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Histological Changes in Epididymis of Albino Rats by Graded Doses of Cyclophosphamide

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Abstract

Background: Cyclophosphamide is widely used in the treatment of various neoplastic diseases and diseases associated with altered immunity. Higher doses used for longer duration effects many organs like urinary bladder, lungs, liver, heart and male reproductive organs.

Methods: To study the effect of cyclophosphamide on the micro anatomy of epididymis, sixty eight Albino rats were taken and divided into three groups, group A (control group) of 20 animals, were fed with routine diet, group B (low dose group) of 24 animals, were given cyclophosphamide at the dose of 0.5 mg/100 gms in addition to the routine diet and group C (high dose group) of 24 animals, were given high dose of cyclophosphamide at the dose of 0.7 mg/100 gms of weight of animal in addition to the routine diet. The animals were sacrificed at intervals of 3, 6, 9 and 12 weeks, sections of the tissue from the epididymis were prepared and stained with Haematoxylin and Eosin stain.

Results: In both low dose and high dose groups there was no apparent histological change at 3 weeks of drug administration, however there were absent sperm whorls in some of the tubules of the epididymis of both low dose and high dose groups at this stage. At 6 weeks there was increase in the number of clear cells in the tubules of epididymis of both the groups. While as at 9 weeks there were some atrophic changes in the tubules of group C rats.

Conclusion: In present study we observed certain histological changes in the epididymis of cyclophosphamide treated albino rats. These were in the form of absent sperms, increase in number of clear cells, increase in peritubular space and atrophic changes.

Keywords: Cyclophosphamide, Epididymis, Clear cells.

1. Introduction

Cyclophosphamide one of the alkylating agents is used in the treatment of various neoplastic diseases like lymphomas, chronic lymphocytic leukemia, solid tumors and breast cancer [1,2]. It was approved by FDA in 1959 as the 8th anticancer agent [3]. Because of its immune-suppressive properties, cyclophosphamide is used in the treatment of many autoimmune diseases either as a single drug or in combination with glucocorticoids. Cyclophosphamide is given orally in the form of tablets. It is also given parentally for which IV route is preferred. Cyclophosphamide is metabolized in liver by hepatic cytochrome P450 to an active metabolite 4-hydroxycyclophosphamide which is in study state with the cyclic tautomer aldophosphamide [4-6]. Aldophosphamide cleaves spontaneously to generate phosphamide mustard and acroline. The main action of cyclophosphamide is due to phosphamide mustard which acts by forming irreversible DNA cross-links both between and within the strands at guanine N-7 position [7]. Cyclophosphamide causes many side effects like pulmonary fibrosis, hepatic veno-occulusive syndrome, hemorrhagic cystitis, irreversible azospermia etc [8,9]. Secondary malignancies are seen with use of high dose of cyclophosphamide [10]. Exposure of the male germ cells to cyclophosphamide during spermatogenesis and sperm maturation can interfere with

the development of embryo [11]. Because of its potential to cause sterility and effect on sperm maturation, the present study is aimed to see the effect of cyclophosphamide on the histology of epididymis in albino rats to correlate the findings in human beings.

2. Materials and methods

The present study was conducted in the department of Anatomy, Government Medical College, Srinagar.68 Albino rats weighing on an average 100 grams were taken from animal house Department of Pharmacology, Government Medical College, Srinagar. Approval was sought from institutional animal ethics committee. The animals were housed under uniform husbandry conditions and were divided in 3 groups: group A (Control Group) of 20 rats were fed with routine diet and water, group B (low dose group) of 24 albino rats were given cyclophosphamide at the dose of 0.5 mg/100gms of weight of rat besides the routine diet. Group C (high dose group) of 24 rats was given high dose of cyclophosphamide the dose of 0.7mg/100gms weight of rat besides the routine normal diet. The drug was given by mixing it with pellets of flour. The animals were kept in different cages labelled as A, B and C.

Dose of the drug: Since the daily oral therapeutic dose of cyclophosphamide for human beings is 5mg/kg body weight so from this dose the dose for albino rats was calculated as 0.5mg/100gms weight of albino rat as low dose group and 0.7mg/100 grams weight of albino rat as high dose. The process of administration was continued up to 12 weeks regularly. To study the effects of the drug, the animals were sacrificed in intervals of 3, 6, 9 and 12 weeks. In each sitting 5 rats from group A and 6 rats each from group B and C were taken. The animals were anaesthetized with chloroform, scrotal incision was given epididymis was identified and excised. The organ was put in between blocking papers. Standard histological techniques were used for processing the tissues, 5 to 7 micrometer thick sections of the tissues were made, stained with haematoxylin and eosin, observed under compound light microscope and observations were recorded.

3. Results

The present study was conducted in the department of Anatomy, GMC- Srinagar to study the histological changes in the epididymis of albino rats by graded doses of Cyclophosphamide. The animals were divided into Group A (Control), Group B (Low dose group) and Group C (High dose group). The administration of the drug was continued for 12 weeks and the animals were sacrificed in the intervals of 3, 6, 9 and 12 weeks. In both low dose and high dose groups there was no apparent histological change at 3 weeks of drug administration, however there were absent sperm whorls in some of the tubules of the epididymis of both low dose and high dose groups at this stage (Figure 1 & 2). At 6 weeks there was increase in the number of clear cells in the tubules of epididymis of both the groups (Figure 3 & 4). At 9 weeks of drug administration there was increase in number of clear cells in the epididymis of both low dose and high dose groups with atrophic changes in the tubules of group C animals and also there were wide peritubular spaces (Figure 5 & 6). At 12 weeks the changes in the low dose group were similar to those at 9 weeks but in high dose group in addition to increase in the number of clear cells there were diffuse atrophic changes in the tubules.

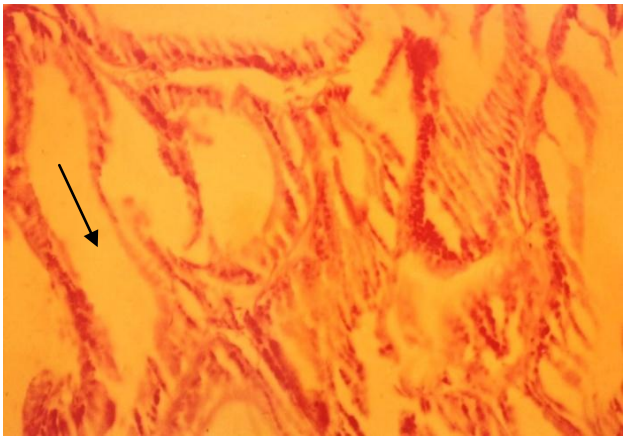

Figure 1: Photomicrograph of Group B animals at 3 weeks showing absent sperm whorls (arrow).

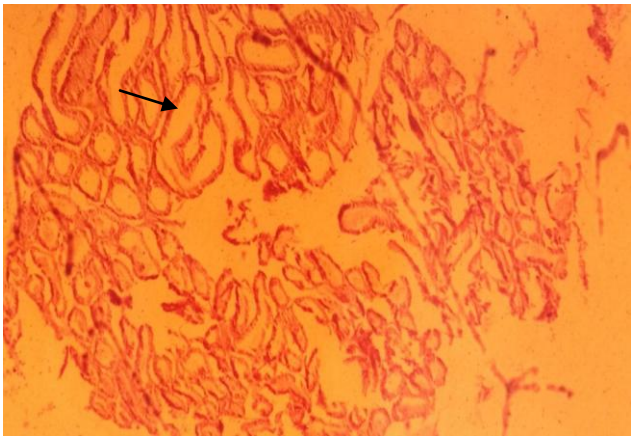

Figure 2: Photomicrograph of Group C animals at 3 weeks showing absent sperm whorls (arrow).

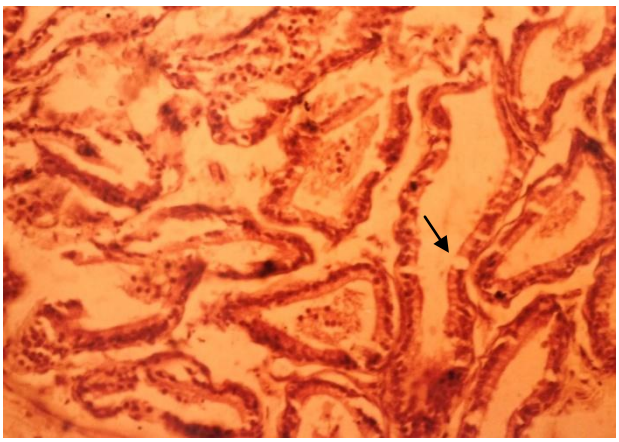

Figure 3: Photomicrograph of Group B at 6 weeks showing increase in the number of clear cells.

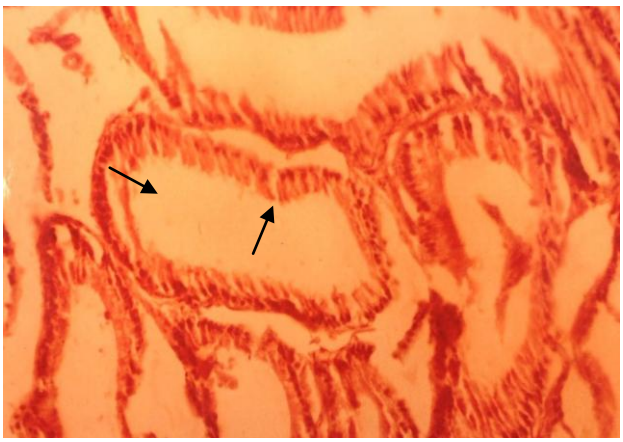

Figure 4: Photomicrograph of Group C at 6 weeks showing increase in the number of clear cells.

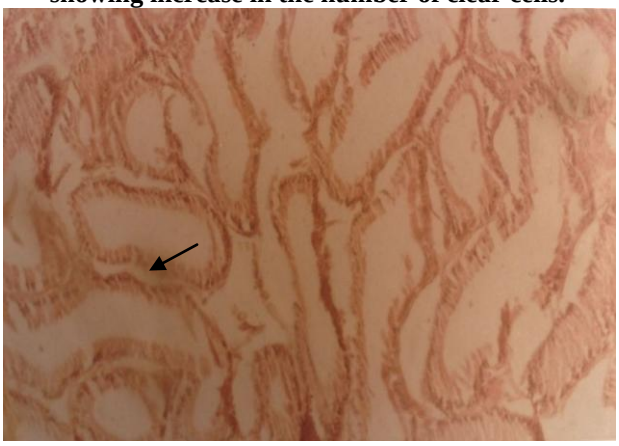

Figure 5: Photomicrograph of Group B at 9 weeks showing interlobular spaces (arrow).

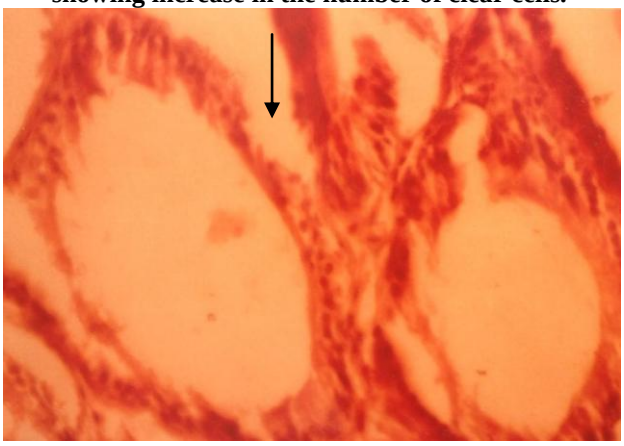

Figure 6: Photomicograph of Group C at 9 weeks showing atrophic changes in tubules (arrow).

4. Discussion

Cyclophosphamide, an alkylating agent is widely used in the treatment of the various neoplastic diseases and diseases associated with altered immunity. It has adverse effects in many organs and organ systems. The present study was conducted in order to see effects of cyclophosphamide in the histology of epididymis. The changes were in the form of absent sperm whorls in some of the tubules in the initial stages of drug administration. With prolonged use of drug there were histological changes in the tubules of the epididymis. These were in the form of increase in number of clear cells. There were also some atrophic changes in some of the tubules with prolonged use of higher doses of the drug. Not much work has been done on the effect of cyclophosphamide on the epididymis of animals. However Trasler *et al* [12] observed histological changes in the testis and epididymis of Sprague-Dawley rats by treating them with cyclophosphamide. In the epididymis there was increase in number and size of clear cells. In the present study the observations like increase in the number of clear cells in the epididymis correlate with the observations made by the earlier workers. Das and Mallick [13] observed the effect of cyclophosphamide on the epididymis of rats. Microscopically there were atrophic changes in the epididymis of cyclophosphamide treated rats. In the present study the observations like atrophy of epididymis of cyclophosphamide treated rats correlates with their observations. Moshtak *et al* [14] while seeing the effect of cyclophosphamide on spermatogenesis in rats observed that some of the tubules of the epididymis showed interlobular space and presence of edematous fluid in some of the tubules. In our present study some of the tubules of epididymis of rats treated with cyclophosphamide showed interlobular spaces at 9 weeks of drug administration.

5. Conclusion

Patients treated with cyclophosphamide develop irreversible azospermia. This could be partly due to its effect on testis and partly due to its effect on epididymis. In present study we observed certain histological changes in the epididymis of cyclophosphamide treated albino rats. These were in the form of absent sperms, increase in number of clear cells, increase in peritubular space and atrophic changes. Thus it is concluded that male patient receiving cyclophosphamide must undergo routine semen analysis.

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