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MALE GONADAL SUPPRESSION BY THE NEEM OIL AFTER SUB-ACUTE, SUB-CHRONIC AND CHRONIC ORAL ADMINISTRATION OF NEEM OIL

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ABSTRACT

This research was undertaken to observe the subacute, subchronic and chronic effects of the neem oil on gonadal morphology and function after oral administration. Forty eight male albino rats were used in this daily for a period of three, six, twelve and twenty four weeks. The animals were administered with three different doses of neem oil orally
The animals were divided in 4 different groups and each group was further divided into 4 subgroups o the basis of durations. Animals were sacrificed at the end of the experimental period. Testes were observed for gross and microscopic findings and the serum levels of Testosterone and gonadotrophins were studied
This study records a dose and duration dependent morphological and functional suppression of male gonads in albino rats.

Key-words: Neem oil, Chronic toxicity, Testosterone, Testis , Seminiferous tubules

INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization. *Azadirachta indica* A. is a medicinal plant commonly known as the neem tree. Neem is a fascinating tree and known for almost last 5000 years (Naqvi, 1998). In India neem leaves are used to suppress libido during meditation (Naqvi, 1998).. Recently researchers have reported antifertility effect of neem in both sexes. Its Spermicidal effect has been reported by Sinha *et al*. (1984); Riar *et al*. (1991). A significant decrease in serum testosterone level has also been reported by Parshad *et al*. (1994) and Aladakatti *et al*. (2001) and a significant but reversible dose dependent decrease in weights of the testes, epididymis and seminal vesicles in albino rats (Raji *et al*. 2003). Reversible antifertility effects of neem oil in mice were reported by Yin *et al*. (2005) and similar findings were found by Shaikh *et al*. (2009 a and b) in both male and female albino rats.

Sub acute, sub chronic and chronic are types of toxicity on the basis of repeated exposure to a chemical for one month or less, for one to three months and for more than three months respectively (Hayes, 1982).

This study aims to analyze the male gonadal suppression done by the neem oil after sub-acute, sub-chronic and chronic oral administration.

MATERIALS AND METHODS

PLANT AND ANIMAL SOURCES

Fresh neem oil was obtained from Hussein Ebrahim Jamal Research Institute of Chemistry (H. E. J. R. I. C), Karachi University, Pakistan. The material was stored in a closed container in a cool dry place.

Fort eight adult albino male rats of Wistar Strain were obtained from the animal house, Baqai Medical University (BMU), Karachi. This Wistar strain albino strain is being bred at BMU for the last 14 years. BMU obtained from Jinnah Post Graduate Medical Institute Karachi (JPMC) and JPMC Karachi originally obtained from Charles River Laboratory, Brooklyn, Massachusetts, USA and was cross bred.

These albino rats (150-200 g) were housed in the central animal house in separate cages with paddy husk bedding and maintained on standard pellet diet and drinking water ad-libitum until the time of use.

Throughout the experiment, husk beddings were renewed every 3-4 days. The animal room was maintained at 22 to 30 centigrade and with a 12:12 light-dark cycle. At the end of the experimental period the animals were anaesthetized with ether and then sacrificed by terminal dose of the same. Animals were randomly allotted into four experimental groups. After drug administration, free access to food and water was allowed.

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GROUPS

Low Dose

A_1	=	3	Rats	A_2	=	3	Rats
A_3	=	3	Rats	A_4	=	3	Rats

Medium Dose

B_1	=	3	Rats	B_2	=	3	Rats
B_3	=	3	Rats	B_4	=	3	Rats

High Dose

C_1	=	3	Rats	C_2	=	3	Rats
C_3	=	3	Rats	C_4	=	3	Rats

Control

D_1	=	3	Rats	D_2	=	3	Rats
D_3	=	3	Rats	D_4	=	3	Rats

These 48 albino rats were divided in four experimental groups A, B, C and D. Each comprised of 1, 2, 3 & 4 subgroups. Each subgroup contained 3 animals.

Subgroup A_1, A_2, A_3 and A_4 treated with a dose of 0.5ml/Kg/day orally (low dose) for a period of 3,6,12 and 24 weeks respectively. Subgroup B1, B2, B3 & B4 (medium dose), were administered neem oil with dose of 1.0 ml/Kg/day orally for a period of 3,6,12 and 24 weeks respectively. In subgroup C_1, C_2, C_3 & C_4 were administered with dose of 2.0 ml/Kg/day orally (high dose) for a period of 3,6,12 and 24 weeks respectively. In group D (control), 24 animals were given peanut oil with dose of 2.0 ml/Kg/day orally and were assigned as subgroups, D_1, D_2, D_3 and D_4.

At the end of experiment the animals were anesthetized by deep ether anesthesia. Blood removed from the heart by intracardiac puncture (Allen *et al*., 2001) and then sacrificed (Inauwa and Williams, 1995). Testes were removed and fixed in 37% formaldehyde for microscopy (Culling,1974).

The slides were examined under the microscope and digital scans of all glass slides were created with a Scan Scope scanner (Aperio Technologies, Inc.). Pathological changes were graded according to the severity from mild, moderate to severe.

The testicles were evaluated with respect to the tubular diameter (morphometry), maturation of the germinal epithelium (spermatogenesis, different stages of sperm maturation, sertoli cells), integrity of the tunica propria and interstitial components including leydig cells.

In morphometric analysis the tubular diameter of at least 10 randomly chosen tubular cross-sections per testis (specimen) was determined, using the linear measurement tool of Aperio's ImageScope software (Aperio Technologies, Inc.). Only round or nearly round tubules were used for diameter measurement to minimize the error due to oblique cutting of tubules. Also to minimize any error, diameter was measured at two points per seminiferous tubule and averages were obtained (Fig. 1).

Blood removed from the animals by intracardiac method was centrifuged at 2000 Revolution per minute (RPM) to separate the serum for the gonadotrophins and Testosterone.

The quantitative determination of hormones was done by using Enzyme Immunoassay Method (ELISA).

QUANTIFICATION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

During this research 2695 Water Alliance system, having binary pump system, connected with 2487 UV detector and fitted with auto sampler, Zorbax C18, 4.6x150 mm, a reversed phase Column Was Used. The Empower Software Was Used To Produce Analysis.

Sample Preparation

One ml of the serum was taken in centrifuge tube and added 1 ml of the acetonitrile, 1 ml of internal standard was added to the same centrifuge tube, 1 ml of precipitating reagent was also added to the centrifuge tube. Sample was sonicated for the 10 minutes and mechanically shook for 15 minutes. Centrifuged at 13000 RMP for 15 minutes, used the supernatant solution, and filtered through 0.45Um Acrodisc syringe filter, directly in to HPLC amber vial for HPLC analysis (Ramesh and Balasubramanian,1999; Mahmoud *et al*., 2011).

STATISTICAL ANALYSIS

All results were expressed as mean $\pm$ SD for the indicated number of experiments. Statistical analysis was performed by SPSS software version 22.0. The data was analyzed using one-way analysis of variance .The p value less than 0.05 was considered as significant.

RESULTS

FOLLICLE STIMULATING HORMONE (FSH)

At 3 week mean serum FSH concentration in male rats at low dose was 42.03±1.77 (IU/L), at medium dose was 54.37±7.18 (IU/L) and at high dose was 62.89± 3.11 (IU/L) while the FSH concentration in control rats was 46.15±3.05 (IU/L). The FSH level in treated males at low dose was statistically insignificant (P>.005) when compared with control group at 3 weeks .However , the FSH level in other two groups received medium and high dosed found statistically significant (P<.001) when compared to untreated males of this group (Table-1).

At 6 week mean serum FSH concentration in male rats at low dose was 50.58±1.12 (IU/L), at medium dose was 66.88±2.48 (IU/L) and at high dose was 68.96±1.75 (IU/L) while the FSH concentration in control rats was 46.67±3.34 (IU/L). The FSH level in treated males at low dose was statistically significant when compared with control at 6 weeks (p<0.005). The groups received medium and high dose of neem oil were statistically highly significant (P<.001) when compared to control. (Table-1)

At 12 week mean serum FSH Concentration in male rats at low dose was 70.32±1.15 (IU/L), at medium dose was 78.74±2.24(IU/L) and at high dose was 90.33±1.59 (IU/L) while the FSH concentration in control rats was 44.92±3.40 (IU/L). The FSH level in treated males at all doses was statistically highly significant (P<.001) when compared to control of this group (Table-1)

At 24 week mean serum FSH Concentration in male rats at low dose was 70.28±2.55 (IU/L), at medium dose was 78.85±4.87 (IU/L) and at high dose was 90.52±3.27 (IU/L) while the FSH concentration in control rats was 45.56±3.48 (IU/L). The FSH level in treated males was statistically highly significant (P<.001) when compared to control group (Table 1)

Table 1.Comparison of Serum Concentrations of Follicle Stimulating Hormone (IU/L) In Treated and Control Male Rats.

WEEKS	Low Dose (Mean± SD)	Medium Dose (Mean± SD)	High Dose (Mean± SD)	Control (Mean± SD)
3	42.03±1.77	54.37± 7.18	62.89±3.11	46.15±3.05
6	50.58±1.12	66.88±2.48	68.96±1.75	46.67±3.34
12	70.32±1.15	78.74±2.24	90.33±1.59	44.92±3.40
24	70.28±2.55	78.85±4.87	90.52±3.27	45.56±3.48

COMPARISION OF CONTROL GROUP WITH TREATED GROUPS

3 WEEK	Low Dose	P>0.05	Non Significant
	Medium Dose	P<0.05	Significant
	High Dose	P<0.01	Highly Significant
6 WEEK	Low Dose	P<0.05	Significant
	Medium Dose	P<0.01	Highly Significant
	High Dose	P<0.01	Highly Significant
12 WEEK	Low Dose	P<0.01	Highly Significant
	Medium Dose	P<0.01	Highly Significant
	High Dose	P<0.01	Highly Significant
24 WEEK	Low Dose	P<0.01	Highly Significant
	Medium Dose	P<0.01	Highly Significant
	High Dose	P<0.01	Highly Significant

LUTEINIZING HORMONE (LH)

At 3 week mean serum LH concentration in male rats at low dose was 1.30±0.03 (IU/L), at medium dose was 1.32± 0.06 (IU/L) and at high dose was 1.31±0.12 (IU/L) while the LH concentration in control rats was 1.32± 0.03 (IU/L). The LH level in treated males statistically non significant (P> .05) when compared to control males of this group (Table 2).

At 6 week mean serum LH concentration in male rats at low dose was 1.31±0.03 (IU/L), at medium dose was 1.31±0.08 (IU/L) and at high dose was 1.36±0.08 (IU/L) while the LH concentration in control rats was 1.33± .04 (IU/L). The LH level in treated males statistically non significant (P> .05) when compared to control males of this group (Table 2).

At 12 week mean serum LH concentration in male rats at low dose was 1.33±0.06 (IU/L), at medium dose was 1.35±0.07 (IU/L) and at high dose was 1.36±0.04 (IU/L) while the LH concentration in control rats was 1.35± 0.04 (IU/L). The LH level in treated males statistically non significant (P> .05) when compared to control males rats of this group (Table 2).

At 24 week mean serum LH concentration in male rats at low dose was 1.32±0.02 (IU/L), at medium dose was 1.30±0.04 (IU/L) and at high dose was 1.30±0.08 (IU/L while the LH concentration in control rats was 1.33±0.03 (IU/L). The LH level in treated males statistically non significant (P> .05) when compared to control males of this group (Table 2)

Table 2. Comparison of Means of Serum Levels of Luteinizing Hormone (IU/L) In Treated and Control Male Rats

WEEKS	Low Dose (Mean± SD)	Medium Dose (Mean+ SD)	High Dose (Mean+ SD)	Control (Mean+ SD)
3	1.30±0.03	1.32± 0.06	1.31±0.12	1.32±0.03
6	1.31±0.03	1.31±0.08	1.36±0.08	1.33±0.04
12	1.33±0.06	1.35±0.07	1.36±0.04	1.35±0.04
24	1.32±0.02	1.30±0.04	1.30±0.08	1.33±0.03

COMPARISION OF CONTROL GROUP WITH TREATED GROUPS

3 WEEK	Low Dose	P>0.05	Non Significant
	Medium Dose	P>0.05	Non Significant
	High Dose	P>0.05	Non Significant
6 WEEK	Low Dose	P>0.05	Non Significant
	Medium Dose	P>0.05	Non Significant
	High Dose	P>0.05	Non Significant
12 WEEK	Low Dose	P>0.05	Non Significant
	Medium Dose	P>0.05	Non Significant
	High Dose	P>0.05	Non Significant
24 WEEK	Low Dose	P>0.05	Non Significant
	Medium Dose	P>0.05	Non Significant
	High Dose	P>0.05	Non Significant

TESTOSTERONE HORMONE

At 3 week mean serum testosterone concentration in male rats at low dose was 6.98±0.45 (ng/dl), at medium dose was 6.62± 0.47 (ng/dl) and at high dose was 3.14±0.27 (ng/dl) while the testosterone concentration in control rats was 7.36±0.23 (ng/dl). The testosterone level in treated males at low dose was statistically non significant when compared with control at 3 weeks (p>0.005) but treated groups with medium and high dose were statistically significant (P<.001) when compared to control of this group (Table 3).

At 6 week mean serum testosterone concentration in male rats at low dose was 6.11±0.47 (ng/dl), at medium dose was 4.51±0.46 (ng/dl) and at high dose was 2.31±0.20 (ng/dl) while the testosterone concentration in control rats was 7.47±0.40 (ng/dl). The testosterone level in treated males at all doses were found statistically significant when compared with control at 6 weeks (p<0.005) (Table 3).

At 12 week mean serum testosterone concentration in male rats at low dose was 4.28±0.42 (ng/dl), at medium dose was 2.69±0.19 (ng/dl) and at high dose was 1.86±0.11 (ng/dl) while the testosterone concentration in control rats was 7.40±0.50 (ng/dl). The testosterone level in treated males at all doses were found statistically highly significant when compared with control at 12 weeks (p<0.001) (Table 3).

At 24 week mean serum testosterone concentration in male rats at low dose was 4.05±0.11 (ngdl), at medium dose was 2.22±0.20 (ng/dl) and at high dose was 1.79±0.16 (ng/dl) while the testosterone concentration in control

rats was 7.27±0.18 (ng/dl). The testosterone level in treated males at all doses were found statistically highly significant when compared with control at 24 weeks ($p<0.001$) (Table 3).

Table 3. Comparison of Serum Concentrations of Testosterone (ng/dl) in Treated and Control Male Rats.

WEEKS	Low Dose (Mean± SD)	Medium Dose (Mean± SD)	High Dose (Mean± SD)	Control (Mean± SD)
3	6.98±0.45	6.62± 0.47	3.14±0.27	7.36±0.23
6	6.11±0.47	4.51±0.46	2.31±0.20	7.47±0.40
12	4.28±0.42	2.69±0.19	1.86±0.11	7.40±0.50
24	4.05±0.11	2.22±0.20	1.79±0.16	7.27±0.18

COMPARISION OF CONTROL GROUP WITH TREATED GROUPS

3 WEEK	Low Dose	P>0.05	Non Significant
	Medium Dose	P<0.05	Significant
	High Dose	P<0.01	Significant
6 WEEK	Low Dose	P<0.05	Significant
	Medium Dose	P<0.01	Highly Significant
	High Dose	P<0.01	Highly Significant
12 WEEK	Low Dose	P<0.01	Highly Significant
	Medium Dose	P<0.01	Highly Significant
	High Dose	P<0.01	Highly Significant
24 WEEK	Low Dose	P<0.01	Highly Significant
	Medium Dose	P<0.01	Highly Significant
	High Dose	P<0.01	Highly Significant

MICROSCOPIC OBSERVATIONS

HISTOLOGY OF TESTIS

The sections of the testes from control groups (D) showed normal histological features with the cross section of the convoluted seminiferous tubules showing stratified epithelium consisting of two distinct populations of cells; the spermatogenic cells and the sertoli cells. The leydig cells within the supporting tissues in the interstitial spaces between the tubules were all visible (Fig.2 and 3).

There were no apparent morphological differences in the gonads between the treated groups A (low dose) and B (medium dose)and the control (D) animals on the sub acute exposure. Animals exposed to the high dose for any of the duration have shown a reduction in seminiferous tubular diameter. Some of the tubules were showing normal spermatogenesis from spermatogonia to spermatocyts, to round spermatids and to elongated spermatids (Fig.2 and 3) while an impaired / hypo-spermatogenesis was noted in nearly $1/3^{rd}$ of the sections (Fig.4). The seminiferous tubules contained mainly spermatogonia and primary spermatocyts however, no evidence of degeneration and resorption or sloughed cell and vacuoles was seen. The Sertoli cells and Leydig cells were normal in treated groups A and B at all durations whereas in animals treated at high dose (group C) showed hyperplasia in sections with impaired spermatogenesis (Fig.5). Tunica propria was intact with normal thickness. There was no leukocytic infiltration in the interstitium in animals of any group.

MEAN SEMINIFEROUS TUBULAR DIAMETER (MSTD)

At 3 week mean (MSTD) of rats at low dose was 331.67±2.08 (µm), at medium dose 325.00± 1.73 (µm) and at high dose was 314.00±1.00 (µm) while the MSTD in control rats was 337.33±1.52 (µm). The MSTD in treated males at low dose was statistically insignificant ($p>0.05$) when compared to control group. The MSTD of groups treated with medium and high dosed were statistically highly significant ($P<.001$) when compared to control animals of this group (Table 4).

Table 4. Comparison of Mean of Seminiferous Tubular Diameter (µm) in Treated and Control Male Rats.

WEEKS	Low Dose (Mean± SD)	Medium Dose (Mean± SD)	High Dose (Mean± SD)	Control (Mean± SD)
3	331.67±2.08	325.00± 1.73	314.00±1.00	337.33±1.52
6	325.00±4.00	313.33±2.08	297.33±5.51	336.33±2.08
12	311.67±2.08	302.67±7.57	270.00±4.00	337.67±1.52
24	313.00±2.00	303.00±4.36	268.00±3.60	336.33±2.51

COMPARISION OF CONTROL GROUP WITH TREATED GROUPS

3 WEEK	Low Dose	P>0.05	Non Significant
	Medium Dose	P<0.05	Significant
	High Dose	P<0.01	Highly Significant
6 WEEK	Low Dose	P<0.05	Significant
	Medium Dose	P<0.01	Highly Significant
	High Dose	P<0.01	Highly Significant
12 WEEK	Low Dose	P<0.01	Highly Significant
	Medium Dose	P<0.01	Highly Significant
	High Dose	P<0.01	Highly Significant
24 WEEK	Low Dose	P<0.01	Highly Significant
	Medium Dose	P<0.01	Highly Significant
	High Dose	P<0.01	Highly Significant

Table 5.Comparison of Mean Weights (g) in Treated and Control Rats.

	N	Weight (g)Mean ± SD	p-value
Base line			
Low Dose (A)	24	227.0±28.4	
Medium Dose (B)	24	200.1±22.7	0.09
High Dose (C)	24	185.4±39.4	
Control (D)	24	217.3±58.1	
At 3 week			
Low Dose (A)	24	236.0±38.7	
Medium Dose (B)	24	205.8±39.6	0.09
High Dose (C)	24	191.4±49.0	
Control (D)	24	225.4±78.3	
At 6 week			
Low Dose (A)	18	252.0±43.0	
Medium Dose (B)	18	227.2±42.5	0.16
High Dose (C)	18	203.1±45.0	
Control (D)	18	261.3±54.1	
At 12 week			
Low Dose (A)	12	264.3±48.3	
Medium Dose (B)	12	255.8±43.9	0.06
High Dose (C)	12	214.1±43.7	
Control (D)	12	290.5±59.6	
At 24 week			
Low Dose (A)	6	292.8±72.1	
Medium Dose (B)	6	290.0±65.8	0.562
High Dose (C)	6	260.8±42.5	
Control (D)	6	327.8±74.2	

At 6 week mean seminiferous tubular diameter of rats at low dose was 331.67±2.08 (µm), at medium dose 325.00± 1.73 (µm) and at high dose was 314.00±1.00 (µm) while the MSTD in control rats was 337.33±1.52 (µm). The MSTD in all treated males was statistically significant ($p<0.05$) when compared to control group (Table4).

At 12 week mean seminiferous tubular diameter of rats at low dose was 325.00±4.00 (µm), at medium dose 313.33±2.08 (µm) and at high dose was 297.33±5.51 (µm) while the MSTD in control rats was 336.33±2.08 (µm). The MSTD in all treated males was statistically significant ($p<0.05$) when compared to control group (Table 4).

At 24 week mean seminiferous tubular diameter rats at low dose was 311.67±2.08 (µm), at medium dose 302.67±7.57 (µm) and at high dose was 270.00±4.00 (µm) while the MSTD in control rats was 337.67±1.52 (µm). The MSTD in all treated males was statistically significant ($p<0.05$) when compared to control group (Table 4).

Table 6. Comparison of Mean Testis Weights (g) In Treated and Control Rats.

		N	Weight (g) Mean ± SD	p-value
Low Dose	(A)	12	4.3±0.5	
Medium Dose	(B)	12	4.0±0.5	0.4
High Dose	(C)	12	3.8±0.6	
Control	(D)	12	4.2±0.6	

DISCUSSION

Mammalian organ systems work in great harmony, they are interdependent structurally and functionally therefore to obtain the full therapeutic benefit of a pharmacologic agent maximum natural interplay amongst them is needed.

According to the findings of our study the weights of animals (Table 5) and testis (Table 6) did not show any significant change when compared to their controls in any of the group in this study.

One very significant finding recorded in this study is the suppression of the male gonads on sub-acute, sub-chronic and chronic exposure after oral administration of neem oil. Sub-acute suppression was seen at high dose while the sub-chronic and chronic suppressions were recorded at all doses in this study. Seminiferous tubules are associated with spermatogenesis (production of the spermatozoa from primitive germinal cells). These cells constitute the lining of the tubule and as they mature, move to next stage and towards the lumen and finally they are released into the lumen as spermatozoa (Hall, 2010). Seminiferous tubular diameters were significantly reduced in the treated animals in this study. Spermatogenesis is androgen dependent (Moore *et al*., 2011) . Testosterone levels estimated in the low and medium doses treated animals at the end of three weeks in this study have not shown any significant difference with their controls while the high dose treated animals at the end of three weeks and all the other treated animals of any of the group (sub-acute, sub-chronic & chronic) have shown a significant decline in the testosterone levels (Table 3). Release of testosterone from Leydig cells of testes is dependent on LH which comes from Pituitary gonadotrophs (Young *et al.,* 2006). According to our findings LH levels were not affected in any of the animal treated at any of the dose or duration. Decline in testosterone level in the presence of unaffected LH is surprising since the quantity of testosterone available increases approximately in direct proportion to the amount of LH available. LH releases from pituitary in a pulstile manner every 1-3 hours (Hall, 2010) . May be the time of sample collection in our study did not match with this may be a possible explanation for insignificant levels of LH or alternately some local gonadal inhibition of the Leydig cells may possibly be involved. Sertoli cells secrete an androgen-binding protein, which transports testosterone and dihydro-testosterone to the seminiferous tubule. Production of this binding protein is believed to be dependent on the FSH (Young *et al.,* 2006). Serum levels of FSH, when compared to their controls were found significantly raised in this study. Lower testosterone levels may

have resulted in an increase in pituitary FSH through feedback in an attempt to produce more androgen-binding protein for transport of androgens. This finding of present study is in agreement with the findings of Parshad *et. al.*, (1994) who have reported a significant decrease in serum testosterone level and Shaikh *et al.* (2009-b) who have reported a decline in the levels of testosterone and LH.

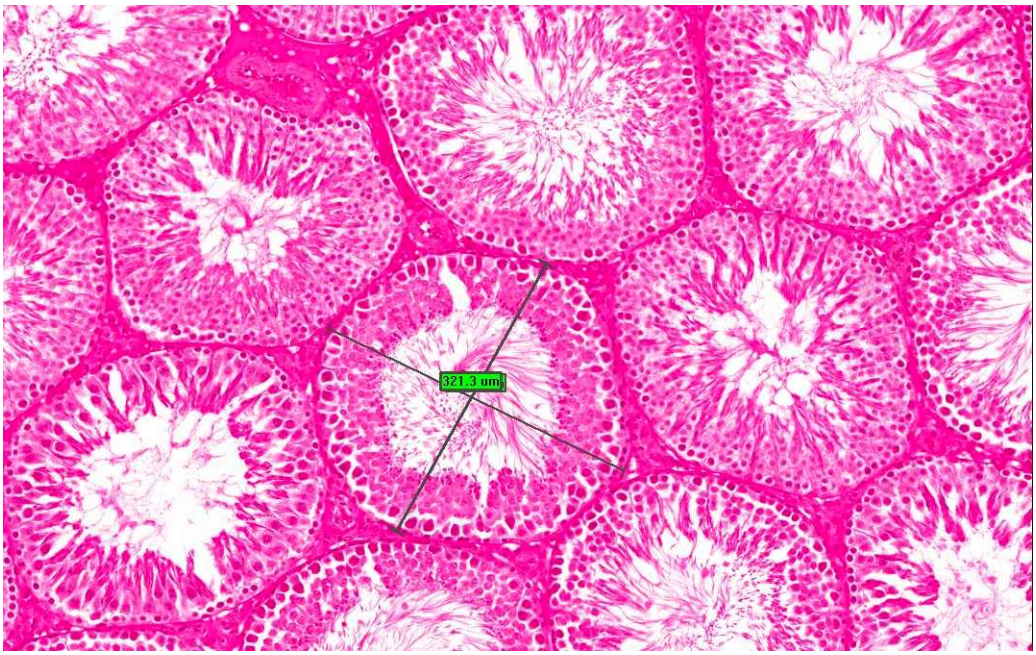

Fig-1. Seminiferous Tubular diameter. Magnification 6X (H & E)

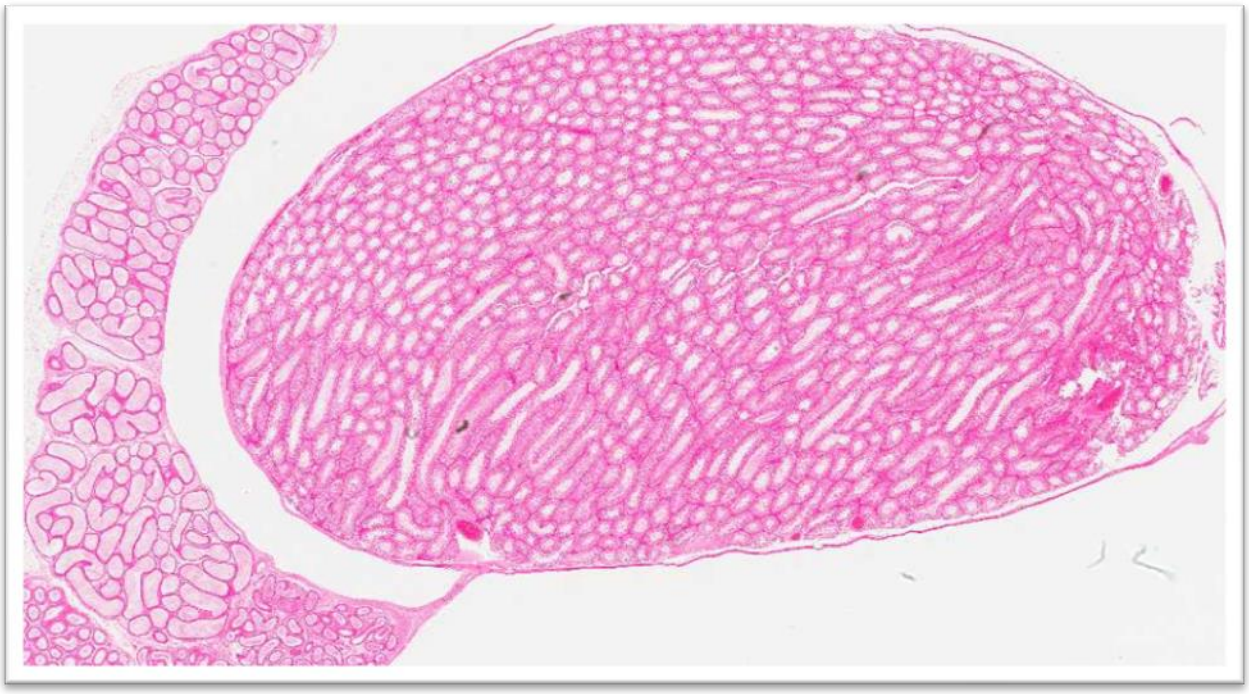

Fig-2.Photomicrograph of a whole mount 5 µm thick horizontal section from a control male rat showing normal Testis and Epididymis. Original magnification 5X (H & E)

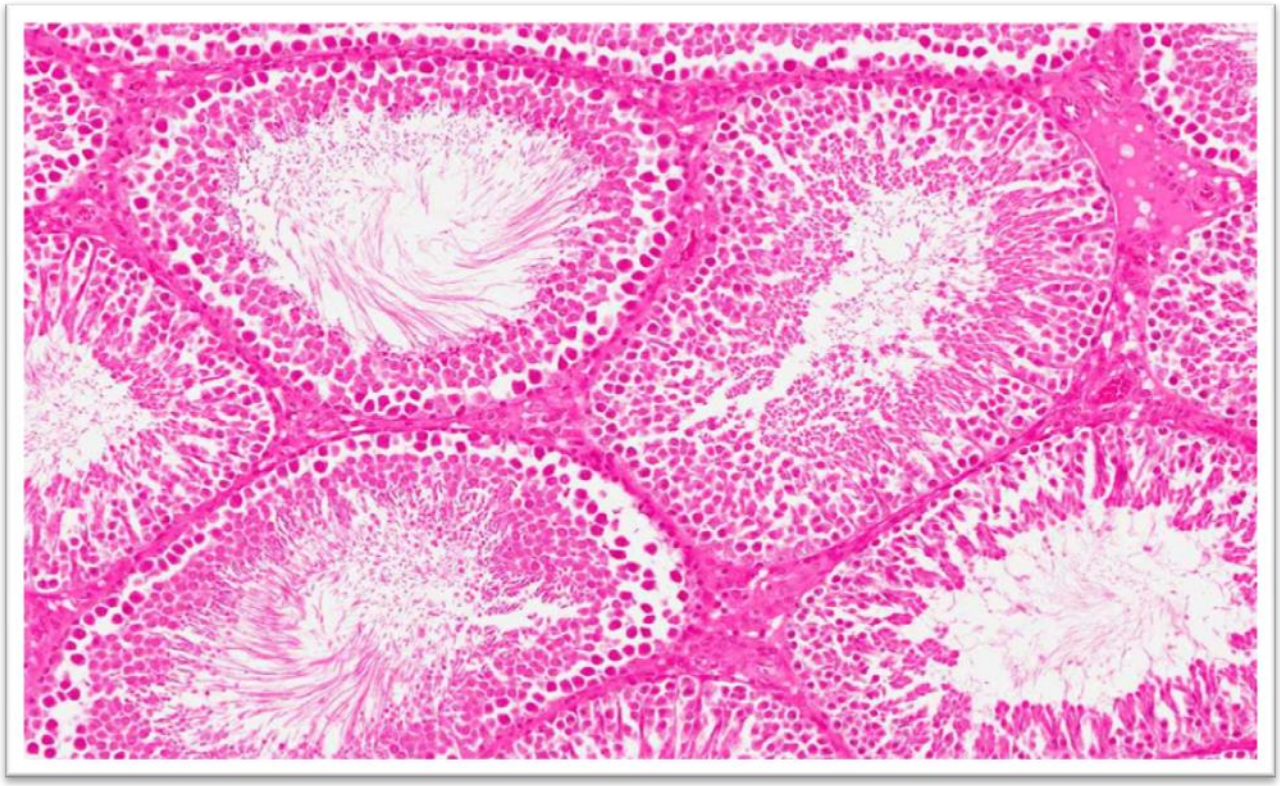

Fig-3. Photomicrograph, of a 5 µm thick horizontal section of the testis from a control group male rat showing seminiferous tubules & interstitial spaces with connective tissue and cells. Regular rounded lumen, stratified lining epithelium of the tubules and spermatozoa in the tubular lumens can be seen. Original magnification 20X (H & E).

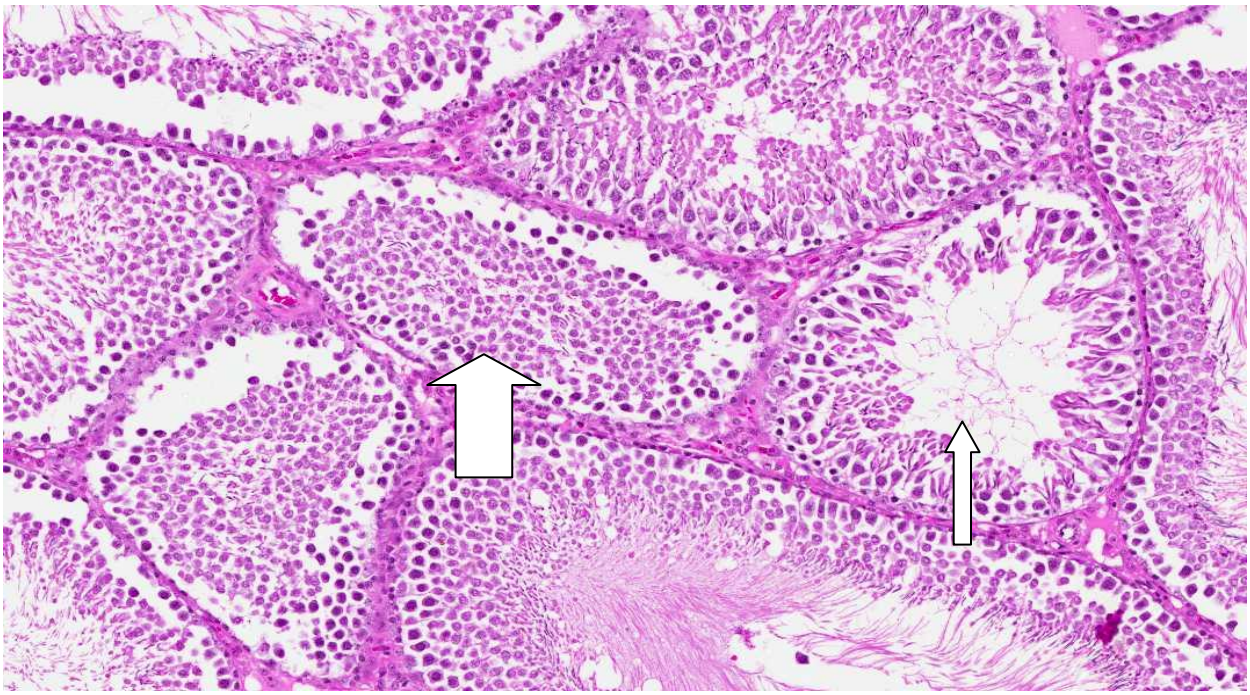

Fig-4. Photomicrograph, of a 5 µm thick horizontal section of the testis from a male rat treated with high dose at 24 weeks showing mild hypoplasia (stratification) with presence of earlier stages of spermatogenic cells. Mild to moderate decrease in the luminal spermatozoa (narrow arrow) is evident some of the tubules have no spermatozoa in lumen (wide arrow). Original magnification 20X (H & E).

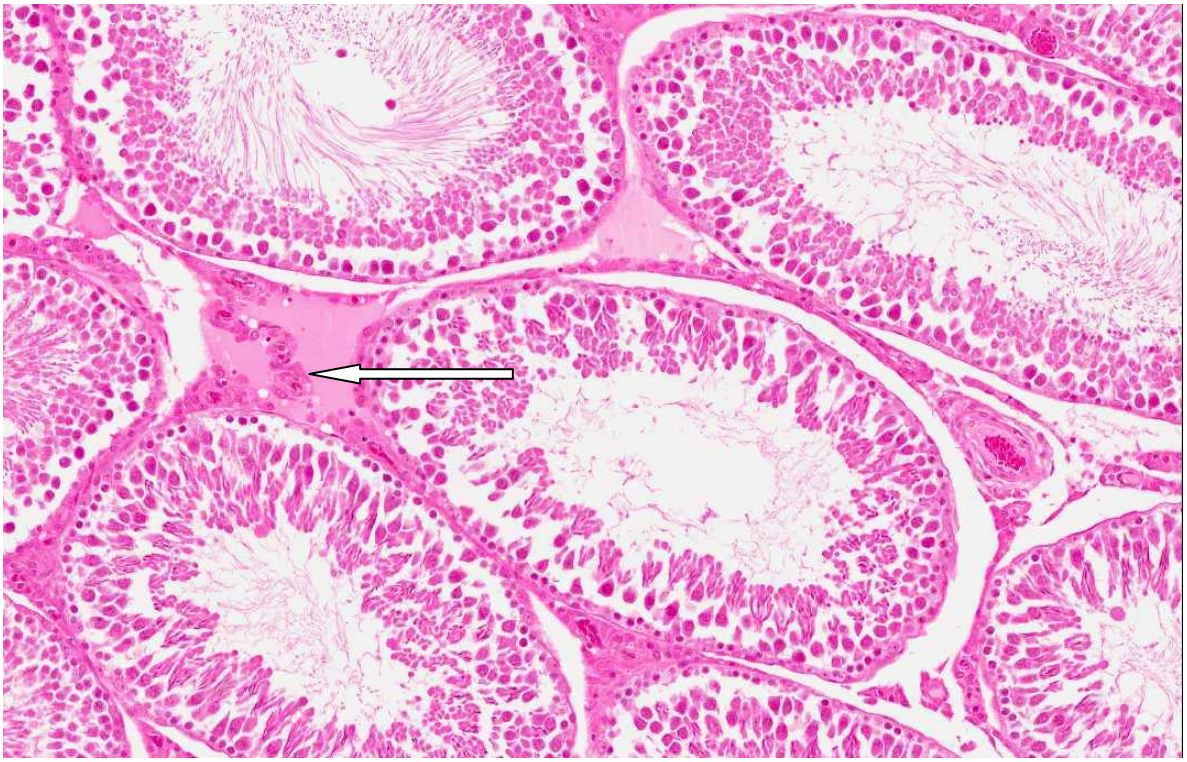

Fig-5. Photomicrograph of a 5 µm thick horizontal section of the testis from a male rat treated with high dose at 24 weeks showing seminiferous tubules & interstitial spaces with connective tissue and prominent Leydig cells (narrow arrow). Original magnification 40 X (H & E).

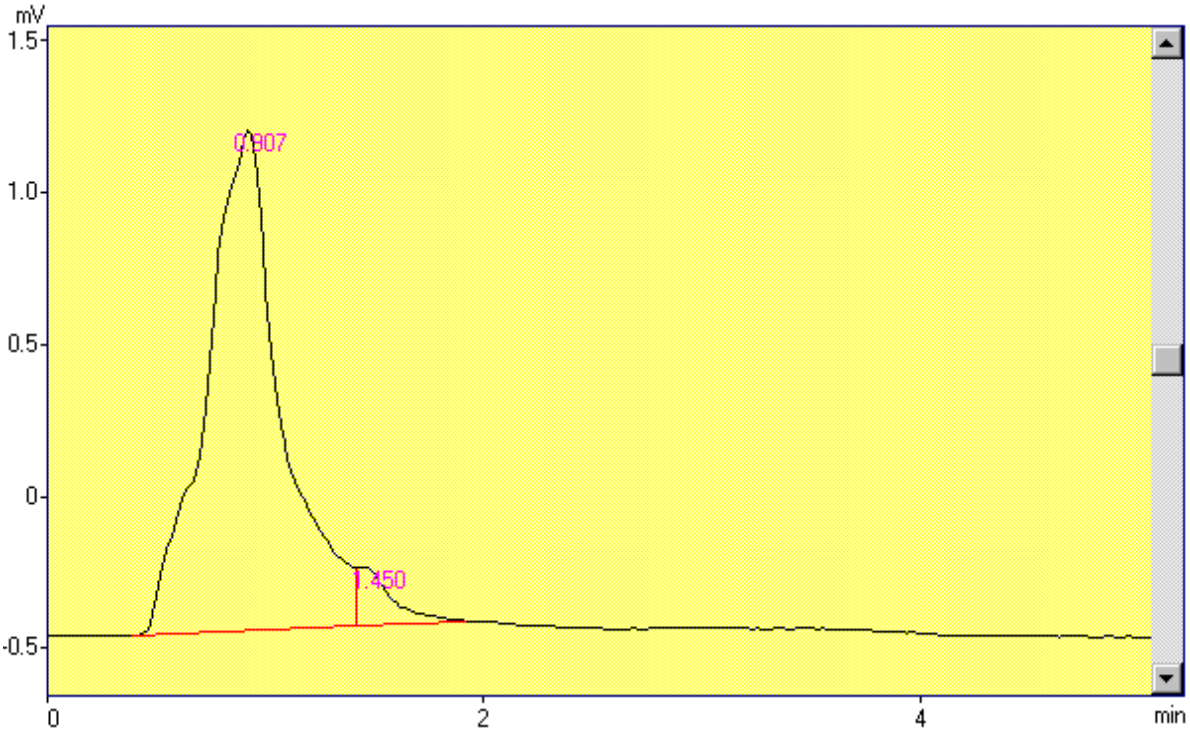

Plate 1. Peanut oil extracted with acetonitrile, chromatograms of control group D2 at 24 week.

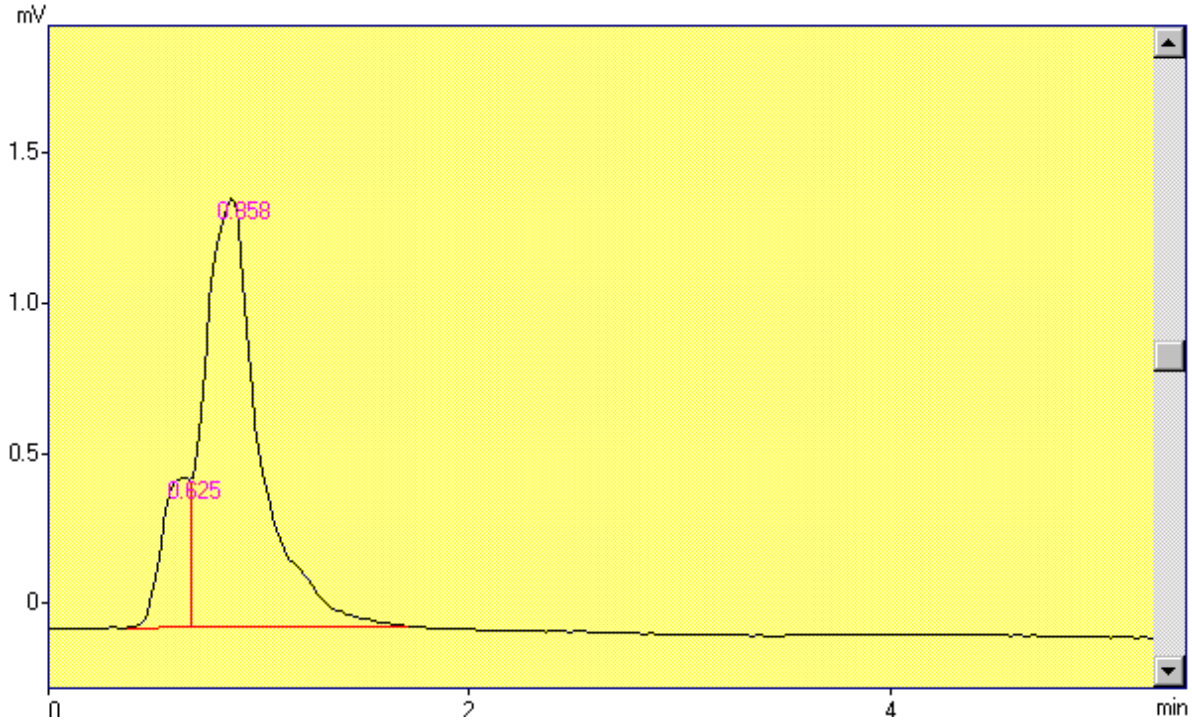

Plate 2. Neem oil extracted with acetonitrile, chromatograms of group C4 (High Dose at 24 week).

Peaks report (Plate 1)

Peak No	TIME(min)	AREA	HEIGHT	CONC
1	0.907	39424	1652	95.1512
2	1.450	2009	192	4.8488
TOTAL		41433	1843	100.0000

Peaks report (Plate 2)

Peak No	TIME(min)	AREA	HEIGHT	CONC	NAME
1	0.625	4616	498	15.2772	
2	0.858	25599	1430	84.7228	
TOTAL		30215	1928	100.0000	

These hormonal findings are very much supported by the histological section of the testes which have shown decrease in the diameter and stratification of the lining epithelium of the seminiferous tubules (Table 4 and Fig. 3-5). Some of the tubules did not show any spermatozoa in the lumen (Fig. 5). Though this effect was visible on sub-acute exposure but maximum effects were seen in the animals treated for 12 and twenty four weeks (sub chronic &chronic exposure). HPLC done using serum also supports our findings (Plates 1&2). Microscopic sections from the animals treated at high dose at 24 weeks (chronic exposure) have shown a little hyperplasia of the Leydig cells (Fig. 4). The only explanation to this may be that elevated FSH levels for such a time may have resulted in the release of the higher amount of the factor from the sertoli cells which regulate the function of the leydig and peritubular cells (Moore *et al*., 2011). This further supports our point that instead of gonadal-pituitary axis some local gonadal

mechanisms are involved in altering the reproductive hormone profile in male rats. Present findings are partly in agreement with the findings of Shaikh *et al.* (2009-b) who have reported a decline in Testosterone and LH and rise in FSH level while Raji *et al.* (2003) have reported reduced Testosterone and LH with unaffected significantly FSH level.

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