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Research Article

HISTOLOGICAL STUDIES ON TESTIS OF ALBINO MICE TREATED WITH SILDENAFIL CITRATE (VIAGRA)

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ABSTRACT
Sildenafil citrate, a new class of orally active drug proven effective for the treatment of erectile dysfunction, is a selective inhibitor of the enzyme Phospho diesterase type (PDE5). In order to study the impact of the drug on histoarchitecture of Testis, Albino mice were treated with Sildenafil citrate (Viagra). Thirty healthy male Albino mice (*Mus musculus*) were selected on weight basis and divided into five groups, each consisting of six animals in it. Group A animals served as the control while other four groups were treated as the experimental ones. Control animals were treated with conductivity water (@1µg/g body wt.) while the experimental animals were treated with Sildenafil citrate (@1µg /g body wt.) and sacrificed after 1 hour, 4 hours and 24 hours. The last group, namely, Group E animals were served with a single dosage of the chosen drug daily for 15 days and sacrificed after 4 hours of the last dosage. A vertical ventral midline incision was made in the abdominal wall to collect the testis samples. Damaging effects on the histoarchitecture of Testis were prominently observed in the case of long term Sildenafil citrate treated animals. Therefore, the prolonged use of Sildenafil citrate on Albino mice will have adverse effects on the structural components as well as the function of Testis.

***Keywords*:** *Albino Mice, Testis, Viagra, Histoarchitecture, Oedema*

INTRODUCTION
The stimulation of sexual function of the animal organism, especially, the stimulation of spermatogenesis and ovogenesis, has acquired a greater biological and medical significance since it is related with the problem of preserving the sexual potential of male and female individuals. However, in recent times, Erectile dysfunction (ED) has become a common and multifactorial disease that strongly impairs the quality of life in man (Benet & Melman, 1995; Burnett, 1998; Gauv, 1995; Korenman, 1998). Sildenafil citrate (Viagra) is a breakthrough, the first of a new class of orally active drug proven effective for the treatment of ED.

The onset and duration of action of Sildenafil citrate for the treatment of ED has been studied by earlier researchers (Eardley *et al.*, 1999; Eardley *et al.*, 2002). Systematic review and meta- analysis on the safety and efficacy of this drug for male ED have been made by many workers (Fink *et al.*, 2002; Hatzichristou, 2002; Padmanathan *et al.*, 2002). Though this drug has been reported to be well tolerated, it has some mild to moderate side effects. As Viagra has become the drug of attraction even among common men, the impact of this drug on the structure and vital functions of Testis of Albino mice, is worth studying. The present study was conducted to study the long-term effects of Sildenafil citrate on histological components of Testis of Albino mice.

MATERIALS AND METHODS
Permission for the study was obtained from the Institutional Animal Ethics Committee and all etiquettes were adhered to.

Thirty healthy male Wistar Albino mice were selected on weight basis and acclimatized for a period of seven days before starting the study. Standard experimental conditions such as temperature (24±2°c), humidity (60-70%) and 12 hours of light/ dark cycle were maintained. Feed and water were allowed *ad libitum* under strict hygienic conditions.

These animals were divided into five groups (A, B, C, D & E), each consisting of six animals in it. Here, Group A served as the control while the rest of the four groups served as the experimental ones. Group A animals were treated with a single dosage of conductivity water while the experimental animals belonging to Group B, C and D were treated with a single dosage of the chosen drug (@ 1µg/g body wt.) and sacrificed after 1 hour, 4 hours and 24 hours of the last dosage. Group E animals were treated with a single dosage of the chosen drug (@ 1µg/g body wt.) daily for 15 days and sacrificed after 4 hours of the last dosage.

'Drug´ here refers to Sildenafil citrate (Viagra from M/s Pfizer Company, USA) purchased from the market. Chloroform anaesthesia was used and a vertical ventral midline incision was made in the abdominal wall to collect the Testis samples. The organs were preserved in 10% formalin, processed and stained with Eosin and Haematoxylin stains.

RESULTS AND DISCUSSION

Results

The outcome of the present investigation clearly indicates the following facts:

In the case of Group E (15 days) animals, there were remarkable changes in the behaviour of the animals. At the time of drug administration, the animals were very ferocious for half an hour to one hour. Then, their usual activities were diminished and they came in close to lie down together.

A reduction of body weight to the order of 6 to 10 gm. was noticed for 15 days experimental animals. In the case of testicular weight, a reduction of 50% of the initial weight was observed for the experimental (15 days) animals (Table-1).

Normal features of seminiferous epithelium and interstitium of Testis were noticed for all the control animals (Figure 1).

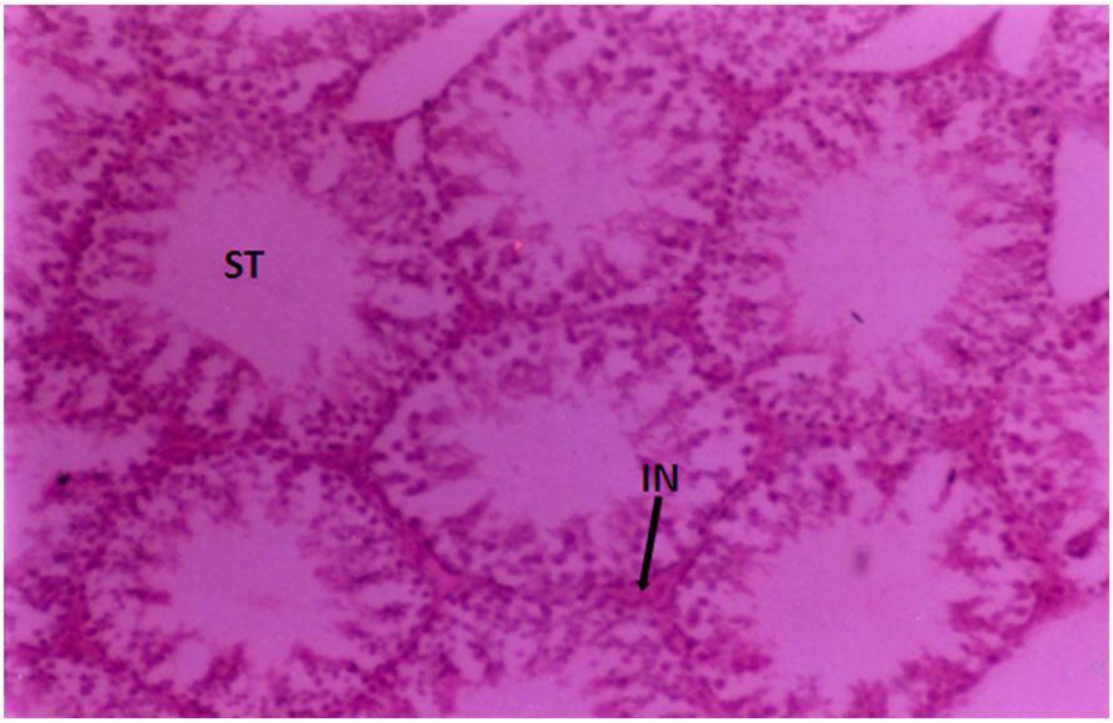

Figure 1: Photomicrograph of Testis of Albino mice- Group A (Control). ST- Normal feature of Seminiferous tubules, IN- Normal feature of Interstitial space. Eosin- Haematoxylin

Normal features of seminiferous epithelium accompanied by an increase in Interstitial space was observed for Group B, C and D animals (Figure 2, 3).

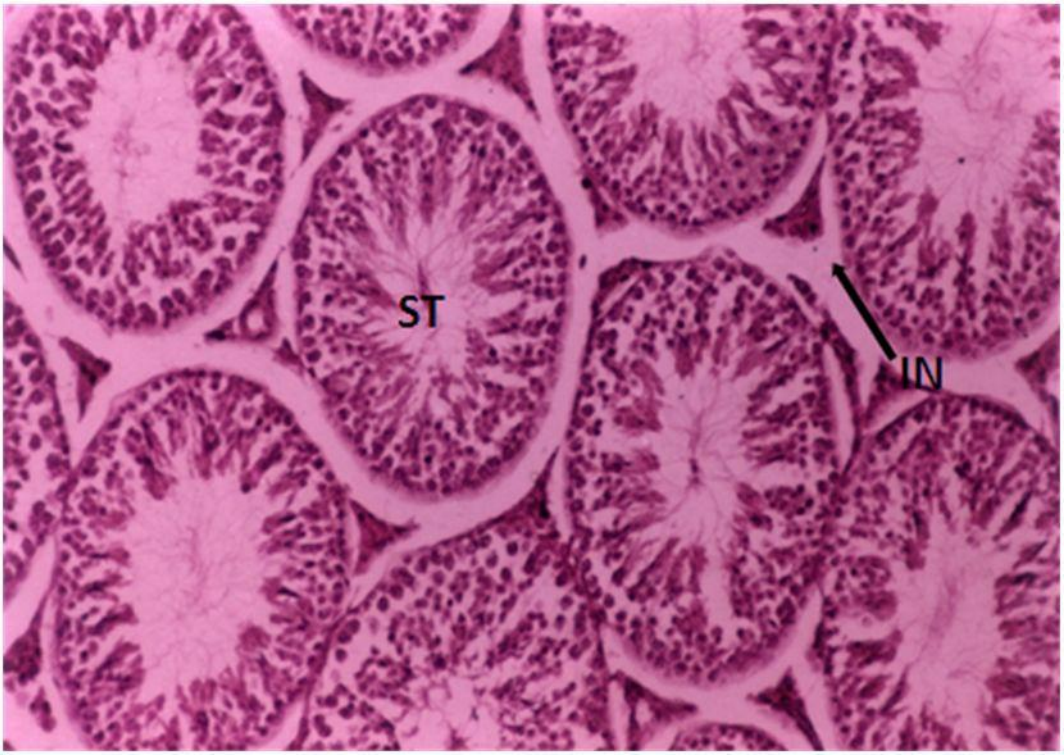

Figure 2: Photomicrograph of Testis of Albino mice- Group B (1 hour). ST- Normal feature of Seminiferous tubules, IN- Slight increase in Interstitial space. Eosin- Haematoxylin

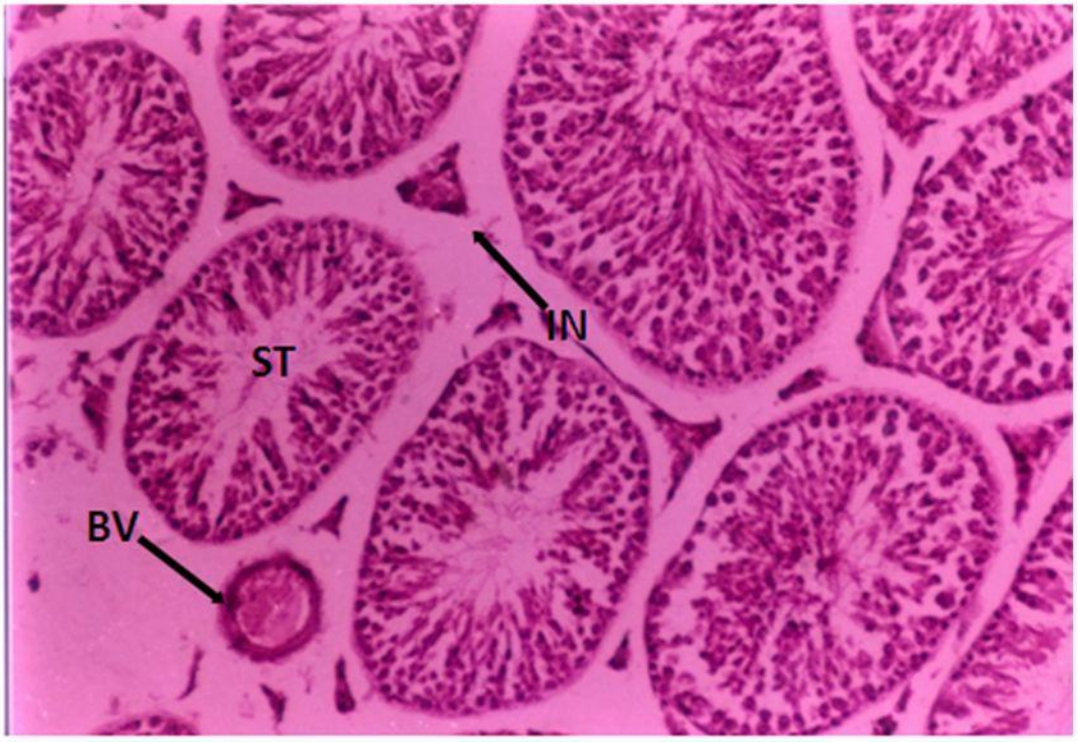

Figure 3: Photomicrograph of Testis of Albino mice- Group C (4 hours). ST- Normal features of Seminiferous tubules, IN- Increase in Interstitial space, BV- Markedly dilated blood vessel. Eosin-Haematoxylin

However, the blood vessels in Interstitium in the case of Group C (4 hours) were remarkably dilated in comparison with that of the Group A (control). Deformation of the shape of the walls of seminiferous tubules, reduction in the size of the seminiferous tubules and an increase in Interstitium were predominantly observed in the case of 15 days experimental animals (Figure 4).

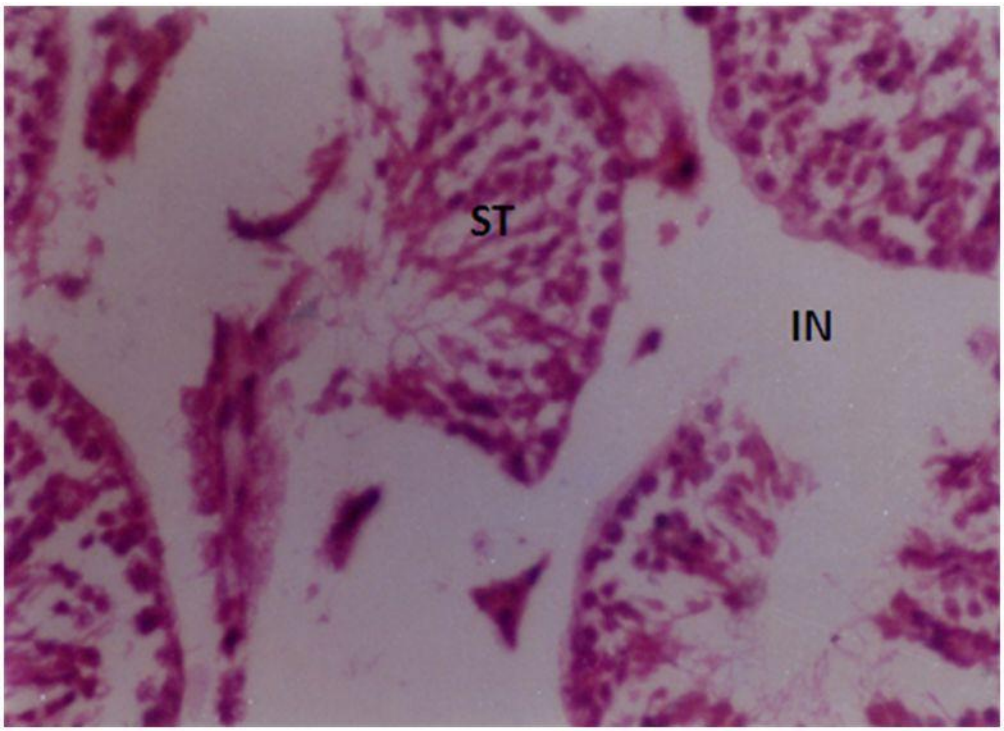

Figure 4: Photomicrograph of Testis of Albino mice- Group E (15 days). ST- Deformation of the shape of the walls of Seminiferous tubules, IN- Prominent increase in Interstitial space. Eosin-Haematoxylin

Mean volume (in mm^3) and the mean diameter of the seminiferous tubules (in microns) have been observed to decrease significantly in all groups of experimental animals gradually. However, it is most prominently noticed in the case of Group E animals (Table-1).

Table 1: Effect of Sildenafil Citrate (Viagra) on testis of albino mice ($^@$ Mean value of six replicates; p value< 0.001)

Sample	Mean testicular weight$^@$ (mg)	Mean volume of the seminiferous tubules$^@$ (mm^3)	Mean volume of interstitium$^@$ (mm^3)	Mean diameter of seminiferous tubules$^@$ (µm)
S_1	165.3±8.27	0.7795±0.0206	0.2198±0.0208	202.27±11.91
S_2	168.3±5.89	0.7788±0.0153	0.2202±0.0160	202.66±6.33
S_3	168.3±8.42	0.7353±0.0139	0.2642±0.0139	205.90±3.24
S_4	149.0±7.74	0.6744±0.0111	0.3254±0.0111	200.88±8.18
S_5	85.8±3.86	0.4999±0.0086	0.4999±0.0090	161.31±4.19

S_1- Group A (Control), S_2- Group B (1 hr.), S_3- Group C (4 hrs.), S_4- Group D (24 hrs.), S_5Group E (15 days)

Discussion

The histological studies on the impact of Sildenafil citrate (Viagra) on testis of Albino mice, as carried out in the present investigation, result in many fascinating findings.

The behavioural changes in Group E (15 days) animals indicate the fact that prolonged use of Sildenafil citrate (Viagra), though administered at normal dosage levels, results in restlessness and ferocity.

The marked reduction in body weight and testicular weight of Group E animal's crystal clearly portrays the fact that the long term drug administration induces physiological alterations which are in accordance with that of drug induced Diabetes mellitus (Arulmoli, 1991). Moreover, the basement membrane thickening in testis of experimental animals was observed in the present study as a constant qualitative change. Robbins and Cotran (Robbins & Cotran, 1979) have reported the basement membrane thickening as one of the qualitative changes in the case of diabetes mellitus. Therefore, Viagra induced changes in the metabolism of Albino mice are to be observed as similar to that of drug induced diabetes mellitus.

From the morphometric studies, it has been observed that there is an increase in intertubular area which may be due to intertubular oedema in the Testis. For all the experimental animals, the seminiferous tubules are widely separated from each other and there is a decrease in volume and diameter of seminiferous tubules. It may be inferred that this state of the seminiferous tubules is due to intertubular oedema which disturbs the nutrition supply to the seminiferous epithelium and may ultimately result in lysis of spermatogenic cells. It has been reported by earlier workers (Mehta & Lakshmanan, 1982) that spermatids are more sensitive, among the spermatogenic cells, to any physiological alterations. Therefore, the metabolic changes, as noticed in the present investigation, may induce qualitative and quantitative changes in spermatids.

General reduction in diameter of seminiferous tubules and accumulation of oedematous fluids in testis has been observed in the case of long-term Viagra treated animals. Similar histological and biochemical changes have been noticed in the case of testis of rats treated with the leaf powder of *Azadirachta indica* (Joshi *et al.,* 1996) and *Andrographics paniculata* (Akbarsha *et al.,* 1990), the latter being known for its anti- fertility activities.

The intertubular oedema may make the interstitial cells inefficient in their function, resulting in impotency. However, this statement has to be substantiated with hormonal assay and enzyme assay. As Sildenafil citrate (Viagra) is exclusively used for the treatment of ED and impotency, the inference that long-term Sildenafil citrate (Viagra) treatment may bring about reversal effect is to be closely scrutinized.

Conclusion

The Histological studies, as carried out in the present investigation, clearly portray the fact that the long-term Sildenafil citrate (Viagra) treatment in the case of Albino mice brings in adverse effects and completely alters the function and the architecture of testis.

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