissue. It is the principal androgenic hormone that plays a crucial role in spermatogenesis, maturation of spermatozoa, and secondary sexual functions. Therefore, the impairment of testosterone biosynthesis can result in significant reproductive dysfunction (Mohamed et al., 2017).

The results reflected a significant decrease in serum testosterone levels in cocks given fluconazole when compared with the control group. The histopathological changes and the significant decreased in CYP17A1 gene expression that induced by the drug supporting the obtained result.

The obtained data are supported by the result recorded by many authors. Clissold (1997) and de Coster et al. (1989) stated that in mammalians tissues, fluconazole has inhibited lanosterol conversion to cholesterol, this lead to inhibition of testosterone synthesis from both Leydig's cells and adrenal gland. Similarly, El-Medany and Hagar (2002) stated that oral administration of fluconazole to sexually mature male rabbits induced a significant decrease in serum testosterone level. In addition, administration of ketoconazole has also been reported to induce a significant reduction in the level of serum testosterone in male rats (Shin et al., 2006; Amin, 2008). Furthermore, ketoconazole

administered at relatively high dosages, has inhibited adrenocortical steroidogenesis by blocking steroidogenic enzymes (Ohlsson et al., 2010). Moreover, there was a significant decreased in testosterone level after fluconazole treatment as mentioned by van der Pas et al. (2012). In the same direction, a testicular damage and reduced testosterone level in serum of ketoconazole-treated males has also been reported by Abdelraouf et al. (2014). Similar finding has reported that administration of fluconazole at both therapeutic and double therapeutic doses induced a significant decrease in serum testosterone level (Hammad et al., 2018). Moreover, Mohamed et al. (2020) found that ketoconazole administration induced a prominent decrease in serum testosterone level in treated rabbits.

Table 2: Effect of linseed oil (60ml/kg) and fluconazole (5mg/kg b.wt) on total serum testosterone levels (ng/ml) of clinically healthy adult cocks. (M± S.E) (n=5).

In 3rd week post dosing	G1	G2	G3	sig
Total serum testosterone (ng/ml)	4.10±.025[a]	2.73±0.13[b]	4.14±0.13[a]	0.001

The different small letters in the same column means that there were significant changes at P <0.05.

Table 3: Effect of the administrated linseed oil (60ml/kg) and fluconazole (5mg/kg b.wt) in 3rd week post fluconazole administration on semen picture of clinically healthy adult cocks.

Parameter	G1	G2	G3
Mass motility %	85[a]	55[b]	60[b]
Abnormal sperm %	3[a]	20[b]	15[b]
Live/dead sperms ratio	80[a]	60[b]	60[b]

The different small letters in the same raw means that there were significant changes at P <0.05.

Table 4: Effect of linseed oil (60ml/kg) and fluconazole (5mg/kg b.wt) in 3rd week post fluconazole administration on fertility related genes of clinically healthy adult cocks. (M± S.E) (n=5).

Genes groups	In 3rd week post dosing		
	LH receptor	Aromatase	CYP17A1
G1 Control (Non treated)	0.89±0.058[a]	4.28±0.61[a]	1.178±0.17[a]
G2 (Treated with Fluconazole 5mg/ kg b.wt)	0.29±0.061[b]	15.35±1.10[b]	0.45±0.09[b]
G3 Fluconazole (5mg/kg b.wt) then Linseed oil (60ml/kg)	0.52±0.058[b]	9.09±1.16[cd]	0.62±0.17[ab]
Sig	0.002	0.001	0.02

The different small letters in the same column means that there were significant changes at P <0.05.

In the current study, serum testosterone levels were significantly improved in linseed oil supplemented group after fluconazole medication when compared with that treated with FCZ only. Hence, the values of testosterone hormone become nearly similar the normal range of the control group. This may be attributed to omega 3 that increased the production of luteinizing hormone that increased the production of testosterone inside Leydig cells or increases the number of Leydig cells or repair of their structure.

These findings are in agreement with Goyal et al. (2001) who stated that, plasma testosterone concentration and LH concentration were increased by addition of omega 3 to diet of male rabbits. Similarly, Veldhuis et al. (2009) and Yan et al. (2013) reported that a balanced n-3/n-6 PUFA ratio will improve male reproduction in rats through increasing sperm characteristics and enhanced the structure integrity of testis and sperm, which may be related to the elevation of testosterone hormone level. Moreover, Bostani et al. (2014) found that, testosterone levels significantly increased in the experimental male rats receiving omega 3 in the form of walnut oil.

Supplementation of breeder feed with n-3 and n-6 PUFA have significantly increased gonadotropin-releasing hormone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone hormone levels as mentioned by Feng et al. (2015). Moreover, male rabbits treated with linseed oil (1 and 2%) showed an improvement in levels of testosterone level (Abu-Heakal et al., 2016). Also Elsawy et al. (2018) reported that linseed supplemented buck rabbits showed a significant elevation in serum testosterone. Recently, Al-Kadhi et al. (2020) stated that linseed oil in combination with bicalutamide (anti-androgenic drug) induced a non-significant decrease in the level of testosterone, FSH and LH in treated male mice when compared to the control group.

It has been known that lipid composition of the spermatozoa plasma membrane is responsible for mobility characteristics, viability and membrane integrity (Robinson et al., 2006). The recorded results revealed that, there were a significant decrease in mass motility % and live/dead ratio while abnormal sperm% was significantly increased in fluconazole medicated group when compared with the control group. This finding could be explained in the light that fluconazole might have reduced the sensitivity of steroids receptors to androgens (Baulieu, 1984) which supported by the recorded significant decrease in serum testosterone level and the histopathological alterations in testes and epididymis from the obtained data.

The detected data are in accordance to El-Medany and Hagar (2002) who mentioned that fluconazole revealed

a significant decrease in testosterone level, semen volume, sperm count and percentage of viable sperms in male rabbits. Also, fluconazole has been shown to decrease sperm cells motility and progressivity in boar (Ciornei et al., 2009). Furthermore, Drobnis and Nangia (2017) stated that oral administration of ketoconazole could induce inhibition in cauda epididymal sperm motility post-dosing and arrest in the epididymal spermatozoa motility. Similarly, Amer et al. (2018) revealed that, there were a significant decrease in the sperm count in fluconazole medicated cocks. Moreover, Mohamed et al. (2020) declared that ketoconazole administration induced a marked decrease in semen volume, sperms concentration, live sperms % and sperm motility % and increase the morphological abnormalities in sperms of treated rabbits.

The obtained data in LIN oil supplemented group post FCZ medication reflected a non-significant increase in mass motility %, and non-significant decrease in abnormal sperm % while live/dead ratio was not significantly changed in comparison with the semen picture of group treated with FCZ only. This result is supported by Mourvaki et al. (2010) who recorded that flaxseed supplementation increased PUFAs content in sperm tail of supplemented rabbits. The PUFAs in LIN oil involved in flagellar movement of sperm which in turn increased the motility percentage. The addition of a moderate ratio of ω-3: ω-6 fatty acids showed an improvement in sperm motility, progressive motility, membrane functionality, and viability; also the testosterone level increased; and a higher fertility rate was declared by Al-Daraji (2001). Also, Rooke et al. (2001) stated that the use of n-3 and n-6 PUFAs in the pig diet resulted in improving spermatozoa characteristics. Similarly, Vizcarra et al. (2010) said that spermatogenesis and steroidogenesis in the avian testis are increased with the addition of omega3 to their diets. Moreover, Naji (2013) showed that, intake of omega3 was potentially useful in increasing the fertility of male rabbits by increasing sperm concentration, motility, grade activity, viability, and reduced abnormality. Similar results were recorded in ram sperm (Jafaroghli et al., 2014) and bovine sperm (Moallem et al., 2015). In addition, Abu-Heakal et al. (2016) found that, linseed oil (1% and 2%) increased sperm count, sperm livability, sperm mass motility and individual motility in treated male rabbits. Furthermore, Elsawy et al. (2018) recorded an improvement in semen picture of linseed supplemented buck rabbits (elevation in sperm motility %, sperm concentration and fertility rate and reduction in dead sperm % and abnormal sperm %).

Testicular tissues of fluconazole treated group showed widening in the lumen of seminiferous tubules which contained a large amount of exfoliated germ cells and vacuolated spermatocytes. These findings are supported by Johnson (2014) who declared that, the toxic metabolite of azole derivatives caused depolymerization of Sertoli cell microtubules which induced the detachment and sloughing of the seminiferous epithelium into the seminiferous tubule lumen. Also, Amer et al. (2018) reported that testes of fluconazole medicated cocks showed vacuolated spermatocytes with more widening diameter of seminiferous tubule and loss of spermatocytes inside the dilated lumen of seminiferous tubules. Similarly, Mohamed et al. (2020) stated that, the testes of ketoconazole treated animals showed marked interstitial fibrosis and edema with severe tubular degeneration and desquamation of germ cells in lumen and arrested spermatogenesis. Testes of linseed oil supplemented group for 2 weeks after fluconazole administration showed widening in luminal diameter of seminiferous tubules, besides, mildly vacuolated spermatocytes in testes of treated cocks. The obtained data are in agreement with many authors. Yan et al. (2013) stated that the dietary supplementation with n-3/n-6 PUFAs improved the development of testis and the morphological structure of spermatozoa in rats. Also, Feng et al. (2015) detected that n-3 and n-6 PUFA have increased the spermatogonial development and germ cell layers in testes of supplemented rooster. Moreover, Abu-Heakal et al. (2016) and Vaškas et al. (2017) reported a histopathological improvement in the examined testis of 2% linseed oil treated male rabbits that showed a prominent increased in lumen diameter of seminiferous tubules and thickness of interstitial tissue. Epididymal sections from fluconazole treated group showed marked decrease in diameter of epididymal duct and decreased amount of spermatozoids in lumen with presence of large amount of exfoliated germ cells and round bodies in the lumen. Moreover, hyperplastic alteration in the lining epithelium forming infolding or cribriform change and pseudo-glandular structures besides hyperplasia of clear cells were observed in the epididymal sections of fluconazole treated group. These findings are in agreement with those obtained by Hammad et al. (2018) who revealed that the epididymis of fluconazole medicated cocks showed, desquamation of epithelial lining inside lumen with leukocytic cells infiltration and dilated capillaries. Also epididymis showed focal epididymitis characterized by focal aggregation of inflammatory cells with low density of sperms in its lumen. Moreover, Mohamed et al. (2020) found that the epididymis of ketoconazole-treated rabbits showed severe changes characterized by hyalinization of sperms in lumen of ducts, absence of sperms, and vacuolization of epithelial lining ducts.

As showed in the obtained data, linseed oil supplemented group after fluconazole medication showed a slight increase in amount of spermatozoids in epididymal lumen with slightly decreased hyperplastic alteration in the lining epithelium, besides presence of few exfoliated germ cells and round bodies in the epididymal lumen. Similar

findings were obtained by Vaškas et al. (2017) who said that epididymis has normal epithelium lining in linseed supplemented rams. The LH receptor (LHR) is critical for mammalian male sexual development and reproductive function. LHR signaling is responsible for androgen production by Leydig cells and recent studies have demonstrated that LHR activation necessary for androgen synthesis as well as proliferation/survival of Leydig cells (Yamashita et al., 2011).

From the obtained data, it was recorded that fluconazole treated group showed a prominent significant decrease in LHR gene expression in the testicular tissue of treated cocks. This findings could be collectively explained in the light that fluconazole might have reduced the sensitivity of steroids receptors to androgens as declared by Baulieu (1984). Therefore, there was a concomitant decrease in testosterone as stated by El-Medany and Hagar (2002).

On the other hand, the obtained data recorded a non-significant elevation on the expression of LHR gene in linseed supplemented group after fluconazole medication when compared to FCZ only treated group. Therefore, the value still below the value of the control group. As fluconazole might have reduced the sensitivity of steroids receptors to androgens as declared by Baulieu (1984). Linseed oil has solved this induced problem as mentioned by Feng et al. (2015) who stated that PUFAs in linseed oil could regulate the expression of hormone receptors and steroid acute regulator protein (StAR). Moreover, Abu-Heakal et al. (2016) who found that all treated male rabbits with linseed oil (1 and 2%) have showed an improvement in levels of testosterone level, FSH and LH regarding to control group. Furthermore, Elsawy et al. (2018) who reported that linseed supplemented buck rabbits showed a significant elevation in serum testosterone, FSH and LH.

Aromatase is a cytochrome P450 enzyme (CYP19A1 isoform) able to catalyze the conversion of androgens to estrogens (Linardi et al., 2017).

In the light of the study, Cocks of fluconazole medicated group showed a prominent significant elevation in aromatase gene expression. Our result is in agreement with Munkboel et al. (2019) who stated that fluconazole administration inhibited CYP17A1-lyase activity.

As a result of linseed supplementation, the results showed that there was a significant decrease in aromatase gene expression when compared with FCZ only treated group. But the value still above the normal value of the control group.

Linseed oil could be considered as aromatase inhibitors. So administration of aromatase inhibitors, is associated with an increase in levels of LH, follicle-stimulating hormone (FSH) and testosterone. Therefore, aromatase inhibitors have been suggested as a tool to increase testosterone levels in men with low testosterone levels T'Sjoen et al. (2005). Polyunsaturated fatty acids could regulate the expression of hormone receptors and steroid acute regulator protein (StAR). PUFAs significantly increased the mRNA levels of all hormone-related genes (GnRHR, FSHR, LHR, and StAR mRNA levels) as mentioned by Feng et al. (2015).

CYP17A1 is a member of the cytochrome P450 superfamily of enzymes (Storbeck et al., 2011). CYP17 is the pivotal enzyme in the biosynthesis of steroidal hormones and responsible for the conversion of pregnenolone and progesterone to DHEA and androstenedione, respectively, which are the direct precursors of testosterone (DeVore and Scott, 2012).

The obtained data revealed a marked significant decrease in the expression of CYP17A1 gene in testicular tissue of fluconazole treated cocks.

The obtained data are in accordance to Clissold (1997) and de Coster et al. (1989) who stated that in mammalians tissues, fluconazole has inhibited lanosterol conversion to cholesterol, this lead to inhibition of testosterone synthesis from both Leydig's cells and adrenal gland. Also, El-Medany and Hagar (2002) reported that fluconazole has androgen suppressive properities through inhibiting CP450 mixed function oxidase enzymes, so it can inhibit steroidogenesis by inhibiting C17-20 layase enzyme, which involved in steroid hydroxylation "such as progestins turned into androgens".

Therefore, fluconazole considered as an inhibitor for LHR and CYP17A1 genes and this explanation is supported by Zhu and Kyprianou (2008). Moreover, Goetz et al. (2009) stated that triazole antifungals reduced levels of testosterone by 40–68% in the adult and neonatal testis culture, and altered steroid production in a manner that indicated CYP17A1 inhibition at the highest concentration tested. Also, clotrimazole induced an inhibition to cytochrome P450 (CYP) enzymatic activities, included several steroidogenic CYP (Baudiffier et al., 2013). In addition, Munkboel et al. (2019) stated that fluconazole administration inhibited CYP17A1-lyase.

Linseed oil supplementation after treatment with fluconazole, reflected a significant elevation in the expressed amount of CYP17A1 gene in the tecticular tissue of treated cocks when compared with FCZ only treated group. This result explained by DeVore and Scott (2012) who stated that CYP17 is a key enzyme in the steroidogenic synthesis pathway. Moreover, Cardozo et al. (2012) observed an increase in estradiol after a prolonged

intake of 25% flaxseed. Furthermore, Corrêa et al. (2017) found that the prolonged exposure to flaxseeds induced a significant increase in serum estradiol levels in rat.

CONCLUSIONS AND RECOMMENDATIONS

It could be concluded that, administration of linseed oil after fluconazole medication, capable to ameliorate the fluconazole induced fertility disorders by prompting the antioxidant defense mechanism and reversing the disturbance of steroidogenic genes expression.

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AUTHOR'S CONTRIBUTION

NKH performed the experiment and wrote the original draft. YYE semen analysis. AEH and STE edited final draft and supervision. MSA and YYE wrote, reviewed, data curation and prepared the manuscript for publication. All authors read and approved the final manuscript.

List of abbreviations

CYP17a1: Cytochrome P450 17A1, LHR: Luteinizing hormone receptor. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase. FSH: Follicle-stimulating hormone. DHEA: Dehydroepiandrosterone. StAR: Steroid acute regulator protein. PUFAs: Polyunsaturated fatty acids.

Conflict of interest

The authors have declared no conflict of interest.

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