

Androgenic and Anabolic Activities of Some Newly Synthesized Epiandrosterone and Progesterone Derivatives.

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Abstract

Derivatives of epiandrosterone and progesterone were synthesized. The androgenic and the anabolic activities of some of them were investigated on prepubertal male albino rats of 21 days old by: i- determining the weight gain of the body, levator ani muscle, ventral prostate gland, testis, seminal vesicles, vas deferens and epididymis, ii- estimation of serum luteinizing (LH) hormone, iii- histopathological examination of the testis and ventral prostate glands. The results from this study showed that the presence of an appended substituted 2-aminopyridine ring at the C- 17 of testosterone gave the maximum androgenic activity, whereas the presence of a substituted piperidine ring fused to ring D of 5 α - androstane exhibited the maximum anabolic activity. However, fusion of a pyrazoline moiety with the ring D of 5 α -androstane led to a compound with considerable androgenic and anabolic activity.

Keywords: epiandrosterone, progesterone, androgenic activity, anabolic activity, prepubertal rat, male sex accessory glands, luteinizing hormone, histopathology

Introduction

Androgens are a class of steroids responsible for the primary and secondary sex characteristics of the male. In addition, these steroids have been found to possess potent anabolic promoting properties. The androgens are formed by the Leydig cells of the testis which is regulated by the gonadotropic luteinizing hormone (LH). The latter is secreted by the β - cells of the anterior pituitary gland under the control of the hypothalamic gonadotropin - releasing hormone. LH polypeptide β -chain is biochemically unique and confers the LH biological and immunological specificity ⁽¹⁻³⁾.

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Testosterone is the predominant circulating male sex hormone that is produced mainly by the testis. In addition to its androgenic properties, testosterone also exhibits anabolic characteristics⁽⁴⁾.

More extensive presentation of the topic of androgens as well as the anabolic and androgenic properties evoked by many epiandrosterone and progesterone derivatives are cited in several published treatises⁽¹⁻⁸⁾.

The results issued from these studies encouraged the biological investigation of some newly synthesized epiandrosterone and progesterone derivatives, having different chemical functionalities at rings A, B and C, with the aim to find new compounds exhibiting selective androgenic with minor or no anabolic properties.

Materials and Methods

Materials:

Animals:

Pharmacological tests were conducted on prepubertal male albino rats of 21 days old for the screening and bioassay of androgenic and anabolic activities. Animals were obtained from Animal House Colony of National Research Center, Cairo, Egypt. All animals were allowed free access to water and kept on a constant standard diet.

Drugs and chemicals:

Testosterone (BDH, England) was used in this study. All other chemicals were of analytical grade.

Experimental design:

Groups of immature male albino rats (n=8) of 21 days old received, subcutaneously the individual target compounds as well as testosterone as a reference standard at a total dose level of 0.7 mg/kg according to the following design:

Group (1): received the vehicle (7% tween-80)

Group (2): received the standard reference

Group (3): this group was subdivided into four subgroups, each subgroup received individually one of the newly synthesized compounds.

The dose of testosterone was given as reported by Laurence and Bennett (1982)⁽⁹⁾.

Methods:

Evaluation of the androgenic and anabolic activities:⁽¹⁰⁾

The methods described by Hershberger *et al.* was followed for the investigation of the androgenic as well as the anabolic activities of the synthesized compounds (s-13d), (s-33g), (s-25c) and (s-7h). Groups of prepubertal male albino rats (n=8) of 21 days old, were kept on constant diet and tap water. Each animal was given daily subcutaneous injection of

one of the test compounds as well as testosterone as reference standard at a dose level of 0.1 mg/kg for seven days. On the 8th days (22 to 26 hours after the last injection) the animals were weighed than sacrificed. Blood samples were collected. Dissection of the levator ani muscle, ventral prostate gland, testis, seminal vesicles, vasdeferens and epididymis were carried out and were weighed. Samples of testis and prostate tissues were fixed in Bouin's solution for histopathological examination.

The ratio of the weight gain of the levator ani muscle to the weight gain of the ventral prostate gland was calculated, where the gain in weight of the levator ani muscle indicates the anabolic effect and the gain in weight of the ventral prostate gland shows the androgenic effect of the tested compound.

Serum preparation for hormone assay:

The blood was collected from rats, serum was separated by centrifugation at 200 r.p.m. for 10 minutes. The serum was stored frozen at -20°C until used for luteinizing hormone estimation.

Evaluation of serum luteinizing (LH) hormone:^(11,12)

Coat-A- count LH- IRMA is an immnuoradiometric assay designed for the quantitative measurement of luteinizing hormone. The assay based on monoclonal and polyclonal anti- LH antibodies: I¹²⁵-labeled anti - LH polyclonal antibodies in liquid phase, and monoclonal anti- LH antibodies immobilized to the wall of a polystyrene tube. In the procedure:

- LH captured between monoclonal anti- LH antibodies immobilized on the inside surface of the polystyrene tube and the radio- labeled polyclonal anti- LH tracer.

- Unbound I¹²⁵- labeled anti- LH antibodies were removed by decanting the reaction mixture and washing the tube, this reduces nonspecific binding to a very low level, and ensures excellent low- end precision.

- The LH concentration is directly proportional to the radioactivity present in the tube after the wash step. The radioactivity is counted using an automatic gamma counter (Riastar - Packard, Canberro Company), which is a sodium iodide solid centillator counter for hormonal assay. The concentration of LH in the sample is obtained by comparing the sample counts per- minute with those obtained for the set of calibrators provided.

Histopathological technique:

At the end of the experiment (8 days) samples of rats' testis were fixed in Bouin's solution. The tissues were blocked into paraffin wax. However, prostatic specimens were fixed in 10% formaldehyde. Sections of 5µm thickness were obtained and stained with hematoxyline and eosin (H&E)⁽¹³⁾ and Masson trichrome stain⁽¹⁴⁾.

Several methods have been proposed for quantitatively assessing the germ cell elements in the testicular biopsies¹⁵. Two methods were applied in this study:

A- Score of 1 to 10, each tubule cross section was examined according to the following criteria

10: Complete spermatogenesis and perfect tubules

9: Many spermatozoa present but disorganized spermatogenesis.

8: Only few spermatozoa present.

7: No spermatozoa but many spermatids present.

6: Only few spermatids present.

5: No spermatozoa or spermatids present but many spermatocytes present.

4: Only few spermatocytes present.

3: Only spermatogonia present.

2: No germ cells present.

1: No germ cells or Sertoli cells present.

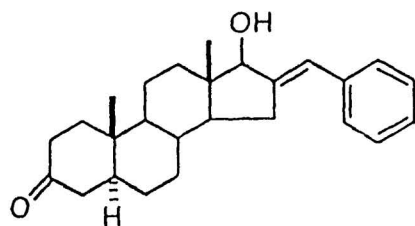
The mean score count should be at least 8.9 with an average of 9.38 and 60% or more of the tubules should score at 10¹⁶.

B- Estimation of the degree of spermatogenesis in testicular biopsy by establishing the germ cell: Sertoli cell ratio which is relatively constant at about 13:1. An average of twelve Sertoli cells per tubular cross section is considered normal, approximately half the germ cell elements should be in the spermatid stage¹⁷.

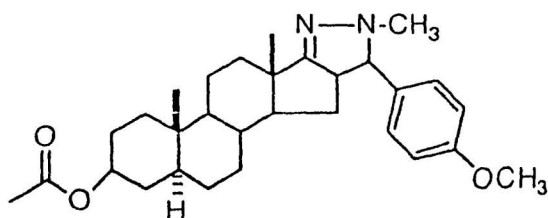
The data collected from different groups of rats were statistically analysed. Statistical analysis for significance difference between the mean value were performed by means of Students't⁽¹⁸⁾ - test. P values lower than 0.05 were considered to be significant.

Results and Discussion

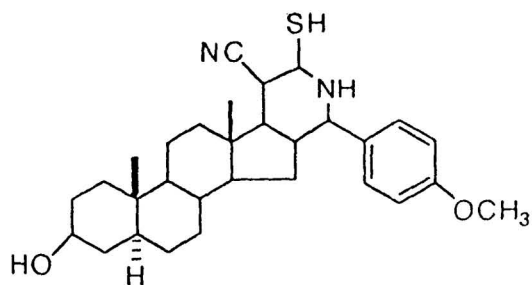
The present work was undertaken to test the androgenic and anabolic activities of compounds (s-13d), (s-33g), (s-25c) and (s-7h)⁽¹⁹⁾ in prepubertal male albino rats.



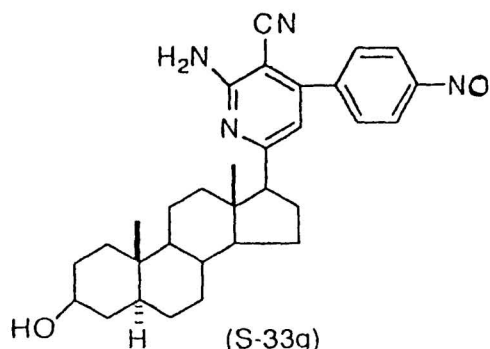
(S-7h)



(S-13d)



(S-25c)



(S-33g)

From this study it is deduced that, these compounds, at a total dose level of 0.7 mg/kg, exhibit significant ($p \leq 0.05$) androgenic as well as anabolic effects which are illustrated through the following investigated parameters: -

A) Androgenic activity (Table 1):

A significant ($p \leq 0.05$) effect was observed on the testis and the male secondary sex organ weights of prepubertal rats following treatment with the tested compounds (s-13d, s-33g, s-25c and s-7h).

Subcutaneous administration of compound (s-13d) exhibited a significant increase in weights of epididymis, seminal vesicles and ventral prostate by 134, 47 and 205% respectively, compared to control value.

Regarding compound (s-33g), it exerted a significant increase in weights of testis, epididymis, seminal vesicles, vasdeferens and ventral prostate by 100, 189, 58, 77 and 230% respectively, compared to control value. On the other hand, there was no significant increase above the control value in weight of testis, epididymis, seminal vesicles as well as vasdeferens of prepubertal rats injected (s.c.) with compound (s-25c) at a total dose level of 0.7 mg/kg. At the same time, compound (s-25c) showed a significant ($p \leq 0.05$) increase in weight of ventral prostate by 90% with respect to control value. In addition, the newly synthesized compound (s-7h) produced a significant increase in weight of epididymis, seminal vesicles and ventral prostate with respect to control value by 117, 11 and 60% respectively.

Serum luteinizing hormone level (Table 2, Fig. a):

Systemic administration of testosterone at a total dose level of 0.7 mg/kg to the prepubertal male rats was accompanied by a significant ($p \leq 0.05$) decrease in LH level by 47% with respect to control value. Furthermore, the results present in table (2) and Fig. (a) showed that compound (s-33g) at the same dose level induced a significant ($p \leq 0.05$) inhibition in serum LH level by 38% with respect to control value. However, no significant effect was detected on serum LH level following subcutaneous administration of compound (s-13d), (s-25c) or (s-7h).

B) Anabolic activity:-

1- Body weight gain: (Table 1)

Administration of compounds (s-13d) and (s-33g) each at a total dose level of 0.7 mg/kg did not significantly increase body weight of prepubertal male rats above control value. However, compounds (s-25c) and (s-7h) at the same dose level produced a significant ($p \leq 0.05$) increase in total body weight: (55.11 and 59.89g respectively) compared to control

value (47.81g). Moreover, results in table (1) showed that the percent increase in body weight of rats given compound (s-25c, 0.7mg/kg) was 15.269 and 12.676 relative to control and testosterone value respectively; while body weight gains in prepubertal rats given compound (s-7h) at the same dose level was increased by 25 and 22 % with respect to control and testosterone value respectively. Thus compound (s-7h) displays a pronounced increase in body weight of prepubertal rats than compound (s-25c).

2- Levator ani muscle weight: (Table 1)

Subcutaneous injection of compounds (s-13d), (s-33g), (s-25c) and (s-7h) at a total dose level of 0.7 mg/kg produced a significant increase in weight of levator ani muscle by 203, 148, 248 and 296% respectively, compared to control value. Furthermore, a significant increase was detected in weight of levator ani muscle of prepubertal rats treated with the tested compounds, by 165, 116, 204 and 246% respectively, relative to testosterone value.

In conclusion, it could be deduced from the results shown in table 1 and 2 as well as fig. (a), that compound (s-33g) at a dose level of 0.7 mg/kg exhibited the most potent androgenic activity with least anabolic effect. Whereas, compound (s-7h) evoked a pronounced anabolic effect with least androgenic property.

Histopathological examination

The histopathological examination of the rats' testis injected with compound (s-33g) showed regular distribution of seminiferous tubules, no thickening of basement membrane with normal thickness of the tunica propria. There was average proportions of spermatogonia, spermatocytes and spermatids. Spermatozoa and Sertoli cells showed relative increment. The interstitium showed average number of Leydig cells with no inflammatory cells and no fibrous tissue deposition. There was hyperplasia of the mesothelial cells of the tunica albuginea with no adhesions. Sertoli cell proliferation was moderately greater in treated rats than in control one Fig. (1&2). The score at 10 was 35%; at 9 was 60% and at 8 was 5% of the tubules. Regarding compound (s-13d), seminiferous tubules showed preserved spermatogenesis, there was good layering with increased tubular size. Many spermatozoa were present but with disorganized spermatogenesis. The score at 10 was 25%; at 9 was 65%; at 8 was 5% and at 7 was 5%, of the tubules. Sertoli cell proliferation was mildly greater than that of control Fig. (3&4). Furthermore, compound (s-25c) induced preserved elongated seminiferous tubules with good layering, although there was no mature sperms. Focal areas of hydropic degeneration were evident in some tubules. Leydig cells were usually normal but on occasion were found to

be reduced in size and number. Sertoli cell proliferation was moderately increased. The score at 10 was 10%; at 9 it was 10%; at 8 was 15%; at 7 was 60% and 6 at was 5% of the tubules Fig. (5&6). On the other hand, compound (s-7h) showed a decrease in number and size of seminiferous tubules with evident atrophy and increased thickness of the tunica propria. They showed homogeneous acidophilic secretion and hydropic degeneration of the lining germ cell. There was halt of the maturation sequence at the stage of secondary spermatocytes with sloughing of some. No spermatids or spermatozoa were present despite the presence of abundant cells in division. Atrophy of the interstitium was evident. The tubules were populated by Sertoli cells with thickening of the basement membrane i.e tubular hyalinization, the score at 10 was 2%; at 9 was 3 %; at 8 was 5%; at 7 was 5%; at 6 was 10 %; at 5 was 65%; and at 4 was 10%; of the tubules. The histopathological examination of the rats' prostate injected with compound (s-33g) induced well developed hyperplasia of the glandular component. The glands often contain an inspissated secretion, the nuclei were regular and centrally located. Papillary infoldings were common. A continuous basal cell layer was seen immediately above a well-developed basement membrane (Fig.9). Stromal proliferation in the periductal and interlobular areas of a concentric or an eccentric quality was evident with compounds (s-13d) and (s-25c). It was formed of more smooth muscle and less elastic tissue than the control (Fig.10). On the other hand, the prostate gland was within normal in case of compound (s-7h). Based on the present investigation it could be concluded that, the presence of an appended substituted 2- aminopyridine ring at the C-17 of testosterone gave the maximum androgenic activity, whereas the presence of a substituted piperidine ring fused to ring D of 5 α - androstane exhibited the maximum anabolic activity. However, fusion of a pyrazoline moiety with the ring D of 5 α - androstane lead to a compound with considerable androgenic and anabolic activity.

Table (2): Effect of, (S-13d), (S-33g), (S-25c), and (S-7h) administration on Serum LH hormone level of prepubertal male rats.

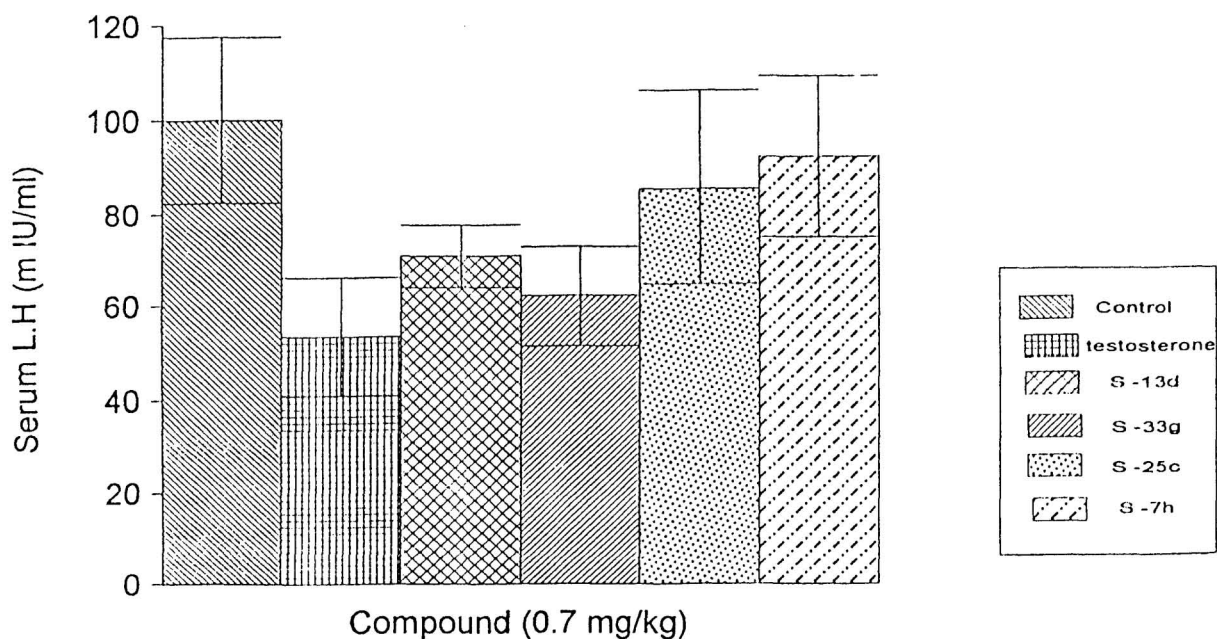
Treatment group	Dose (mg/kg)	Serum LH level (mIU/ml) ^a	
		Absolute value $\pm$ s.e.m	% to control value $\pm$ s.e.m
Control	-	3.941 $\pm$ 0.690	100 $\pm$ 17.508
Testosterone	0.7	2.102 ^b $\pm$ 0.491	53.337 ^b $\pm$ 12.459
S-13d	0.7	2.779 $\pm$ 0.273	70.515 $\pm$ 6.927
S-33g	0.7	2.438 ^b $\pm$ 0.420	61.862 ^b $\pm$ 10.657
S-25c	0.7	3.344 $\pm$ 0.816	84.852 $\pm$ 20.705
S-7h	0.7	3.607 ^c $\pm$ 0.671	91.525 ^c $\pm$ 17.026

a) Data as mean $\pm$ s.e mIU/ml, number of animals in each group (n=8)

b) Significantly different from control value at $p \leq 0.05$

c) Significantly different from testosterone value at $p \leq 0.05$

Fig.(a) Luteinizing hormone (LH) level in serum of prepubertal male rats injected (s.c.) with (0.7 mg/kg) testosterone, S-13d, S-33g, S-25c or S-7h.



***HISTOPATHOLOGICAL EFFECTS OF COMPOUND (S-33g) ON
PREPUBERTAL RATS' TESTIS***

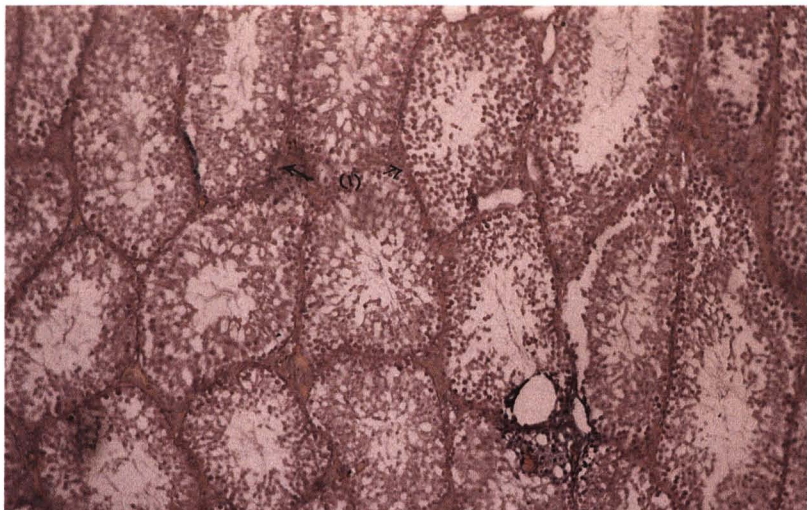


Fig.(1): H & E (X 20)

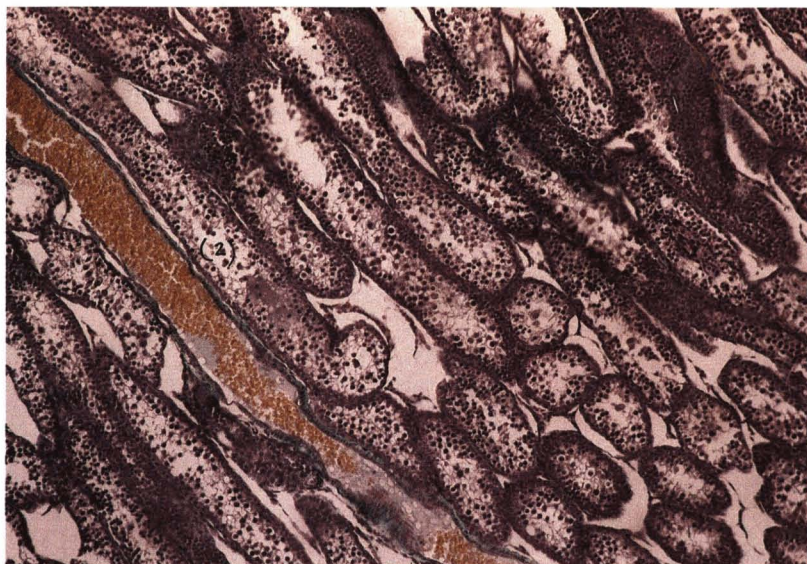


Fig.(2): Masson trichrome (X10)

Compact adhesive tubules (1), alternating with markedly distended ones (2), thick layering with average proportions of spermatogonia, spermatocytes, spermatids and spermatozoa.

***HISTOPATHOLOGICAL EFFECTS OF COMPOUND (S-13d) ON
PREPUBERTAL RATS' TESTIS***

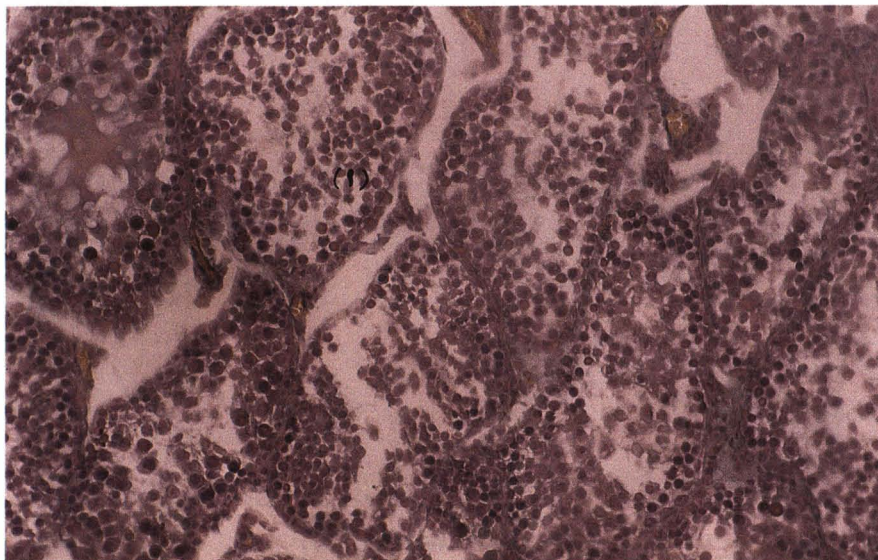


Fig.(3): H & E (X 40)

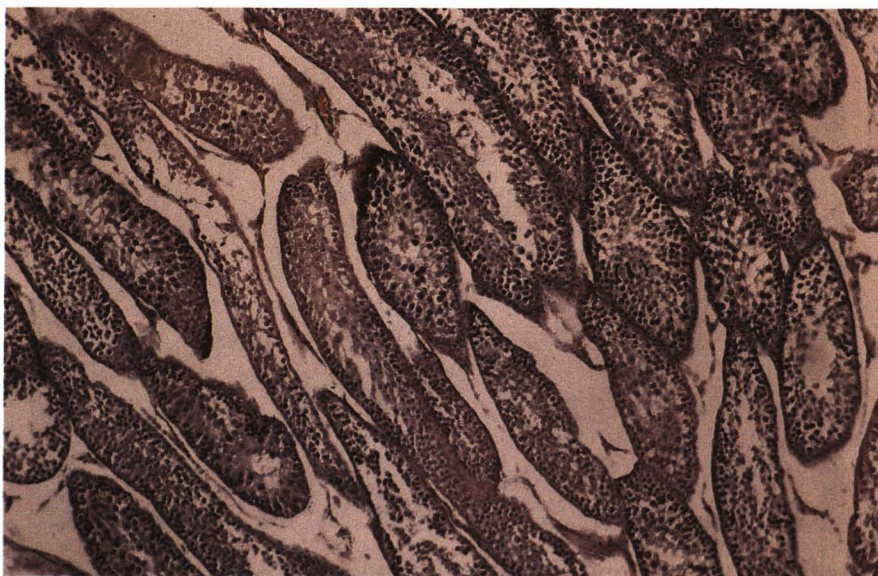


Fig.(4): Masson trichrome (X10)

Preserved disorganized spermatogenesis with good layering (1).

***HISTOPATHOLOGICAL EFFECTS OF COMPOUND (S-25c) ON
PREPUBERTAL RATS' TESTIS***

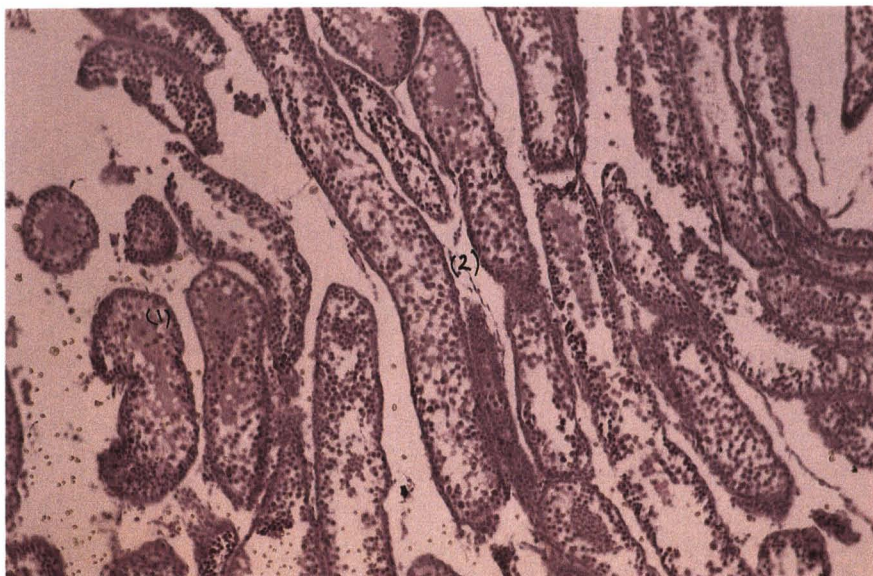


Fig.(5): H & E (X 10)

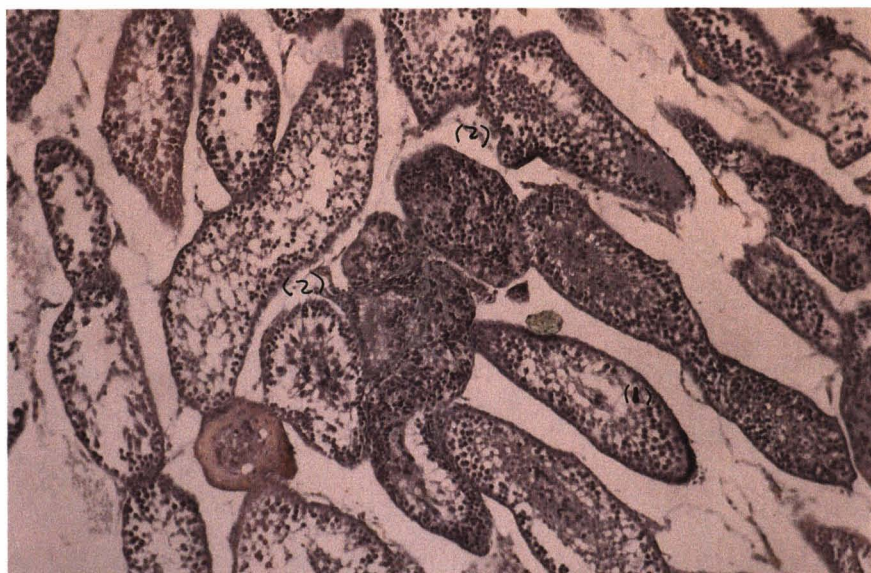


Fig.(6): Masson trichrome (X10)

Preserved elongated seminiferous tubules with focal hydropic degeneration (1). There is a decrease in the interstitial tissue (2).

***HISTOPATHOLOGICAL EFFECTS OF COMPOUND (S-7h) ON
PREPUBERTAL RATS' TESTIS***

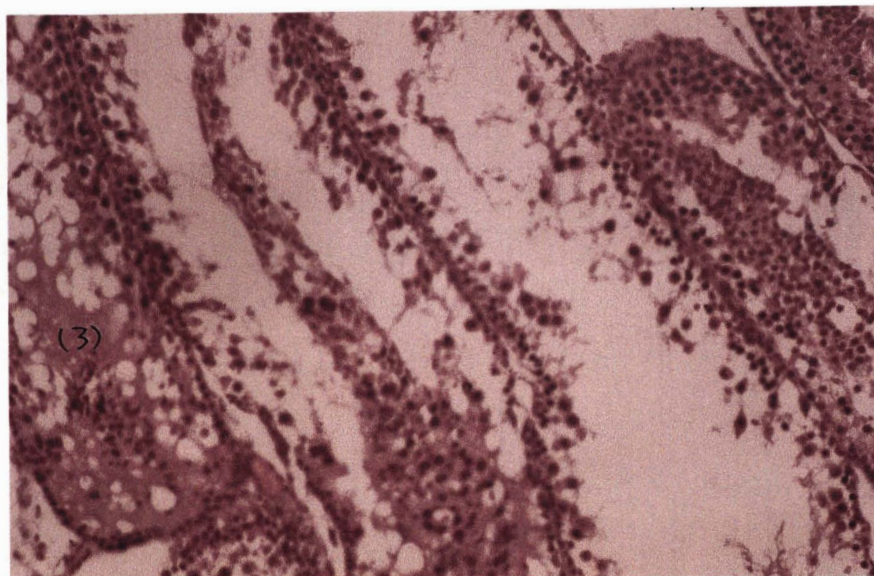


Fig.(7): H & E (X 40)

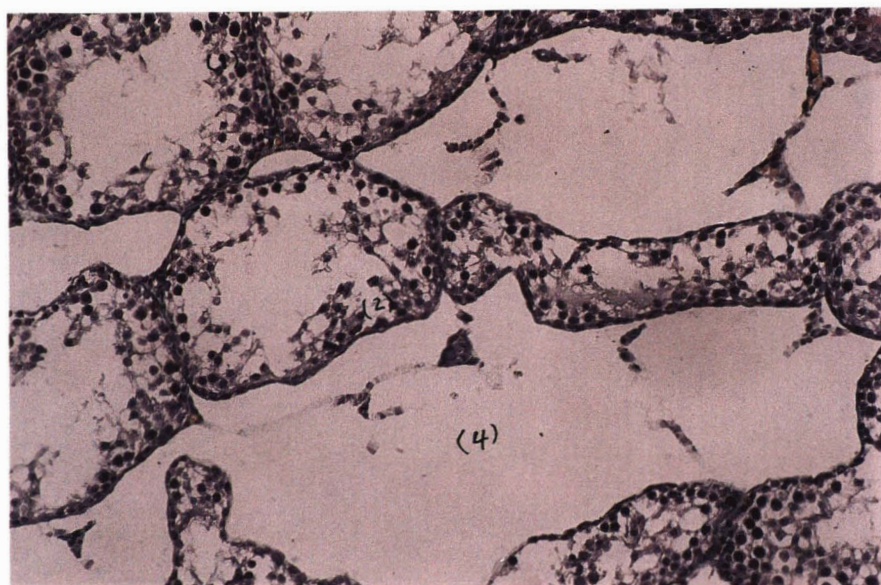


Fig.(8): Masson trichrome (X40)

Hypospermatogenesis up to secondary spermatocytes (1), hydropic degeneration of lining spermatogonia (2), homogeneous acidophilic secretion (3) and atrophy of interstitial cells (4).

HISTOPATHOLOGICAL EFFECTS OF COMPOUND (S-33g) ON PREPUBERTAL RATS' PROSTATE.

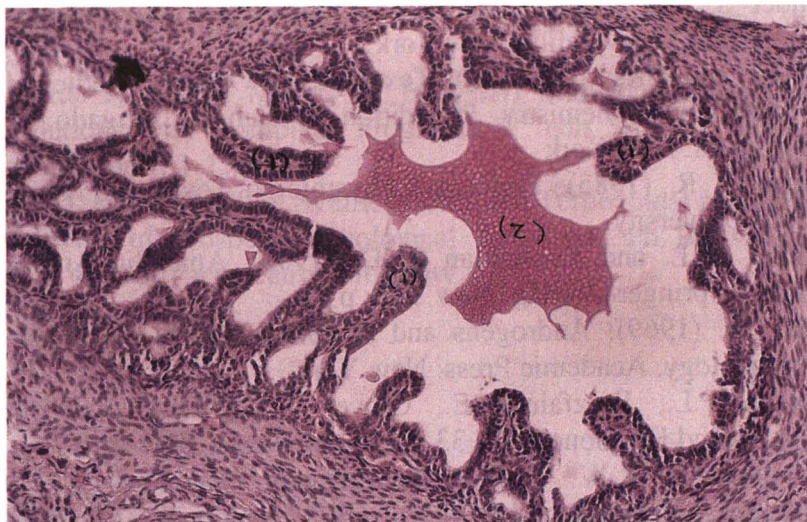


Fig.(9):Prostate showing glandular hyperplasia. The glands are enlarged, form intraluminal papillary budding (1) and contain intraluminal acidophilic secretion (2). (H & E X20).

HISTOPATHOLOGICAL EFFECTS OF COMPOUNDS (S-13d) AND (S-25c) ON PREPUBERTAL RATS' PROSTATE.

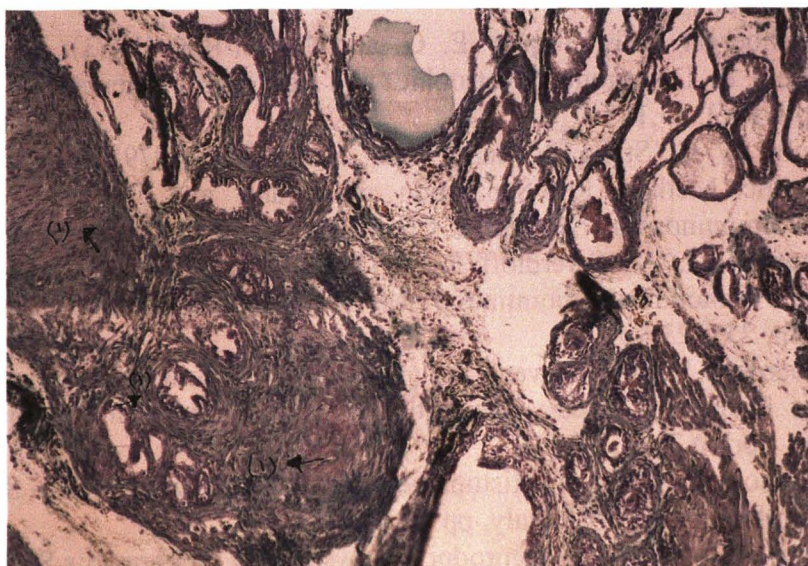


Fig.(10): Stromal hyperplasia of the prostate showing increased fibromuscular element (1). (Masson trichrome X10)

References

1. Vander S. and Luanovo H. (1990): Human Physiology. International edition, Mac Crow - Hill : New York.
2. Wilson and Grisvold (1991): Textbook of Organic Medicinal and Pharmaceutical Chemistry, 9th edition by Jamine, Deigado, William and Ramers: New York.
3. Martin C. R. (1985): Endocrine Physiology Text book, New York, Oxford University.
4. Landau R.L. and Kocliokian C. D. (1967): Anabolic Androgenic Steroids. Springer- Verlag: New York p 48
5. Vida J.A. (1969): Androgens and Anabolic Agents Chemistry and Pharmacology. Academic Press: New York.
6. Munson P.L., Diczfalusy E., Glover J. and Olsen R.E. (1975): Vitamins and Hormones, Vol 33, Academic Press, New york.
7. Brueggemeier R.W. (1986): Androgenics, Anabolics and Anti-androgens. Verderame M. (ed.), CRC Handbook of Hormones, Vitamins and Radiopaques, CRC Press; Boca Raton.
8. Gilman A.G., Rall T.w., Neles A.S. and Tylor P. (1990): The Pharmacological Basis of Therapeutics Androgens. Wilson J. D. (ed.) Pergamon Press: New York, p. 1413.
9. Laurence D. R. and Bennett P.N. (1982): Clinical Pharmacology, 5th edition, ELBS. English Language Book Society/ Churchill Livingstone, p 764
10. Hershberger L.G., Shipley E. G. and Meyer R.K. (1953): A model System to Determine Myotrophic and Androgenic Agents Proc. Soc. Exptl. Biol. 83,175.
11. Wood W. G., Stalla G., Muller O. A., Scriba P.C. (1979): A rapid and Specific Method for Separation of Bound and Free Antigen in Radioimmunoassay Systems. J. Clin. Chem. Clin. Biochem. 17: 111-4
12. Peterson M. A., Swerdloff R. S. (1979): Separation of Bound from Free Hormone in Radioimmunoassay of Lutropin and Follitropin. Clin. Chem. 25: 1239-41
13. Bancroft J.D. and Stevens A. (1975) : Histopathological Stains and their Diagnostic Uses. Churchill Livingstone Edinburgh, London and New York p 7, 11, 25
14. Massaon P. (1994). In: Histopathology Kits (Bio- Optica), Bio- Stain, Viacollamarini (Publ.), Italy, pp. 42-43
15. Rosai, J. (1996): Male Reproductive System, In. Ackerman's Surgical Pathology, 8th edition pp 1257-1263.
16. Johnson L., Petty C.S., Neaves W.B. (1980): The Relationship of Biopsy Evaluation and Testicular Measurements to Over -all Daily Sperm Production. Fertil Steril. 34; 36-40.

17. Silber S. J., Rodriguez-Rigan L.J. (1981): Quantitative Analysis of Testicular Biopsy, Determination of Partial Obstruction and Prediction of Sperm Count after Surgery for Obstruction. *Fertil. Steril.* 36:480-485.
18. Tallarida R. J. and Murray R. B. (1986): *Manual of Pharmacologic Calculations with Computer Programs*. 2nd edn. Springer Verlag Inc.: New York pp 131-134
19. Abdulla M. M. (1998): *Synthesis of Some Terpenoidal and Steroidal Derivatives of Prospected Biological Activities*. Ph. D. Dissertation, Faculty of Pharmacy, Cairo University.

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