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Study of the testicular damage induced by dianabol and its effect on morphological and histological changes in albino male rats

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Abstract:
Began sports and physical stimulants used controversial by thanking by *the therapeutic use of by athletes and non-athletes. Anabolic androgenic steroids (AAS) have been associated with several side effects range from hypogonadism to cardiac and hepatic dysfunction and alteration of blood lipid levels. This research was undertaken to observe the harmful effect of androgenic anabolic steroids over the male reproductive system. Mature male rats of Sprague Dawley strain, weighing 300-400 g b.w. each and 12-14 weeks old were obtained from the Laboratory Animal Colony, Kufa University. Twenty-four male rats were randomly divided into 4 groups, each with 6 animals. Group (1) was given 0.5 ml distilled water/day (vehicle) and kept as control normal. Rats of group (2) (3), (4) were treated orally with 10, 20, 40mg/kg b.w./day for 8 weeks. Semen samples were collected from the cuda epidedemis of sacrificed rats under Phenobarbital anesthesia and semen was used for estimating the sperm characters. The testes and head of epididymus were carefully dissected out and weighed. These organs were then kept in 10% formalin solution pending for histopathological examination. the result shows the decrease of live sperm count & the increased dead sperm in rats treated with Dianabol.on the other hand increase of* ***abnormality*** *(deformity of sperm cell) in rats treated with Dianabol. Histopathological section in the caput epididymal tubules 10mg/kg and 20mg/kg of animals at 7 weeks post-treatment. Severe cystic distension of epididymal tubules with severe hyalinization of spermatied resulting in narrowing of tubular tissue , while Histopathological section in the …40 mg/kg of animal at 7 weeks post-treatment. Variable degree of tubular destruction together with loss of spermatied and evidence of epididymal tubular distortion with hyperplasia. So it is advisable to stay away from these drugs only for therapeutic purposes and a few doses for a limited period*

Keyward: *Anabolic androgenic steroids , dianabol , testestron.*

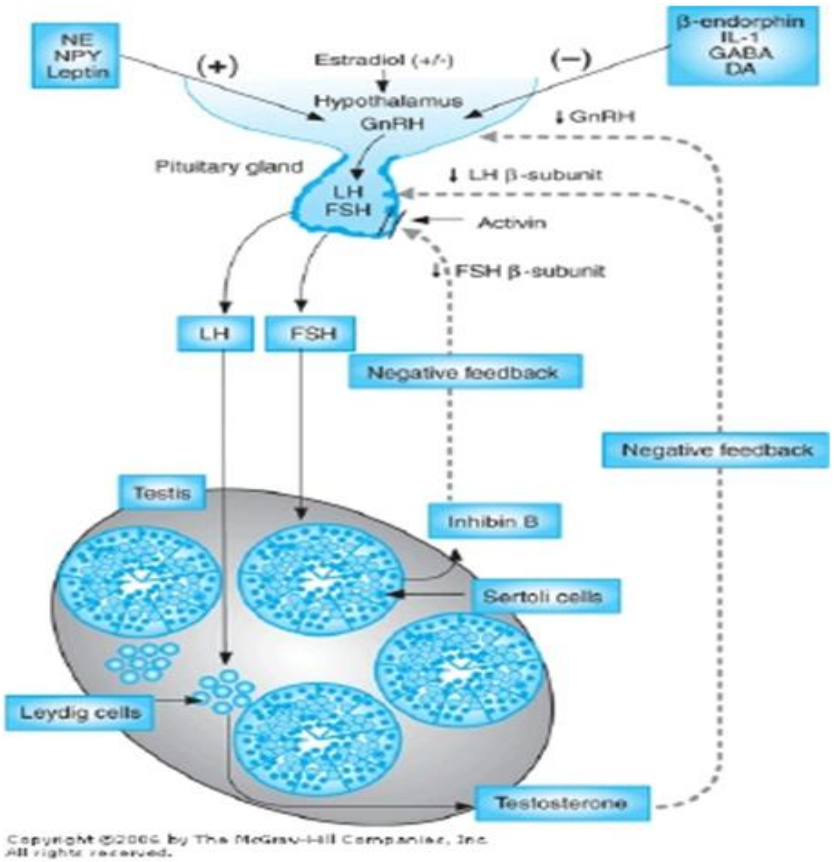
I. Introduction

Anabolic androgenic steroids (AASs) are defined as synthetic derivatives of the endogenous sex hormone testostrone. AASS have relatively small molecules and can passively diffuse into cells of various tissues**. [1, 2].No tissues are devoid of androgen receptors. These receptors belong to the family of nuclear receptor superfamily and different AASS bind to these receptors with different affinities, AASS exert several complementary anabolic effects through pathways such as a psychoactive effect on the brain, glucocorticoid anoffman antagonism, stimulation of growth hormone (GH and insulin-like growth factor 1 (IGF-1) production.** [3 , 4 ,5]. **More than 100 synthetic derivatives of testosterone** have been developed. They are well absorbed from the gastrointestinal tract, then undergo biotransformation during the hepatic first-pass metabolism and partly exerted via bile to the faces. Since they are potential targets for aromatization and reduction, they have various biological properties [6 ,7]. Their clinical indications include treatment of hypogonadism, impotence, delayed puberty (in Turner's syndrome, etc…), catabolic disorders such as muscle wasting and cachexia caused by various cancers and HIV infection, diaphragm atrophy due to catabolic-proteolysis effect of glucocorticoids in chronic obstructive pulmonary disease (COPD), osteoporosis, types of anemia (such as aplastic anemia, fanconi anemia, myelofibrosis, etc), endometriosis and fibrocystic breast disease, alcohol hepatitis, wound and burn healing (by increasing collagen synthesis and the activity of dermal fibroblasts), renal failure (specially in patients on hem dialysis) [8,9,10,11, 12,13]. In general, ergogenic effects of these agents are resulted from an increase in muscle size and strength and reduced muscle damage, increase in protein synthesis, increase in lipolysis and body fat percentage, increase in bone mineral density, increase in erythropoiesis, hemoglobin and hematocrit and increase in glycogen storage, (Karila et al ., 2010) types of androgenic steroids, (15) Injectable Steroids (Testosterone Esters, Nandrolone Esters , Stanozolol Methenolone Enanthate , Boldenone Undecylenate , Trenbolone Acetate) and Orally Active Steroids (Methandrostenolone (dianabol) , Oxandrolone , Stanozolol , Methyl testosterone, Mesterolone) 16.

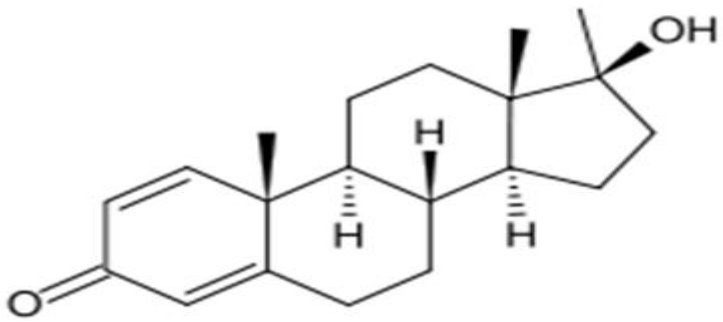
Testosterone is a steroid hormone, synthesized in the body from cholesterol. It serves distinct functions at different stages of life. During embryonic life, androgen action is central to the development of the male phenotype. At puberty, the hormone is responsible for the secondary sexual characteristics that transform boys into men. Testosterone regulates many physiological processes in the adult male, including muscle protein metabolism, sexual and cognitive functions, erythropoiesis, plasma lipid levels, and bone metabolism. The AAS are chemicals, synthetic derivatives of testosterone, modified to enhance the anabolic rather than the androgenic actions.

Testesteron secreted from interstitial (Leydig) cells exerts negative feedback on hypothalamus and pituitary to inhibit LH secretion.[17,18,19].

]It exerts paracrine effects in the seminiferous tubules along with FSH to promote Spermatogenesis. Inhibin secreted from Sertoli cells (support cells within the tubules for spermatogenesis) exerts selective inhibition over FSH secretion.[20 ,9) .Picture 1 refer of **HYPOTHALAMIC-PITUITARY-GONADAL AXIS**



Dianabol (1 7 alpha-methyl-1 7 beta-hydroxil-androsta-1.4 dien-3-on) is an anabolic steroid with moderate to high androgenic qualities. Also called **Methandrostenolone**



Powerful drugs that many people use as a shortcut to boost their athletic performance or improve their appearance. Properly called **anabolic-androgenic steroids**.

Dianabol (is an anabolic steroid originally developed by John Ziegler and released in the US. It was used as an aid to muscle growth by bodybuilders until its ban by the FDA under the Controlled Substances Act. Despite this, ,methandrostenolone continues to be produced in countries such as Mexico under the trade name Reformist-b, and is being manufactured in Russia, as well as Thailand, and subsequently is still seen on the United States black market .Production in most of Western Europe and the United States has ceased. [21]

Dianabol does not react strongly with the androgen receptor, instead relying on activity not mediated by the receptor for its effects. These include dramatic increases in protein synthesis, glycogenolysis, and muscle strength over a short space of time. However, due to its mode of action, it decreases the rate of cell respiration and decreases production of red blood cells. **Dianabol** metabolized into estradiol by aromatase. This means that without the administration of aromatase inhibitors such as Anastrozole or Aminoglutethimide, estrogenic effects will appear over time in men. Harmful side effects (Positive Effects: increase hypertrophy, increase physical performance, increase RBC production in patients with anemia, increase strength (22,23, 24] Males - Testicular atrophy, severe acne, voice-deepening, Gynecomastia, breast development and baldness. In females, toxicity of dianabol refer: menstrual dysfunction, severe acne, enlarged clitoris, breast size, Hirsutism (excessive hair growth in unusual places), baldness, and deepening of voice (22).

II. Materials and Methods

Animals:

Mature male rats of Sprague Dawley strain, weighing 300-400 g b.w. each and 12-14 weeks old were obtained from the Laboratory Animal Colony, Kufa University. The rats were kept under controlled hygienic conditions in plastic cages and fed on basal diet for one week before starting the experiment for acclimatization. Water was provided ad labium.

Experiment and grouping of rats:

Twenty-four male rats were randomly divided into 4 groups, each with 6 animals. Group (1) was given 0.5 ml distilled water/day (vehicle) and kept as control normal.

Rats of group (2) (3), (4) were treated orally with 10, 20, 40mg/kg b.w./day for 8 weeks. Semen samples were collected from the cuda epidedemis of sacrificed rats under Phenobarbital anesthesia and semen was used for estimating the sperm characters. The testes and head of epididymus were carefully dissected out and weighed. These organs were then kept in 10% formalin solution pending for histopathological examination.

Semen Collection: The testes were removed along with the epididymides. The caudal epididymides were separated from the testes, blotted with filter papers and lacerated to collect the semen.

Sperm morphology

A part of sperm suspension was used for preparing smears to evaluate the sperm shape abnormalities[24,25]. The sperm morphology was also determined using Eosin/Nigrosin stain. To test, one drop of 1% eosin Y and nigrosin was added to the suspension and were mixed by gentle agitations. Next, smears were prepared on clean and grease-free glass slides, and allowed to dry in air overnight. Preferably, 400 sperms were examined per animal morphologically at 400 magnification. Morphological abnormalities were classified as amorphous head, hook-less, banana and double headed, coiled with microcephaly, bent at cephalocaudal junction, bent with projecting filaments, microcephaly with tail defect and defective head with duplication of tail [26].

Sperm viability: Sperm viability was evaluated as follows. A 20 µl of 0.5% eosin Y and nigrosin were added into an equal volume of the sperm suspension. After 2 min of incubation at room temperature, slides were viewed by light microscope with magnification of 400. Dead sperms appeared to be pink and live sperms were not stained. In each sample, 400 sperms were counted and viability percentages were calculated [27].

The result

Table (1) shows the decrease of live sperm count & the increased dead sperm in rats treated with Dianabol.

groups	**Viability**
control	83±4.43 a
Dianabol 10mglkg	45.7±1.92 b
Dianabol 20mglkg	58±11.81 b
Dianabol 40mglkg	42.4±12.25 c

Data represent Means ± S.E.

All pretreated groups were compared to the positive control group using Student's test different small letter mean significant at level of ($p<0.05$).

Data in table (1) shows that Dianabol, when given orally to male rats for 7 weeks, decreased livability significantly ($P < 0.05$) than control group which percent of live sperm 83±4.43. On the other hand, the percent of live sperm in group (2,3,4) shows a decrease in percent of live sperm to (45.7±1.92 , 58±11.81 , 42.4±12.25) respectively . Therefore, the result refers to significant ($P < 0.05$) decrease of the percent of sperm cell livability as compared to the control positive group.

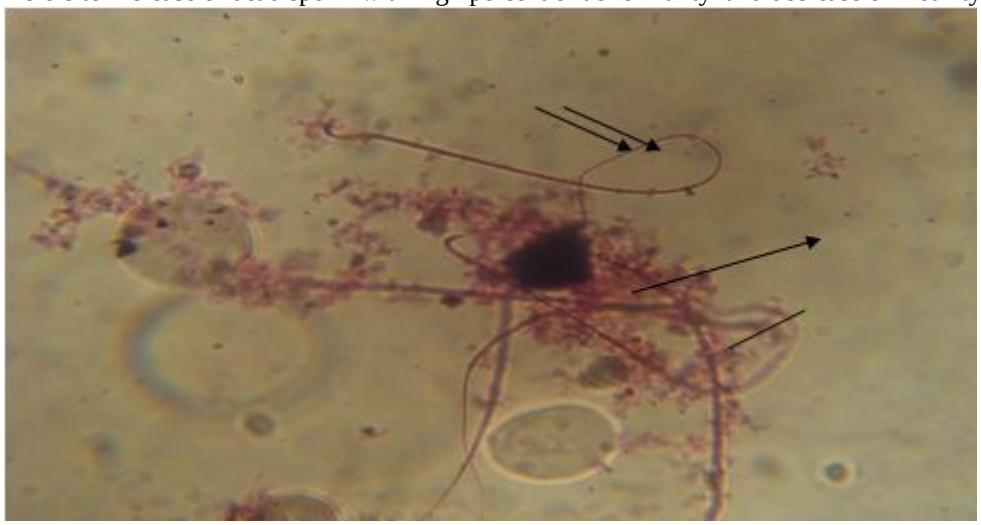
Table(2) shows increase of abnormality (deformity of sperm cell) in rats treated with Dianabol.

Groups	**Abnormality**
Control	9.64± 4.50 a
Dianabol 10mglkg	54.2 ± 4 .26 b
Dianabol 20mglkg	48.2± 18 .41 b
Dianabol 40mglkg	56± 18.16 b

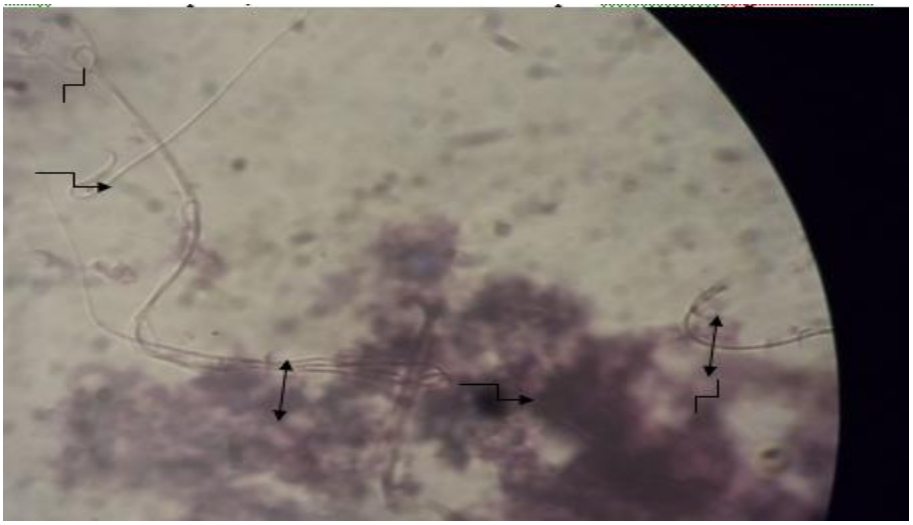
Data in table (2) shows that Dianabol, when given orally to male rats for 7 weeks, increases deformity of sperm cell significantly ($P < 0.05$) than the control group with the percent of abnormality as 9.64± 4.50. On the other hand, the percent of abnormality in groups (2,3,4) show increase in the percent of abnormal sperm to (54.2 ± 4 .26 , 48.2± 18 .41 , 56± 18.16) respectively. Therefore, the result refers to significant ($P < 0.05$) decreases in the normal of sperm cell as compared to the controlled positive group.

Picture 1 refers to increase of dead sperm with high percentage of abnormality. Eosin Y and nigrosin ×10.

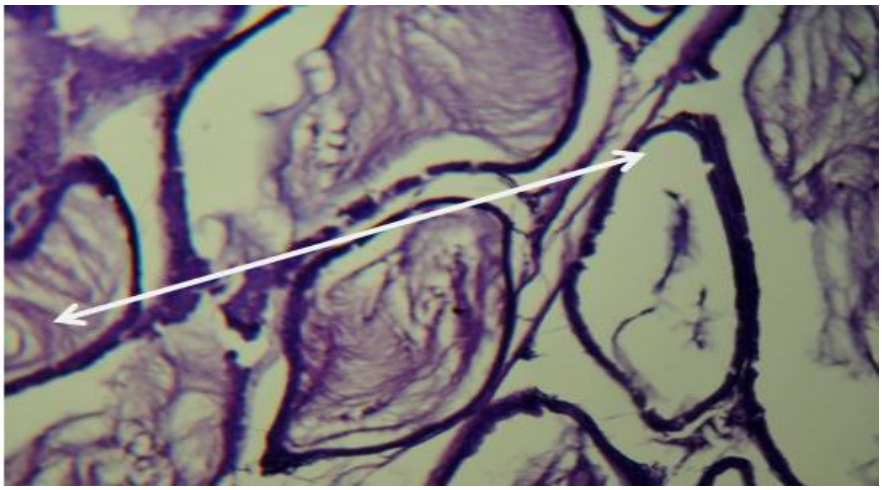
Picture 2 refers to increase of dead sperm with high percent of abnormality and decrease of motility (×10) .



Picture1 : Abnormal sperm, coiled tail and banana shape. Eosin and nigrosin ×40.



Abnormal sperm detached, hook-like head , double tail



(H&E stain 40x)

Fig1: Histopathological section in head of epididymis treated with 40mg/kg characteristic histopathological lesion in the caput epididymis shows severe destruction of basement epithelial lining, with severe degeneration and necrotic and hylain degeneration, tubules with severe loss of sperm.

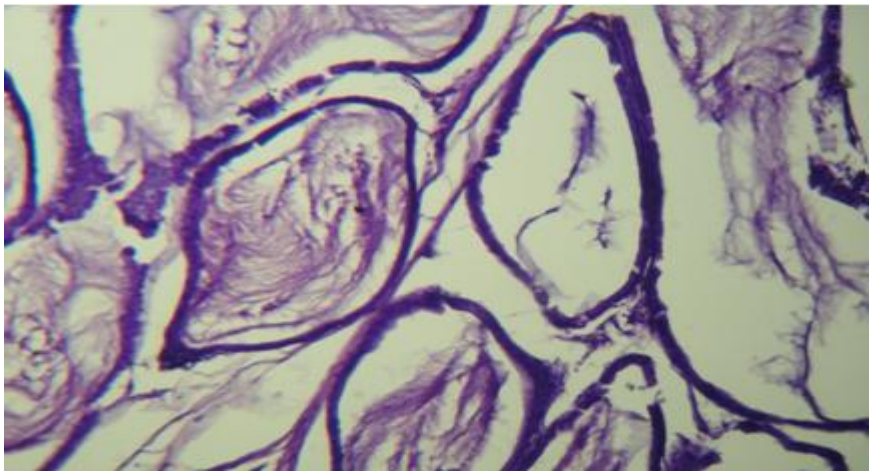


Fig2: Histopathological section in the head of animal 40 mg/kg at 7 weeks post-treatment. caput epidimal tubules cystic destion of tubules with degeneration & necrosis of spermatied (severe destruction of epidiymal parenchyma)

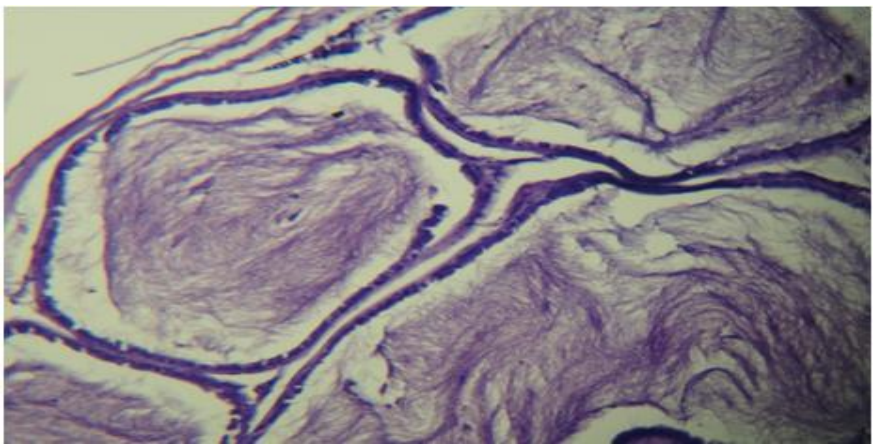


Fig3: Histopathological section in the caput epididymal tubules 20mg/kg of animals at 7 weeks post-treatment. Severe cystic distension of epididymal tubules with severe hyalinization of spermatied resulting in narrowing of tubular tissue

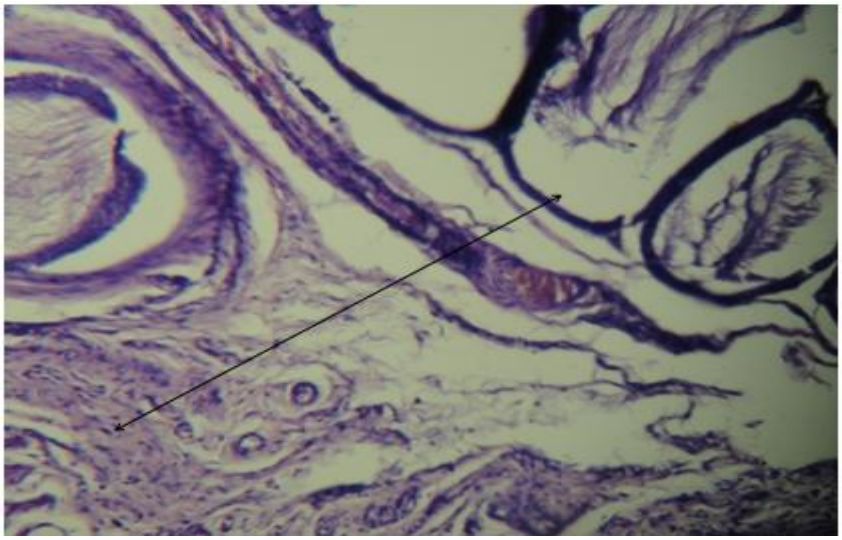


Fig:4. Histopathological section in 10mg/kg.
The histopathological lesion show proliferation interstitial of fibers C.T with slight cellular filtrated together with loss of spermatied in the tubules that appear empty with cystic tubular distention.

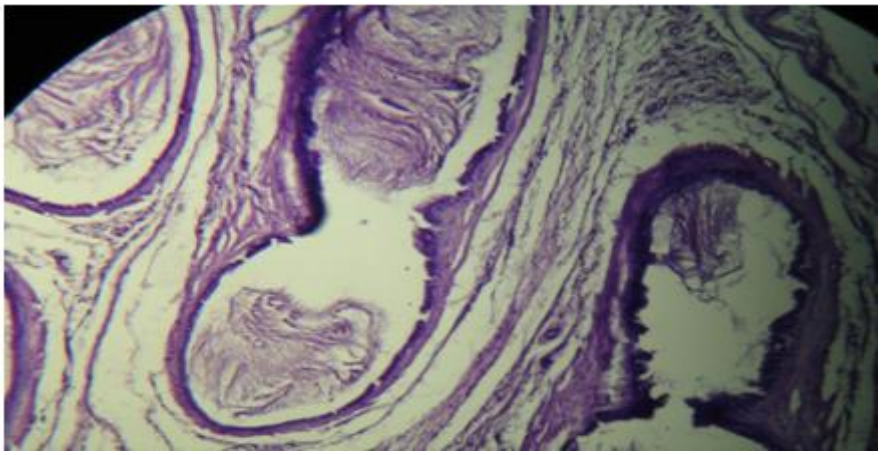


Fig5: Histopathological section in the 40mg/kg of animal at 8 weeks post-treatment. The section showed epithelial hyperplasia of some epididymal duct tubules that show papillary growth in the lumen together with moderate fibrosis in the interstitial tissues

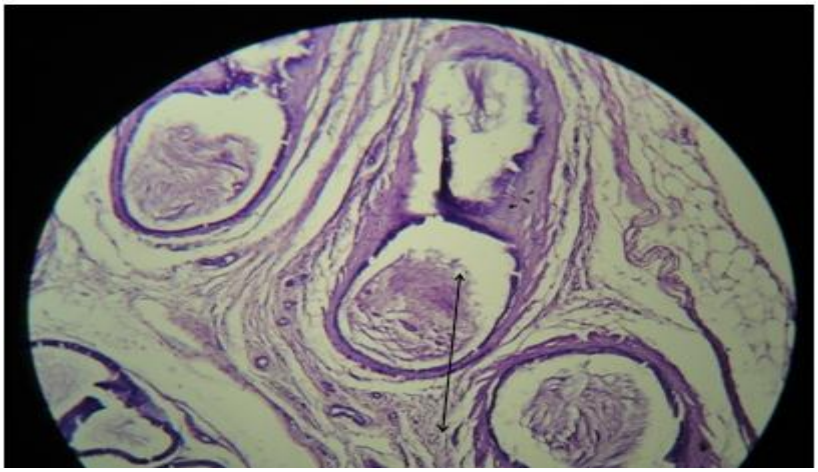


Fig6: Histopathological section in the epididymas of animal at 8 weeks post-treatment with 40mg\kg of Dinabol shows some empty epidermis duct with sperm degeneration, in addition to fibrous connective tissue proliferation in the interstitial tissue H&E stain 40X)

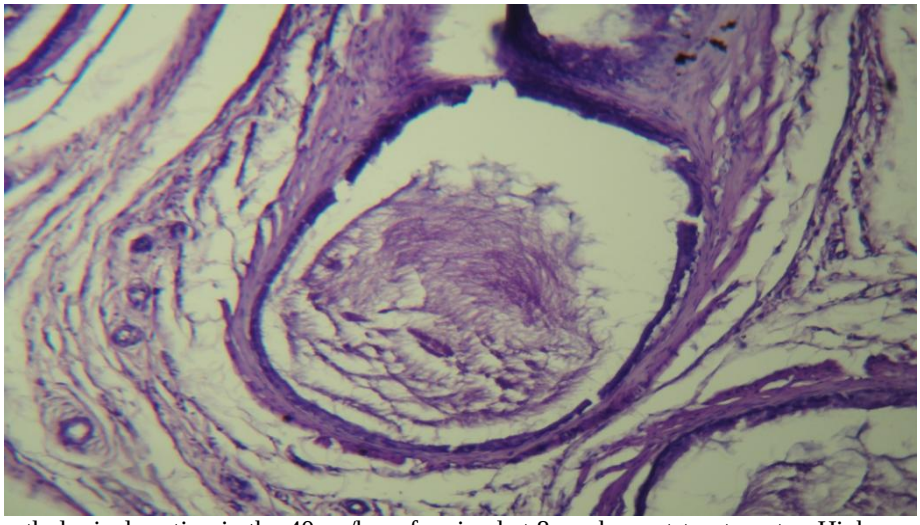


Fig7: Histopathological section in the 40mg/kg of animal at 8 weeks post-treatment. Higher magnification showed epithelial hyperplasia with papillary projection of epididymal of epithelial lining with moderate hyperplasia of fibrous tissue in the interstitial and between tubules.

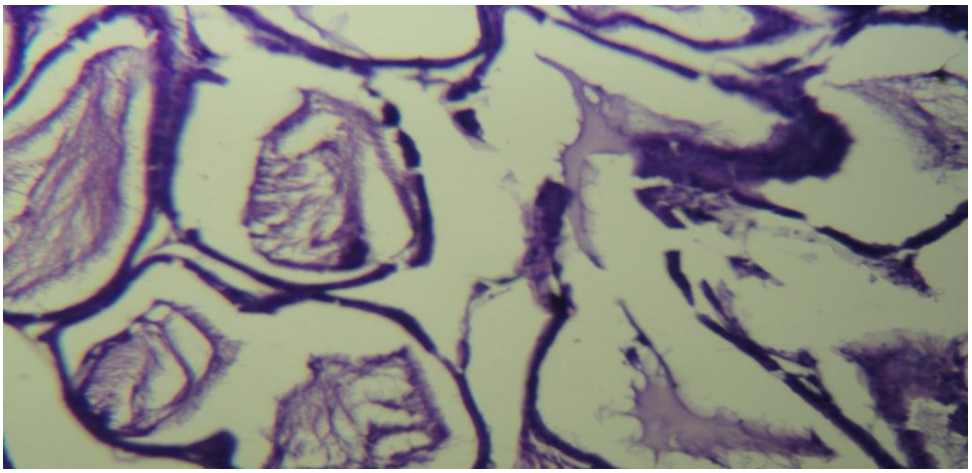


Fig8: Histopathological section in the …40 mg/kg of animal at 7 weeks post-treatment. Variable degree of tubular destruction together with loss of spermatied and evidence of epididymal tubular distortion .

III. Discussion

Dianabol, when given orally to male rats for 7 weeks, decreased livability significantly ($P < 0.05$) than the control group which percent of live sperm was 83±4.43. On the other hand, the percent of live sperm in groups (2,3,4) shows decreases in the percent of live sperm to (45.7±1.92 , 58±11.81 , 42.4±12.25) respectively. Therefore, the result refers to significant ($P < 0.05$) decreases in the percent of sperm cell livability as compared to the controlled positive group.

Table(2) shows an increase of **abnormality**(deformity of sperm cell) in rats treated with Dianabol.

Dianabol, when given orally to male rats for 7 weeks, shows an increase of deformity of sperm cells significantly ($P < 0.05$) than the control group with the percent of abnormality of 9.64 ± 4.50. On the other hand, the percent of abnormality in groups (2,3, and 4) show increases in the percentage of abnormal sperm to (54.2 ± 4.26, 48.2± 18.41, 56 ± 18.16) respectively. Therefore, the results refer to significant ($P < 0.05$) decreases in the normal sperm cells as compared to the controlled positive group.

Results obtained in the present study indicated that Dianabol induces many morphological alterations in the sperm of rats**.** It also increased sperm heads and tail abnormalities. These results indicated the antispermatogenic adverse effect of Dianabol. Abnormal sperm cells induced after treatment of rats with dianabol could be due to the ability of Dianabol to, either, interfere with the spermatogenic processes in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins, which may result in the alteration of spermatogenesis . **Spermatogenesis** is the process by all males spermatogonia to develop into mature spermatozoa. Many studies have shown that Dianabol causes a decrease in serum levels of testosterone [29,30].

According to [31], the process of spermatogenesis is both androgenic and follicle stimulating hormone (FSH) - dependent. In addition, androgen stimulation, as a whole, is responsible for the maintenance of spermatogenesis and secondary sexual characteristics, especially in the male. Thus, the major androgen in the circulation of men and adult males of mostmammalian species, including the rat, is the testosterone (T) that is produced by Leydig cells of the testis. Numerous studies on androgenic anabolic steroids have shown that it possesses potent toxicity on male sexual organs – the testis, by causing the disturbances of spermatogenesis in albino rats [32,33, 31]. The effect of anabolic steroids on the testis results from the negative feedback of androgens on the hypothalamic-pituitary axis and possibly from local suppressive effects of excess androgens on the testis [34] Moreover, testosterone itself is relatively ineffective when taken orally or injected in an aqueous solution because it is susceptible to relatively rapid breakdown by the liver before it can act on the target organ [35]. The androgenic anabolic steroids are known to elevate oxidative stress in the testes [36]. Seminal oxidative stress correlates negatively with sperm concentration, motility and function, adversely affecting fusion events required for fertilization [37].

LH and FSH act on separate components of tests. LH act on leydig cells to regulate testosterone secretion and FSH act on sartoli cell to increase spermatogenesis, testosterone and inhibin differentially influencing the secretary rate of LH and FSH. Testosterone, the product of LH stimulation of the leydig cell act in a negative feed back fashion to inhibit LH secretion . **Inhibin has been postulated to be secreted by Sertoli cells** in **response to the follicle stimulating hormone (FSH) and** in **turn to exert an inhibitory effect on FSH production[38] .[39] indicate that men who received 200 mg testosterone enanthate weekly showed FSH and LH were rapidly suppressed, followed by parallel decline in inhibin B and sperm concentration**. Thus, the decrease of inhibin and testosterone after treatment with dianabol may be the cause of the decrease in spermatogenesis and the increase in the deformity of sperm.

Histopathological sections in the head of epididymis, treated with 40mg/kg chrematistic, histopathological lesion in the caput epididymis showed severe deduction of basement epithelial lining with severe degeneration and necrotic and hyaline degeneration, tubules severe loss of spermatied.(picture 1). On the other hand, the section in picture 2 shows cystic destion of tubules with degeneration & necrosis of spermatied (severe destruction of epidermal parenchyma, epithelial hyperplasia of some epididymal duct tubules that show papillary growth in the **lumen, together with moderate fibrosis in the interstial tissues(**picture 6 ,7). There are degrees of tubular destruction, together with loss of spermatied and evidence of epididymal tubular distortions(picture 8).

Histopathological section in 20mg/kg of animal at 7 weeks post-treatment show severe cystic distension of epididymal tubules with sever hyalinization of spermatied, resulting in the narrowing of tubular tissue, (3). This changes, more less rather than with group 40mg/kg.

Histopathological section in 10mg/kg shows proliferation of interstitial of fibers C.T with a slight cellular filtrate, together with loss of spermatied in the tubules that appear empty with cystic tubular distenstionture(picture 4).the result of this study was agreement with [40]who observed that there was disruption of the somniferous epithelium with broad spaces between the cellular components and the testicular atrophy with shrinkage resulting in decreased diameter of somniferous tubules the somniferous

epithelium of the treated animals with AAS show disrupted with broad spaces between the cellular components showing the presence of copious vacuoles frequently associated with degenerating germ cells[29] .more ever [41,42]reveal that AAS cause reduction in the length of the testis, as a result

there was a reduction in the weight of the testis . that disruption may lead to cessation of mitosis and meiosis. [40] who suggested that the defect may be due to the effect of AAS directly on the Sertoli cells which are responsible for the hormonal, nutritional, and physical support.

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