Original Article

Improvement effect of linseed oil on the activity of testes and physiological parameters in mice treated with Bicalutamide

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ABSTRACT

The study aimed to explore the protective and improvement effect of linseed oil (LSO) on some physiological and histological parameters of male mice treated with Bicalutamide. The study carried out on 25 male mice, all treated orally once daily by gavages needle for 40 days. Animals were allocated randomly to 5 groups each included five at controlled conditions. First group served as control, 2nd group included castrated mice, 3rd group received LSO, 4th group received bicalutamide and 5th group received LSO+ bicalutamide. At the end of experiment, the blood collected and centrifuged to obtain serum and kept at deep freeze (-20 c) until biochemical tests carried out for detection of AST, ALT, ALP, urea, uric acid, creatinin and TSB by semi- auto chemistry analyzer. While testosterone, FSH, and LH were detected with automic fluorescence immune- assay. Histological study carried out after removal of testes to study the diameter and number of seminiferous tubules with number of leydig and sertoli cells. Results of the study recorded a significant elevation ($P<0.05$) in the concentration of AST, ALT, ALP, urea, uric acid, creatinin but significant reduction in the level of FSH, LH and testosterone in castrated and bicalutamide groups. While in the LSO and LSO+ bicalutamide groups not showed any remarkable differences ($P>0.05$) in their concentrations as compared control. However histological study concerned with the diameter of seminiferous tubules with number of leydig and sertoli cells showed significant reduction ($P<0.05$) in the bicalutamide group, while LSO and LSO + bicalutamide groups not recorded remarkable reduction ($P>0.05$). In conclusion supplementation of animals with LSO or co- administrated with bicalutamide can ameliorate and improves some physiological and testicular activities.

Keywords: Linseed oil, Bicalutamide, antiandrogen, hormones, male mice, hepatic enzymes.

Introduction

Linseed (Linumusitatissimum) belongs to the Linaceae family also known as flaxseed, found as seeds or oil forms, for its high nutritional quality recognized asa functional food [1]. Ancient Rome & Greece were used for the cure of different health complaints [2]. The plant is considered one of the richest plant sources forMany trace elements and vitamins as vitamin B, E, and carotene [3]. Also, high levels of omega-3 PUSFAs, specially α-linoleic acid and high levels of vital oils [4], are positively associated with sperm morphology when highly consumed [5].

Essential oil of linseeds biologically precursor to Omega-3 fatty acids such as eicosapentaenoic acid (EPA) [6] are widely used in Asia and Europe for its potential anti-inflammatory effects attributed to the n-3PUSFAs contents that mediate the production of eicosanoids [7] which have antibacterial effects recorded by [8].

However, the plant have been found to have important beneficial effects for reduction concentration of bone biomarkers, plasma cholesterol and LDL-C [9], with high antioxidant capacity for inhibition of lipid peroxidation [10], and significantly decreases atherogenic risks [11]. While in vitro promotes apoptosis and showed inhibitory effects on growth [12]. Plant lignans such as secoisolariciresinoldiglucoside (SDG) found in high concentration in Flaxseed which has been blocked cell proliferation and inhibition tumor growth, possibly by

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modulating estrogen receptor or growth factor-dependent signaling in the experimental models [13] or the culture [14].

Male gonads secrets an important androgen (testosterone)which have sexual activities and beneficial effects for development and functions of the male reproductive system [15]. They are also responsible for prostate tumor growth [16]. 80-90% of prostate cancer is androgen-dependent [17].

Several anti-androgens including Bicalutamide can prevent and inhibits the influences of androgens by antagonist androgen receptors [18], while Finasteride inhibits 5-alpha reductase enzyme [19].

Bicalutamide chemically found as (2 RS)-4-cyano–3–(4-fluorophenylsulphenyl)–2–hydroxy–2–hydroxy–methyl-3(trifluoromethyl)–propionanilide [20], an orally active non steroidal anti-androgen drug are commonly used in the treatment of prostate cancer but causes impairment of spermatogenesis and reduction of testicular weight [21] and behaves as pure anti-androgen that competitively blocks androgen receptors and inhibits both gene expression and cell growth [22]. It also exhibits androgen antagonistic activity [23], thereby inhibiting the negative feedback mechanism that regulates testosterone concentration [24].

The drug has prospective to interfere abnormally with the development as well as with thefunctions of animal and human reproductive system [25] that negatively affects the quality of life [16]. So different attempts were carried out to alleviate adverse effects or minimizing side effects of anti-androgenic drugs and improving the quality of life but without suitable results [26].

So this study was designed to investigate the ability of LSO supplementation for modulation and improvement of some physiological constituents and testicular activities of male mice treated with bicalutamide drug.

Materials and Methods

Materials:

Linseed oil prepared by (Emad company- Baghdad) purchased from a localpharmacy in Kirkuk city stored in a cool place and administrated once in a day 1ml/kg B.W. orally by gavages needle [27]. While Bicalutamide drug is represented as bicalutamide 50 mg under trade name Casodex (AstraZeneca UK.), daily dose 1 tablet as manufacturer prescription.

Experimental design:

Twenty-five male albino Swiss mice, approximately two months old & weighing 26±2 gm were housed (5 mice/cage) at controlled conditions were temperature 24±2 c and daily light / dark 14:10 hrs. Mice were fed rodent chow and tap water ad libitum. Animals of one group were castrated bilaterally under anesthesia while intact mice were used in all other groups.

Castration procedure:

A group of animals was castrated before 14 days of experimentation according to the technique described by [28], were animals injected general anesthesia with diazepam by I/P (10mg/ kg B.W.) followed 5 minutes later by ketamine (50 mg/kg B.W.). After initiation of anesthesia, the scrotal area was clipped then 70 % alcohol applied to disinfect the scrotum.

A small midline incision was made in the scrotal sac and each testis was delivered through the

scrotal incision.Once exteriorized testis was removed after spermatic vessels were tied with 4.0

silk sutures. The incision was sutured an interrupted pattern to close the incision with dusting

penicillin powder locally and I/m injection of penicillin G (2000 IU/kg B.W) for 3 days. Each

castrated mice kept in an isolated cage till recovery.

Grouping and treatment:- Animals were allocated randomly to the following groups:-

1- The intact group that wastreated with normal saline 0.1ml/mice served as a control group.
2- The castrated group that was treated with vehicle 0.1 ml/mice.
3- The intact group that was treated with linseed oil 1 ml/kg B.W/day. [27].
4- The intact group that wastreated with Bicalutamide 0.8 mg/kg B.W.
5- Intact group treated with linseed oil 1ml/kg B.W + Bicalutamide 0.8 mg/kg B.W

All animals were treated orally once daily for 40 days by gavages needle. At the end of theexperiment, blood was collected in jell tubes by cardiac puncture, left for 10 minutes to clot, and centrifuged by Centrifuge (Centrion UK) for 5000 rpm for 10 minutes. Serum was kept in Eppendorf tubes and stored at deep freeze (-20c) until the biochemical analysis was carried out.

Biochemical assays:- levels of hepatic enzymes in the serum, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Renal function tests included urea, uric acid, creatinin, and total serum bilirubin concentration. All biochemical parameters areestimated with commercially available kits AGAPPE (AGAPPE diagnostic Switzerland GmbH) and assayed by Semi-Auto Chemistry Analyzer (Mindray BA-88A) at 540 nm as prescribed by the manufacturer information.

Hormones in the serum of male mice: testosterone, FSH, and LH levels were assessed by commercial kits, and detected with Automic fluorescence Immuno-Assay (AFIAS 6) as depicted by manufacturer information.

For histological studies, the animals were killed by decapitation under basal conditions in a longitudinal fashion. Testes, from each animal, were weighted, immersed in 10% buffered formalin for routinely processed in a tissue processor, and embedded in paraffin. Sections 5 µm were cut by microtome and stained with hematoxylin and eosin stain [29]. Examination of prepared slides

performed by a light microscope with a camera (Optica, Italy). The diameter of seminiferous tubules was carried out by ocular micrometer after calibration with micrometer stage [30], and also,some seminiferous tubules, Leydig cells and Sertoli cells were determined [31].

Statistical Analysis:

Conventional statistical methods were used to calculate means and standard errors. Analysis of variance (ANOVA) was applied to test for any significant differences ($P<0.05$). All statistics were carried out used SigmaPlot (version 12).

Results:

The results of the study recorded alterations of some biochemical parameters concerned with hepatic enzymes and TSB were detected in the serum of treated animals as shown in table 1, werea group of mice treated with the anti-androgenic drug (Bicalutamide) 0.8mg/kg B.W. daily for 40 days recorded a significant elevation ($p < 0.05$) in the concentration of AST (229.4 ± 4.4), ALT (28.4±2.76), ALP (488.8 ± 12.02) and TSB (0.68 ± 0.03) as compared to the control and other treated groups, but castrated group recorded a significant increase ($p < 0.05$) in the level of ALP (386.4 ± 2.85) as compared to the control group. While LSO and LSO + Bicalutamide treated groups recorded non-significant differences ($p > 0.05$) in the activities of hepatic enzymes and TSB as compared to the control group, although LSO treated group recorded significant decrement ($p < 0.05$) in the level of AST (125.8 ± 3.29) as compared to the control group.

On other hand, the other biochemical parameters: urea, uric acid,and creatinine concentration revealed also in table 1,were recorded remarkable elevation ($p < 0.05$) in the group of animals treated with bicalutamide (55 ± 2.64), (362.2 ± 4.42) and (0.62 ± 0.03) respectively as compared control and other treated groups. Whereas the Castrated group recorded a significant increment ($p < 0.05$) in the activities ofurea (45.4 ± 2.52), uric acid (209 ± 2.64), and creatinin (0.52 ± 0.03) as compared to the control, LSO and LSO + bicalutamide treated groups. While LSO and LSO + bicalutamide treated groups recorded a non-significant decrease ($p > 0.05$) in the concentration of estimated biochemical parameters as compared to the control group.

Table 2 shows the results of the hormonal activities, FSH, LH, and testosterone in the serum of control and treated animals were recorded in the castrated and bicalutamide treated groups a significant decline ($p < 0.05$) in the concentration of FSH (0.19±0.01) (0.28 ± 0.02), LH (0.14 ± 0.02) (0.34 ± 0.02) and testosterone (0.66 ± 0.05) (0.72 ± 0.03) respectively as compared to the control other and treated groups.

While LSO treated group recorded a significant increment ($p < 0.05$) in the level of testosterone (2.50 ± 0.1) and non significant increment ($p < 0.05$) in the level of FSH (0.66 ± 0.05) and LH (0.82 ± 0.03) as compared to the control group. Whereas, LSO+ bicalutamide treated group didn't recorded significant decrease ($p > 0.05$) in the activities of FSH (0.50 ± 0.03), LH (0.65 ± 0.03) and testosterone (1.65 ± 0.02) as compared control and LSO treated groups.

Histological parameters of testes shown in the table, 3 and figure, 1 revealedthat the treatment of animals with bicalutamide recorded a significant reduction ($p < 0.05$) in the diameter of seminiferous tubules (258 ± 2.49), number of Leydig cells (27.9 ± 1.43) and Sertoli cells (20.6 ± 0.87) as compared to the control and other treated groups.While LSO treated group not recorded significant increment ($p > 0.05$) in the diameter of seminiferous tubules (289.4 ± 5.28) and number of seminiferous tubules (22.1 ± 1.16), Leydig cells (58.6 ± 1.8) and Sertoli cells (31.8 ± 1.06) as comparedto the control group, but the increment was in above parameters significantly ($p < 0.05$) when compared with the bicalutamide treated group.

On other hand, co-treatment of animals with LSO + bicalutamide recorded a remarkable increment ($p < 0.05$) in the number ofLeydig cells (39.8 ± 1.31) and Sertoli cells (25.8 ± 0.73) but non-significantly ($p > 0.05$) in the diameter and number of seminiferous tubules (278.4 ± 2.5), (21.2 ± 0.8) respectively as compared to the bicalutamide treated group. However,there were no significant differences ($p>0.05$) in the number of seminiferous tubules between all treated groups.

Table 1: Effect of Castration, Bicalutamide, LSO, and LSO+Bicalutamide treatment on the concentration of some biochemical parameters (M±SE) in the serum of male mice.

Groups / Parameters	Control	Castrated	LSO	Bicalutamide	LSO+ Bic.
AST	177.20 ± 2.28	180.6 ± 3.05	125.8 ± 3.29	229.4 ± 4.40	171.8 ± 4.69
U/L	a	a	b	c	a
ALT	15.20 ± 1.06	24.6 ± 1.72	16.8 ± 0.96	28.2 ± 2.76	22.4 ± 1.03
U/L	a	bc	a	c	ab
ALP	338.2 ± 2.69	386.4 ± 2.85	320.8 ± 3.0	488.8 ±12.02	345 ± 4.62
U/L	a	b	a	c	a
TSB	0.34 ± 0.02	0.42 ± 0.03	0.26 ± 0.02	0.68 ± 0.03	0.44 ± 0.05
mg/dl	a	a	ab	c	a
Urea	33.8 ± 1.59	45.4 ± 2.52	25.8 ± 1.49	55.0 ± 2.64	33.0 ± 1.84
mg/L	a	b	a	c	a
Uric acid	181.8 ± 2.65	209.0 ± 2.64	142.0 ± 2.62	362.2 ± 4.42	177.4 ± 2.82
mmol/L	a	b	c	d	a
Creatinin	0.36 ± 0.02	0.52 ± 0.03	0.32 ± 0.03	0.62 ± 0.03	0.36 ± 0.04
mg/dl	a	b	a	b	a

a, b, c: small letters refer to represent significant differences ($p<0.05$) between groups at the horizontal arrows.

Table 2: Effect of Castration, Bicalutamide, LSO and LSO+Bicalutamide treatment on the concentration of some hormones (M±SE) in the serum of male mice

Groups / Parameters	Control	Castrated	LSO	Bicalutamide	LSO+Bic.
FSH mIu/ml	0.53 ± 0.03 a	0.19 ± 0.01 b	0.66 ± 0.05 c	0.28 ± 0.02 b	0.50 ± 0.03 ac
LH mIu/ml	0.72 ± 0.03 a	0.14 ± 0.02 b	0.82 ± 0.03 a	0.34 ± 0.02 c	0.65 ± 0.03 a
Testosterone ng/ml	1.74 ± 0.01 a	0.66 ± 0.05 b	2.50 ± 0.1 c	0.72 ± 0.03 b	1.65 ± 0.02 a

a, b, c: small letters refer to represent significant differences (p<0.05) between groups at the horizontal arrows.

Table 3: Effect of Castration, Bicalutamide, LSO, and LSO+bicalutamide treatment on the diameter and number of seminiferous tubules, Leydig, and Sertoli cells (M±SE) in thetestes of male mice.

Groups / Parameters	Control	LSO	Bicalutamide	LSO + Bic
Seminifer tubules(µm)	298.8 ± 22.8 a	284.4 ± 5.28 a	258.6 ± 2.49 B	278.4 ± 2.5 ab
Seminifer tubules(No.)	20.6 ± 1.20 a	22.1 ± 1.16 a	18.8 ± 0.51 A	21.2 ± 0.80 a
Leydig cells (No.)	53.2 ± 2.15 a	58.6 ± 1.8 a	27.6 ± 1.43 B	39.8 ± 1.31 C
Sertoli cells (No.)	30.0 ± 1.30 a	31.8 ± 1.06 a	20.6 ± 0.87 B	25.8 ± 0.73 C

a, b, c: small letters refer to represent significant differences (p<0.05) between groups at the horizontal arrows.

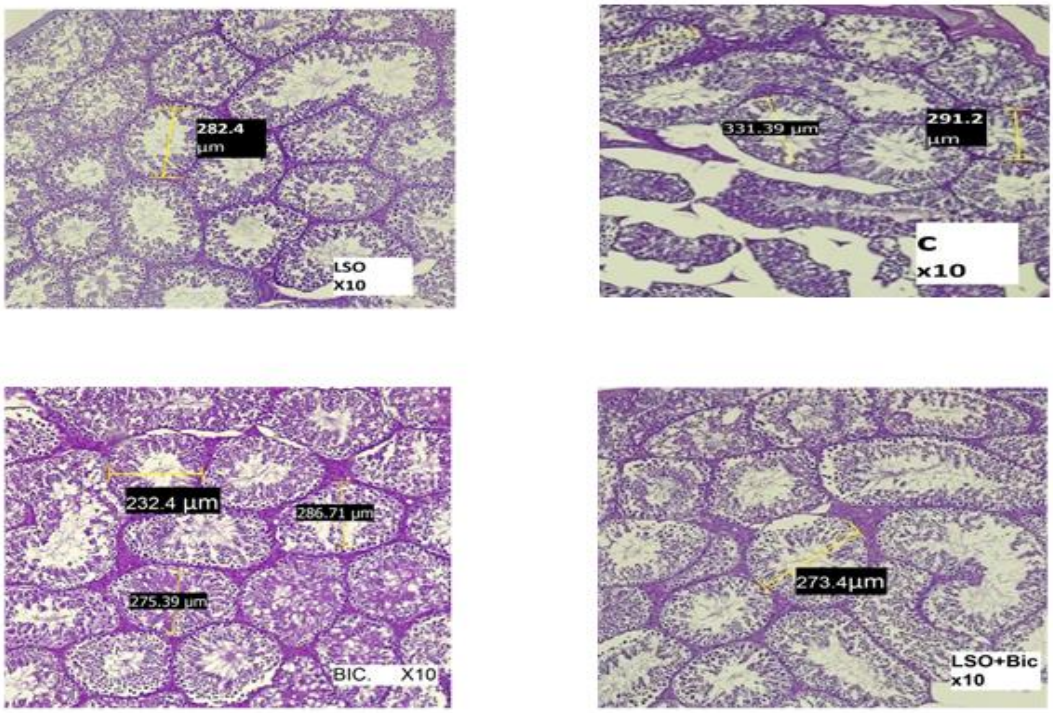

Figure 1: Shows effects of bicalutamide, LSO, and LSO+bic. Treatment following hematoxylin and eosin staining(x10) on the measurement of seminiferous diameter and number with the number of leydig and sertoli cells in the tissues of testes of male mice. C: control group, Bic: bicalutamide treated group, LSO: Linseed oil treated group, and LSO+Bic: group co-treated LSO with bicalutamide.

Discussion:

Treatment of animals for forty days with the anti-androgenic drug(bicalutamide) associated with several side effects that have a significant impact on the quality of life, as revealed from results of the current study were recorded many adverse effects concerned firstly with the biochemical parameters as shown in (table 1) were concentration of AST, ALT andALP enzymes in

addition to TSB and renal function test parameters, urea, uric acid, and creatinin were recorded a significant elevation ($p < 0.05$) as compared to the control and other treated groups. Suggesting that the treatment of animals for a long period with bicalutamide may cause intoxication or oxidative stress which could play an important role in hepato-renal damage. Damages for renal and hepatic cells reflect an elevation in the hepatic enzymes and renal function test parameters in the serum of intoxicated or oxidative stress animals [32].

Results of the current study were compatible with the results of many studies they recorded an increase of hepatic enzymes significantly in rats administrated bicalutamide repeatedly 25 mg/ kg B.W. [18], the same results recorded also in rats exposed to diazinon an organophosphorus insecticide [33]. Increased hepatic enzymes concentration in the serum concerned common adverse effects for bicalutamide administration, so regular liver function tests during treatment must be advised [34].

The impairment of renal function test parameters in the current study as shown in table 1, confirmed with the elevation of urea, uric acid, and creatinin concentration in the serum of treated group were also documented by several studies. The exposure of rats to lead acetate induced damage for renal tissues leads to an elevation of urea, uric acid, and creatinin level [35]. However, animals exposed to diazinon recorded a significant increase in the parameters of the renal function test [5].

Therefore, dietary supplements may be beneficial for animals undergoing anti-androgenic therapy, so the results of this study showed LSO supplementation attenuated the extensive alterations of the biochemical parameters in the bicalutamide treated group. The results of the study are not recorded significant differences ($p > 0.05$) in all biochemical parameters as compared to the control group, moreover, these results were more clear in LSO treated group (table 1). Administration of FXO to rats treated with lead acetate improved levels of urea, uric acid, and creatinin in the serum of animals [35]. Comparative study about Improvement and protective effects between liv.52 and silymarine supplementation to rats induced hepatic damage showed liv.52 was more effective than silymarine [18]. When a group of mice is exposed to 5 Gy of gamma radiation, the induced elevation of hepatic enzymes in which their concentrations declined and normalized in pretreated with FXO for 15 consecutive days during the exposure period [36].

Castration is also one of the methods for deprivation of androgens concentration in the serum and one of the treatment methods for prostate cancer but it is currently limited in developed countries. But this procedure included many deleterious side effects similar to the anti-androgenic drugs particularly that concerned with the biochemical and hormonal criteria (table 1 and 2). So almost the same results were recorded in the castrated group as found in the bicalutamide treated group concerned with hepatic and renal function test parameters, suggesting the castration may cause some damages for tissues around incision site or may generate oxidative stress from castration operation. The destruction of tissues with oxidative stress increases the release of transaminases enzymes and elevates their concentrations in the serum. The generation of ROS and depletion ofthe thiol system mediates hepatic and renal damages increase the concentration of hepatic & renal function test parameters [37]. Results of the castration on primate rhesus monkey recorded a significant increment in the levels of ALT, ALP, urea, and bilirubin [38], while the rats which not recorded significant differences, because these rats replaced with testosterone hormone after castration [39].

Table 2, showed significant decline ($p < 0.05$) in the activities of FSH, LH and testosterone hormones in the serum of castrated and bicalutamide treated animals, where the results were in full agreement with the other studies, suggesting that the regulation of hormones can be interrupted and disrupted at hypothalamo-pituitary level particularly FSH and LH secretion.

Different conformational changes inadult male rats produced by the anti-androgens that competitively block androgenic receptors, therefore, avoided participation of testosterone in the cellular process [40]. Results of the study on the primate rhesus monkey castration recorded a significant decline in testosterone hormone while an increment in the levels of ALT, ALP, urea, and bilirubin in the serum of animals [38].

Cellular mitosis and meiosis of germ cells with the development of the reproductive system in male rats were achieved essentially via interaction of testosterone with androgen receptors on target cells [41]. Treatment with bicalutamide might adversely affects Leydig cells that cause a significant decrease in testosterone synthesis and release [42]. While a slight decline was recorded in the study of [43]. Decrement of testosterone level in rats recorded after oral administration of bicalutamide, these results also achieved in human [44], While not found any changes in the study of [45]. Suppression of anterior pituitary feedback by testosterone in rats was one of the deleterious adverse effects of bicalutamide treatment, which causes a reduction of testicular weight and related changes in testicular tissues [46]. Moreover, exposure of human populations to the anti-androgenic and estrogenic chemicals in the environment can interfere with the level of endogenous sex hormones [47].

Nonetheless, LSO treatment alone or co-treatment with the bicalutamide showed a significant increase ($p < 0.05$) in the studied hormones as revealed in (Table 2). From our results, we found that supplementation of LSO elevated concentration of FSH, LH, and testosterone suggesting the relationship between LSO and the reproductive endocrine system. Furthermore, LSO rich in many active components that may have ameliorative and enhancement effects on testicular tissue or may have a direct role for the regulation of hormones at the hypothalamo-pituitary gonads axis.

Moreover, PUSFAs in the LSO are direct precursors for a large group of compounds that physiologically active [48]. However, saponins may stimulate endogenous testosterone probably by increases the level of LH or may bound to the enzymes involved in steroidogenesis in male rats supplemented with phoenix

doctylifera extract [49]. On other hand, supplementation of rats with Nigella sativa oil recorded a significant increase in the concentration of FSH and LH may be as a result of direct stimulatory effects of oil uponthe hypothalamus to increase GnRH secretion [50]. Similar effects were also observed on the level of testosterone when supplemented male rats with the aqueous extract of Rauvolfia [51].

Table 3, and figure 1, showed histopathological observation deformities at the testicular photometric parameters of bicalutamide treated animals, with serious damages within testis cells and diameters of seminiferous tubules. These results suggest the significant disturbances in the activities of FSH, LH, and testosterone hormones, therefore physiological influences may be interrupted and disrupted at the tissues of the testicular level. Moreover, bicalutamide competitively binds with androgen receptors that inhibit the stimulatory effects of hormones, therefore enhances apoptosis the cellular structures of the primary and secondary male reproductive organs. Whena group of rats exposedto bicalutamide [52] and other groups of rats to flutamide [53] accelerated the apoptotic process in cells associated with the enhances the expression and activation of caspases-3 and caspases-6 enzymes were both are essential elements in the apoptosis of cells. Reduction of testicular weight with significant alterations in the tissues of testis after treatment of rats with bicalutamide were recorded, and adverse effects of bicalutamide may be attributed to the adverse effects the drug [54], with the suppression of testosterone on the anterior pituitary feed-back [55]. In males, secretion of GnRH from the hypothalamus stimulates Leydig cells within interstitial cells of the testes to synthesize and release of testosterone hormones, while FSH stimulates development and differentiation of Sertoli cells and give rise to the processes of spermatogenesis [56]. Retardation growth and development of germ cells in the rat testes were investigated due to the remarked decrease in the level of testosterone when treated rats with bicalutamide [57]. Similar effects were also recorded when treated rats with flutamide [53]. Also, Khursheed, et al 2011, recorded a significant reduction in the diameter and germinal layer thicknesses of the seminiferous tubules in the bicalutamide treated group.

Dietary supplements have remarkable beneficial on subjects undergoing anti-androgenic therapy, so the results of LSO supplementation in this study, revealed the alleviation of the extensive changes in the histopathological and architecture of testicular parameters in animals treated with bicalutamide as shown in (Table 3, and figure 1). These results suggest that the treatment of animals with LSO may act directly or indirectly on the sectional functions of the pituitary gland that stimulates testicular cells which control spermatogenesis and steroidogenesis. Whereas reproductive functions are androgen-dependent, therefore any changes in androgen secretion would reflect and explain alterations in the number of Leydig cells with their functions.

Protective and therapeutic properties of LSO are mostly attributed to the high contents and richness of LSO for many viable USFAs, α- linolenic acid also high contents of lignan precursor responsible for their antioxidant activities [58]. Oxidative damages in testes of rats induced by the cadmium were attenuated & improved by the FXO administration [59]. Improvement of histopathological changes afforded by FXO may belong to the beneficial effects of PUSFAs that may inhibit lipid peroxidation and subsequently alters membrane fluidity [60]. In addition to the high contents of FXO for oleic acid, monounsaturated fatty acids that decrease the susceptibility of the testicular tissue to lipid peroxidation which responsible for integrity defects of seminiferous tubules [61].

Testicular toxicity in male rats induced by sodium valerate was ameliorated and protected by the supplementation with LSO was more successful than Nigella sativa and Celery oils [62]. Moreover, treatment of male rats with lead acetate recorded degenerative alterations in testicular tissue were rescued significantly after treatment with FXO. Cured effect of FXO postulated to the enhancement influences of the plant on the healthy seminiferous tubules filled with different germ cells including Leydig and Sertoli cells [8].

Conclusion: Anti-androgenic drug bicalutamide have several adverse effects and most of them can modulated and ameliorated particularly biochemical, hormonal, and testicular criteria when LSO administrated alone or co-treated with bicalutamide.

Conflict of Interests

The authors of this paper declare that he has no financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper and therefore declare that there is no conflict of interests.

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