

## ORIGINAL RESEARCH

**Microscopic Study of the Effect of Clomiphene Citrate on Prostate**Rohul Afza<sup>1</sup>, Ashfaq ul Hassan<sup>2</sup>, Saba Yaseen<sup>3</sup>, Muzzafar<sup>4</sup>**ABSTRACT**

**Introduction:** Clomiphene citrate is a synthetic analogue of the non-steroidal estrogen chlorotrianisene, 1-(p-(diethylaminoethoxy)-phenyl)-1, 2 diphenyl-2-chloro-ethylene. Clomiphene citrate, has a remarkable structural similarity to Estradiol which enables it to bind to estradiol receptors in various tissues such as the hypothalamus, hypothesis cerebri, ovaries, uterus and cervix.

**Material Methods:** The present study was aimed to determine the effect of clomiphene citrate on reproductive organs of rats. In the present study the experimental animals used were albino rats weighting on an average 150 gms. 64 healthy rats were used for the experimental study. The animals were studied in four groups.

**Result:** There was a general trend to reduction in the heights of the cells and to increased cytological alterations with increasing dose and length of treatment. Clomiphene treatment induced regressive histological changes in the prostate.

**Conclusion:** Clomiphene is a commonly used drug in infertility. The current study is aimed at determining the macroscopic and microscopic effects of Clomiphene on prostate.

**Keywords:** Clomiphene, seminiferous tubule, Hypothalamus, infertility, estrogen, estradiol

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**Conflict of Interest:** None

**INTRODUCTION**

Clomiphene citrate is a synthetic analogue of the non-steroidal estrogen chlorotrianisene, 1-(p-(diethylaminoethoxy)-phenyl)-1, 2 diphenyl-2-chloro-ethylene. Clomiphene citrate, has a remarkable structural similarity to Estradiol which enables it to bind to estradiol receptors in various tissues such as the hypothalamus, hypothesis cerebri, ovaries,

uterus and cervix.<sup>1,2</sup> Clomiphene is a drug used for Infertility<sup>3</sup>

**MATERIAL AND METHODS**

The present study was aimed to determine the effect of clomiphene citrate on reproductive organs of rats.

Here in this study 64 rats were used.

**Group A** - (control group) - in this group 16 rats were used. These were fed with routine food and tap water daily.

In addition to the routine food and tap water 48 other rats were administered clomiphene citrate orally mixed with flour and water as pellets. According to the dose the treated rats were classified into following groups-

**Group B** - It comprised of 16 rats and were administered. 5 mg/ 100 gm daily.

**Group C** - It comprised of 16 rats and were administered 3.5 mg/ 100 mg daily.

**Group D** - It comprised of 16 rats and were administered 5 mg/ 100 gm daily.

Dose of the drug was calculated from human therapeutic dose. The animals were kept in four different cages comprising of group A, B, C and D.

Each day routine diet was prepared for animals in each group. The diet would comprise of different vegetables and gram. Each animal from the cage was taken out fed with its usual food. The group A animals had routine food whereas the group B, C and D animals in addition to routine food were fed with clomiphene citrate mixed with flour as pellets. The process of administration was continued up to twelve weeks regularly. Four rats from each group were killed at intervals of 2, 4, 8 and 12 weeks respectively.

**RESULTS**

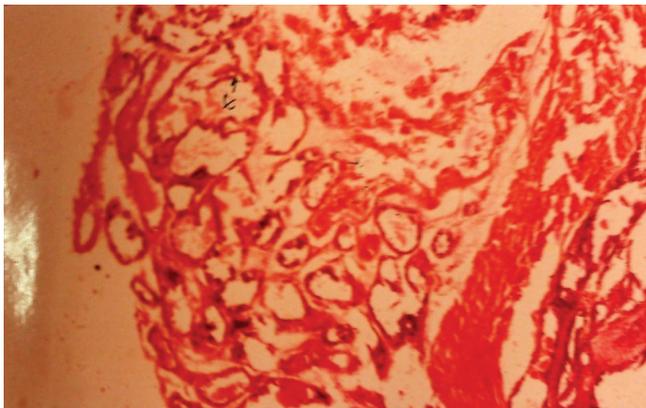
The present study is aimed to observe the effects of clomiphene citrate on reproductive organs of male albino rats. In all 64 animals were used. These animals were grouped in various groups. Group A comprised the control group. Groups B, C and D received drug. Groups were subdivided into subgroups on the basis of duration of treatment. Progressive decrease in rate of weight gain was observed in general (Table-1). The organs namely, Testis, Epididymis, Seminal vesicles and Prostate of male albino rats were studied both macroscopically and microscopically (Figure 1-4).

**Prostate**

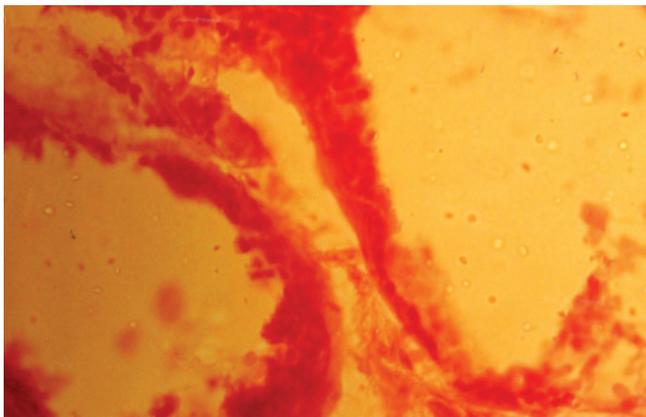
Microscopically the changes commenced from 2<sup>nd</sup> week and were maximum in 12<sup>th</sup> week. The obvious findings were;

Group	Before experimentation	After experimentation			
		2 <sup>nd</sup> week	4 <sup>th</sup> week	8 <sup>th</sup> week	12 <sup>th</sup> week
A	150	198	259	328	350
B	145	150	162	170	183
C	148	155	160	168	172
D	148	150	158	162	170

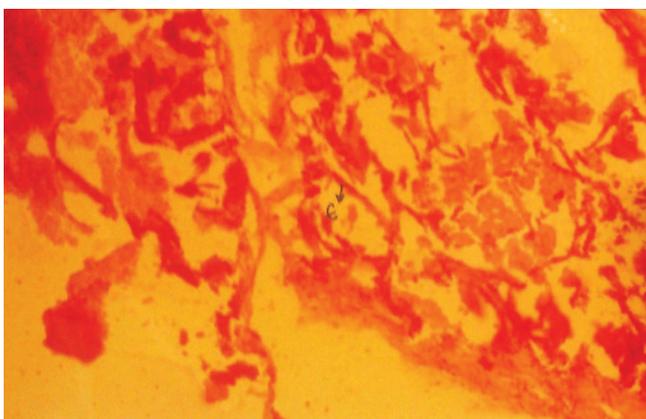
**Table-1:** Average body weight (in gms) of male albino rats



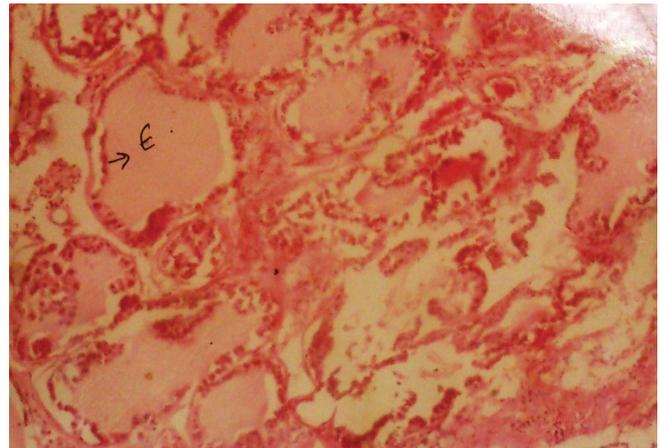
**Figure-1:** Micrograph of Prostate of Rat in Group B3 showing shorter epithelium columnar epithelium.



**Figure-2:** Micrograph of Prostate of Rat in Group D4 showing Completely atrophied epithelium in some acini.



**Figure-3:** Micrograph of Prostate of Rat in group B2 showing prostatic acini lined by columnar epithelium.



**Figure-4:** Micrograph of Prostate of Rat in Group B4 showing More pronounced shortening of columnar epitheliumMagnification

The epithelium was much shorter than in normal, being reduced to in height to a cuboidal shape. In some acini epithelium was completely atrophied. The changes started earlier in 2<sup>nd</sup> week and were more pronounced in group D.

**DISCUSSION**

**Prostate**

Effects of clomiphene were studied in present study on the prostate of albino rats both macroscopically and microscopically.

The mild decrease in weight was noticed from 2<sup>nd</sup> week followed by severe decrease by 8<sup>th</sup> to 12<sup>th</sup> weeks. Microscopic changes were present in the majority of treated animals at the 8 week interval and in all of those treated for 12 weeks. Histologically, the epithelium of the prostate of clomiphene treated rats showed few changes after four weeks, except for an apparent decline in the height of the cells in those rats administered the highest dose of 5mg/100g/ day. Following treatment for eight weeks or more, the epithelia of the prostate were greatly reduced in height from their normal tall columnar shape to a low columnar, cuboidal or even squamous appearance. This effect was particularly pronounced in those rats receiving 5 mg/ 100g/ day for eight weeks and in those that received 3.5mg/100g/day for 12 weeks. Thus there was a general trend to reduction in the height of the cells and to increased cytological alterations with increasing dose and length of treatment.<sup>3,4,5</sup> Holtkamp<sup>4</sup> demonstrated that in immature male rats clomiphene in dose from 1 mg/ kg/day and greater yielded lower relative weights of testis, seminal vesicles and ventral prostates. Degree of lowering was dose dependent. Ventral prostates were lowered more than either seminal vesicles or testis.

Walson observed weights of prostate were decreased at all doses above 0.25 mg / kg and were at hypohysectomy levels at doses of 2.5 mg/ kg and higher. Charles.<sup>6</sup> observed that the average weghts of the prostate were less than those of

control rats and decreased with increasing dose and length of treatment. Microscopically, the normally tall columnar epithelia were reduced to a low columnar or cuboidal shape. Singh<sup>7</sup> observed that clomiphene treatment induced regressive histological changes in prostate accompanied by significant decrease in the weight. Following withdrawal of the drug, full recovery was noticed in the prostate in 28 days as compared to 56 days of full spermatogenic activity and normal epididymal histology.

Thus it is concluded that the prostate manifests both gross and histological changes and are more evident after the 8<sup>th</sup> week of clomiphene therapy.

Prostate: On gross examination a slight to moderate in weight of prostate was noticed from 2<sup>nd</sup> to 12<sup>th</sup> weeks of experiment.

### Microscopic

The structure of prostate seemed to be more readily affected than testis and epididymis, since some animals that showed slight or no alterations in the testis epididymis had microscopic changes in the prostate. When alterations were present in the testis and epididymis, however, changes were also observed in prostate.

**Group B:** In this group rats were administered the lowest dose of clomiphene, 2.5 mg/ 100/day. Microscopic changes in the prostate appeared after eight weeks (B3) of treatment in this group.

B1; Normal histological findings in form of prostatic acini lined by columnar epithelium. No obvious changes

B2; No change from normal

B3; The epithelium was shorter than in normal

B4; More pronounced shortening of epithelium

**Group C:** In this group rats were administered the intermediate dose of clomiphene, 3.5mg/ 100/day. Microscopic changes were present in the majority of treated animals at four week interval (C2) and in all of those treated for 8<sup>th</sup> (C3) to 12<sup>th</sup> week (C4)

C1; No change from normal detected.

C2; Slight change in form of shortening of epithelium than in normal

C3; Changes more pronounced than C2. The epithelium was much shorter than in normal, being reduced to a low columnar shape.

C4; More extensive changes were seen. The epithelium was much shorter than in normal, being reduced in height to a cuboidal shape

**GROUP D:** In this group rats were administered the highest dose of clomiphene, 5 mg/100/day. Microscopic changes were present in most of the treated rats.

D1; Slight change in form of shortening of epithelium than in normal

D2; Changes more pronounced than D1. The epithelium was much shorter than in normal, being reduced to a low columnar shape

D3; The epithelium was much shorter than in normal, being reduced to in height to a cuboidal shape

D4; Findings of D3 were more pronounced in this group. The epithelium was completely atrophied in some acini.

Thus there was a general trend to reduction in the heights of the cells and to increased cytological alterations with increasing dose and length of treatment.

### CONCLUSION

There was a general trend to reduction in the heights of the cells and to increased cytological alterations with increasing dose and length of treatment. Clomiphene treatment induced regressive histological changes in the prostate and ampullary gland accompanied by significant decrease in the weight.

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