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TESTICULAR MORPHOLOGY OF ALBINO RAT; WITHDRAWAL EFFECTS OF CHRONIC ANDROGENIC ANABOLIC STEROID

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ABSTRACT: Withdrawal effects of chronic androgenic anabolic steroid on hormonal and testicular morphology were studied .Forty five male albino rats were divided into 1) Control 2) Chronic group and 3) Withdrawal group. Testoviron was injected at a dose of 400mg/kg body weight intramuscularly once in two weeks for 14 weeks, and then drug was withdrawan for another 14 weeks. Testes were removed and fixed in 10% formalin and processed. Following withdrawal of AAS, testicular and relative testicular weight was restored to control. Increased tubular count also returned near to normal while decreased diameter of seminiferous tubules, thickness of germinal epithelium, count and diameter of leydig cells were also restored near to normal in withdrawal group when compared with the chronically treated group. Histological observations also revealed that degenerated spermatogenic cells were returned to their normal appearance and oedematous vacuoles were reduced. Moreover, decreased level of reproductive hormones, i.e. FSH, LH and testosterone also returned to control level in withdrawal group. These results indicated that chronic AAS has substantial harmful effects on hormonal and testicular morphology. However, these adverse effects gradually restored to normal following withdrawal from AAS.

Key words: androgenic anabolic steroids – testis - testicular hormones - rats.

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INTRODUCTION

Anabolic androgenic steroids (AAS) are the class of steroid hormones that is the synthetic analogue of testosterone.[1] Their medical usage is restricted and it is only used with caution in male hypogonadism, breast cancer, AIDS, anemia, and bone growth.[2-4] AAS are commonly abused by body builders and athletes to increase their strength and muscle mass that may persist for months after drug withdrawal.[5] Long term usage of AAS, cause serious side effects including left ventricular hypertrophy, gynaecomastia, cholestasis, renal failure, elevation of blood pressure, acne, premature baldness, hepatotoxicity and liver cancer.[6-11]

AAS also caused substantial side effects on reproductive systems, both in human and in experimental animals, disturbing the regular endogenous production of testosterone and gonadotrophins.AAS induces hypogonadism that is characterized by reduced testosterone, follicular stimulating hormone (FSH) and leutinizing hormone (LH), altered spermatogenesis and testicular atrophy.[12-16] Decrease sperm production, altered semen quality and infertility have also been reported.[17-19] These effects are due to the inhibitory feedback of AAS on hypothalamus-pituitary-testicular axis and local suppression of excess steroids on testis.[20,21] In a recent study, AAS not only decreased the absolute testicular weight and also altered the architecture of seminiferous tubules of Sprague-Dewley rats.[22] Other studies also confirmed that high dose of AAS also depleted the germ and leydig cell population in testes of rats.[17,23-25]

This study was therefore designed to observe the withdrawal effects of AAS on body weight, testicular and relative testicular weights, hormonal levels and morphology of testes of albino rats with the help of light microscope.

MATERIALS AND METHODS

Forty five male albino rats of wistar strain weighing 180-250 were selected in random manner for this study. All animals were kept in experimental room of animal house of Baqai Medical University, under standard animal house condition of 12 hr dark and 12 hr light cycle and temperature of 30 □C. They were fed with standard laboratory diet and water ad libitum. One week prior to start of experiment, they were held in experimental room in close observations to acclimate them with experimental room. After one week of acclimatization to the laboratory environment, the animals were divided into following groups:

Group 1: These animals were given normal saline and served as controls

Group 2: Chronic group, received inj. testoviron, (i.m) for 14 weeks.

Group3: Withdrawal group, discontinuation after 14 weeks of testoviron injection for another 14 weeks.

Testoviron injection was purchased from Schering AG, Germany. Each ml of testoviron depot is a blend of 110 mg testosterone enanthate and 25 mg testosterone propionate. The drug was introduced intramuscularly at a dose of 400 mg/kg body weight once in two weeks.

At the end of experiment, animals were sacrificed by decapitation and blood samples were obtained by intracardiac puncture. Testosterone, FSH and LH was determined by using ELISA method.

The testes were removed and fixed in Bouin's fluid. They were subsequently embedded in paraffin wax, sectioned at 3µm and stained with haematoxylin and eosin. The stained slides were then studied under light microscope for tubular count and diameter, thickness of germinal epithelium, nuclear count and diameter of leydig cells.

Data were expressed as mean ± SEM and statistical analysis was performed by ANOVA followed by Post hoc Tukey test by using SPSS (19). P value less than 0.05 was considered as significant.

RESULTS

Changes in Body Weight

Final mean body weight of all three groups had highly significantly increased ($P<0.001$) when compared with initial mean body weight. While, the weight gain in withdrawal was significantly higher ($P<0.001$) than to control but significantly less ($P<0.05$) when compared to chronic group (Table-I).

PARA-METER	CONTROL	CHRONIC	WITHDRAWAL
Initial body weight	204.33±3.96	200.80±2.50	201.86±4.26
Final body weight	233.26±4.50	244.93±2.63	242.27±2.97
Weight gain	28.93	44.13	40.4
Testicular weight	12.03±0.33	9.08±0.09	11.50±0.31
Relative testicular weight	5.13±0.15	3.71±0.06	4.82±0.14

Table-I. Body weight, testicular and relative testicular weight of control and treated animals

Testicular and relative testicular weight

The mean testis and relative testis weight of withdrawal group were highly significantly increased ($P<0.001$) when compared with that of chronically treated rats but it was not significant ($P>0.05$) when compared with control (Table-I).

Hormonal Results

The mean serum concentration of testosterone, FSH and LH of withdrawal group were significantly higher ($P<0.001$) than that of chronic group but they were non-significant ($P<0.05$) when compared with that of control group (Table-II).

Histological Results

The mean count of seminiferous tubules of withdrawal group was significantly lower ($P>0.001$) when compared with that of chronically treated group but not significant ($P<0.05$) when compared with that of control (Table-II, Figure-1).

	CONTROL	CHRONIC	WITHDRWAL
HORMONAL			
TESTSTERONE	0.82±0.01	0.25±0.01	0.74±0.01
FSH	10.82±0.09	2.66±0.06	8.25±0.17
LH	5.52±0.10	3.73±0.06	5.20±0.09
SEMINIFEROUS TUBULES			
Count	16.29±0.38	24.42±0.35	16.63±0.35
Diameter	266.41±3.07	213.97±1.31	260.21±2.57
Thickness	93.83±1.76	66.56±1.67	90.19±1.99
INTERSTITIAL CELLS			
Count	13.73±0.56	6.73±0.47	12.13±0.49
Nuclei	4.07±0.05	3.02±0.06	3.94±0.09

Table-II. Hormonal Assays

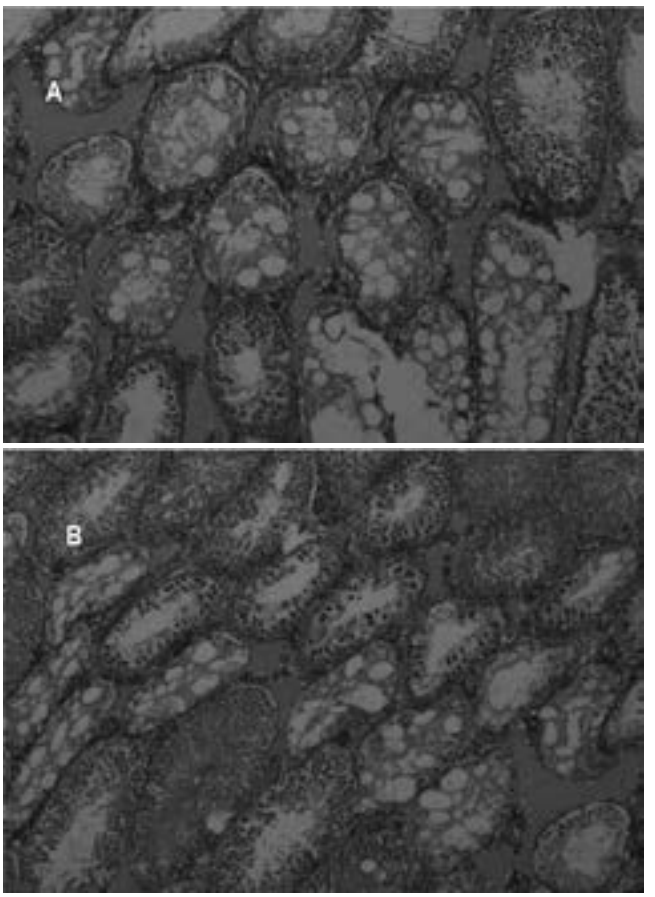

Figure-1: H & E stained 3 μm sections of testis of chronic (A) and withdrawal (B) groups. Figure A shows reduced number of seminiferous tubules, reduced thickening of germinal epithelium and degenerative changes. Figure B shows the partial recovery and increase number of tubules, less degenerative changes and normal thickening of germinal epithelium in most of the tubules (220 X).

The mean diameter of seminiferous tubules of withdrawal group was significantly higher (P>0.001) when compared with that of chronically treated group but not significant (P<0.05) when compared with that of control. Also the mean thickness of germinal epithelium of withdrawal group was significantly higher (P>0.001) when compared with the mean thickness of chronically treated group but not significant (P<0.05) when compared with that of control. (Table-II, Figure-1).

The mean count of interstitial cell nuclei of withdrawal group was significantly higher (P>0.001) when compared with the mean count of chronically treated group but not significant (P<0.05) when compared with the mean count of control. Also the mean diameter of interstitial cell nuclei of withdrawal group was significantly higher (P>0.001) when compared with the mean diameter of chronically treated group but not significant (P<0.05) when compared with that of control(Table-II, Figure-2).

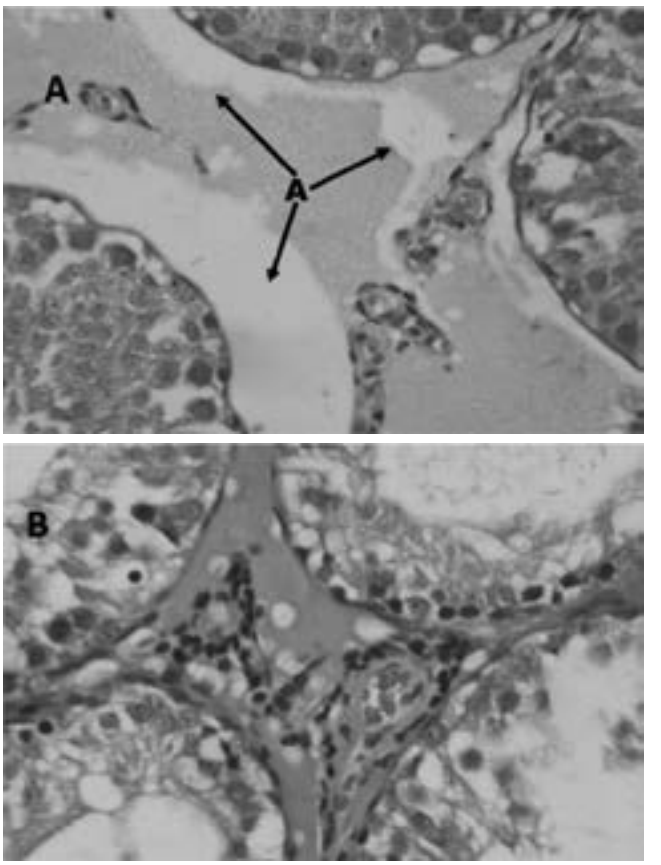

Figure-2: H & E stained 3 μm sections of testis of chronic (A) and withdrawal (B) groups. Figure A shows reduced number and diameter of leydig cells and nuclei. Arrow is indicating the wide space between the tubules. Figure B shows the partial recovery and increase number and diameter of leydig cells and nuclei. Narrowing of the interstitial space between the tubules is also seen (400 X).

A clear reduction of spermatogenic cells with degenerated spermatogonia and spermatocytes which were observed in tubules of chronically treated animal were returned back to the normal in withdrawal group. The tubular lumen was still enlarged but majority of them were found to contain morespermatogonia, spermatocytes and spermatozoa than chronically treated animals (Figure-3). Oedamatous vacuoles which were formed and concentrated mainly opposite to the boundary of the tubules were reduced in numbers in most of the tubules of withdrawal group (Figure-3).

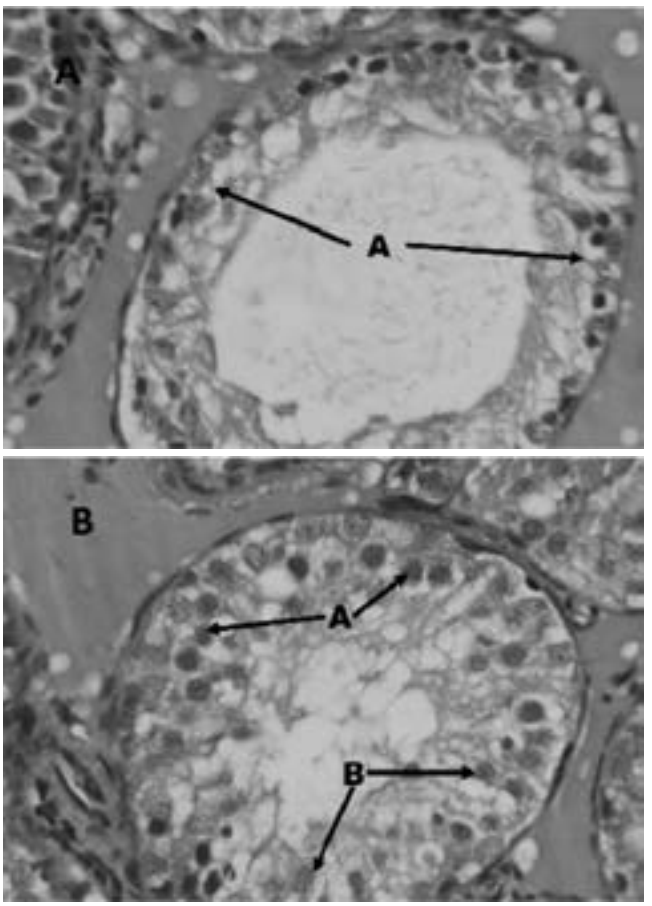

Figure-3: H & E stained 3 μm sections of testis of chronic (A) and withdrawal (B) groups. Figure A shows reduced number of spermatogonia(arrow), primary and secondary spermatocytes and absence of spermatozoa. Figure B shows the partial recovery and increase number of spermatogonia, primary and secondary spermatocytes (arrow A & B) and few spermatozoa (400 X).

DISCUSSION

AAS usage in athletes and in body builders to increase their muscle mass, physical strength and performance is a common practice. Large identifiable studies have shown the potential toxic effects of AAS on male reproductive systems both in experimental animals and human.[13,26]
This study was performed to observe and record the withdrawal effects of AAS on morphology of testes with the help of light microscope.

In the present study, the weight gain in withdrawal group was significantly more than to control but it was significantly lower than to chronic group. This result are in line that following discontinuation of AAS, increase muscle mass and weight gain fade slowly and it may persist up to several weeks and recover partially.[27,28] Similarly, significant recovery in the testicular and relative testicular weight was also noted near to control animals following withdrawal from AAS.[29]

Our result showed the reversibility of FSH, LH and testosterone level near to control following withdrawal of AAS that is contrast to other findings that FSH and LH sustained suppression for a period of one year after withdrawal from AAS.[30] Kanayama *et al* (2015) also found that after 3-26 month from discontinuation of AAS, serum testosterone level retained to lower levels in AAS using weight lifters as compare to non-AAS using weightlifters.[13]

AAS exerts a negative feedback to hypothalamus-testicular axis that results into suppression of testicular function that is characterized by decreased production of testosterone, suppression of spermatogenesis and atrophy of testis.[21,23] In the present study, chronic treatment

of AAS halted the process spermatogenesis as indicated by the decrease number of primary and secondary spematocytes, spermatids and spermatozoa.[25] These changes could be due to reduction of size and shape of seminiferous tubules which halted the smoothness of spermatogenesis.[22] When animals were left untreated for fourteen weeks, decrease number of primary and secondary spematocytes, spermatids and spermatozoa improved to control level. This recovery could be due to recovery of the process after some weeks following discontinuation of AAS.[2]

Our study showed the remarkable reduction of number and size of leydig cells, widening of interstitial space and appearing of fibroblast cells in chronically treated rats.[16,20,31] Moreover, our study showed that number and size of leydig cells was restored near to normal in withdrawal group. Our study is in line with the results of some researchers[32] but contrast with the finding of others that depletion of leydig cells was not reversible after withdrawal of AAS.[17]

AAS had a negative impact on germ cell that resulted into more number of tubules per field.[25] In our study, tubular count of withdrawal group restored to control level. This recovery could be due to the reversal of testosterone to control level because optimal level of testosterone maintains the normal architecture of seminiferous tubules.[32]

The tubular diameter and germinal epithelium thickness were also highly significantly decreased in chronic treated group. The germinal epithelium was disrupted, copious vacuoles were seen and broad spaces appeared between the cellular components. Two layer spermatocytes layer decreased into one layer and four to five layer thick zones of spermatids were decreased into two to three layers thick. These findings are in consistent with the previous reports that chronic AAS alter the testicular function and ceased the maturation of germ cell population with reduction in germ layer thickness. Moreover, disruption of cellular components of germinal epithelium led into decrease diameter and disorganization of seminiferous tubules hence wide spaces appear between tubules.[16,25] However, reversibility of tubular diameter and germinal epithelium thickness to control after withdrawal from AAS were observed. These effects may be due to the restoration of testosterone and LH after withdrawal that helps to mature the germ cells and precede normal spermatogenesis that maintains the tubular diameter.[33,34]

It is concluded that chronic AAS has substantial harmful effects on hormonal and testicular morphology. However, these adverse effects gradually restored to normal following withdrawal from AAS. Further studies are warranted to evaluate the exact mechanism of toxicity and recovery.

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"Even the nicest people have their limits."

Unknown

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2	Dr. M. Rashid Ahmed	Dissection of Rats	
3	Dr. Hafiz Syed Imran Ul Haq	Data collection & Analysis	
4	Syed Naeemul Hassan Naqvi	Critical Review	
5	Rehana Parveen	Revision	