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Regular Article

The effect of Cyclophosphamide on spermatogenesis in rats

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This study aimed to evaluate the effects of cyclophosphamide on spermatogenesis; we used three doses with variable time interval to determine the effect of the low and high doses of cyclophosphamide. The results showed that low doses for long time interval caused a considerable increase in the percentage of sperm head abnormalities (Tertatospermia), without any significant changes in tissue sections, the percentage of sperm head abnormalities was increased to 20.72% with the dose 5mg/Kg, while high doses caused a significant tissue changes in testes, and epididymis as well as head sperm abnormalities but less than the 5mg/Kg dose. The percentage of head sperm abnormalities were 14.75 and 13.19 for the doses 15mg/Kg and 10mg/Kg respectively.

Keywords: Cyclophosphamide, Sperm head abnormalities, Tissue changes.

Cyclophosphamide is one of the alkylating agent which interfere with Nucleic acids transcription and translation, it also affect the cell division because of the cumulative damages of DNA (Davidson et al, 2003). Cyclophosphamide had been discovered by modification of Mechlorethamine compound and this modification allow using it in treating tumor tissues instead of using other chemical based treatments (Young et al, 2006). CP is used for treatment of cancer diseases such as Lymphoma, Myloma, Leukemia, Mycosis, Neuroblastoma, Adenocarcenoma, Retinoblastoma, and Breast carcinoma (Shanafelt et al, 2007). CP Also used as immunospressor after organs transplantation and in autoimmune disease such as Rheumatoid arthritis, Wegeners granulomatosis, and Nephritic syndrome in children (Chabner et al, 2001). Cyclophosphamide metabolized in liver and it activated by multiple oxidation system hepatic microsomal mixed function oxidase cytochromes P 450 (4-OHCP) to convert into 4-hydroxycyclophosphamide which will transform into aldophosphamide which is unstable and split into compound, phosphamide mustard and acroline (Zhang et al, 2005). Cyclophosphamide had been classified as one of the top chemicals which karm health because it's effect on inducing different types of cancers and this drug is found in different common names such as Cytoxan, Indoxan, and Procytox (Irac, 1975).

Materials and Methods

Cyclophosphamide had been supplied from Baxter Corp. as tablet with drug concentration 50mg. The drugs dissolved in ultrapure distil water and the animal has taken the dose orally.

In this study we used white male rats weighing around 300±5 gm and they were divided into the following groups:

- Negative control which take ultrapure water only.
- Treated group which receive 15 mg/Kg for 10 days (it should continue to 15 days but because of the mortality of all animals in day 11 then the period of treatment with the drug has been reduced to 10 days).
- Second treated group which received 10 mg/Kg for 15 days (it should continue to 21 days but because the high mortality rate for the animals the period of treatment with the drug has been reduced to 15 days).
- Third treated group which received 5 mg/kg for 35 days.

At the end of the experiment the animals has been sacrificed to study the sperm head abnormalities as well as Epididymis and Testes tissues. Sperm parameters evaluation was done according (Colos, et al, 1980), and tissue sectioning according to (Humason, 1997).

Results

Table 1 show the sperm head abnormalities of rats treated with different doses of Cyclophosphamide with different time intervals, the percentage of sperm with head defect (no head) has been increased significantly for all doses, also there is a significant increase in other head defect like lobular head, and spherical head.

Table 1: Some types of sperm head abnormalities of rats treated by cyclophosphamide

Treatment mg\kg	Curved head	Globular head	Lobule head	Missed head
N C	0	1.00 ± 0.53	2.66 ± 1.33	14.66 ± 2.18
CP 15	3.66 ± 2.02	*20.00 ± 2.08	*37.33 ± 13.86	*86.66 ±13.09
CP 10	7.00 ± 1.73	*10.00 ± 2.88	*40.33± 6.96	*74.66 ± 14.72
CP 5	*16.66 ±7.79	*24.00± 2.30	*66.66 ± 7.79	*100.00 ± 5.77

Significant at p< 0.05* Me±Se

A significant increase in Hook head abnormality for 5 mg/Kg dose only. Figure 1 show the percentage of all teratospermia (head only), the dose 5 mg/Kg record the highest level in these abnormalities, also other defect had been noticed in sperm such as sperm without tail, double head, and sperm without apical head clamp. Figure F & G, the frequency of these abnormalities was very low so it didn't process in statistic.

Figures A, B, C, D, E the tissue changes in Testes, and Epididymis for the treated rats with 15 mg and 10 mg dose while 5 mg dose didn't introduce any histopathological changes in these tissues. Figure A represent the epididymis tail and it's clearly show the large spaces between the epididymis head section, also the lumen of the epididymis head is filled with edema fluid, the testes tissue in figure B (cross section) clearly show the suspended in spermatogenesis process with a clear necrotic effect of the drug, additionally there are a considerable amounts of spaces between seminiferous tubules and the disappearance of Leydig cells with a regression in the spermatogenesis process.

Figure D show the residual bodies and cells in the lumen of the seminiferous tubules, the edema fluid filled the lumen of the seminiferous tubules. Figure E show the increase of the size of interstitial spaces and the occurrence of edema fluid.

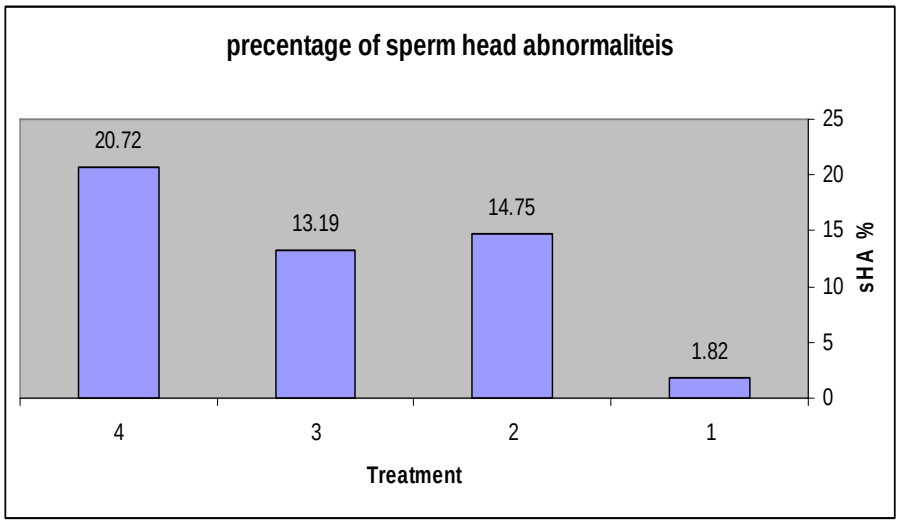

Figure 1: Percentage of sperm head abnormalities in rats treated by cyclophosphamide. 1, negative control ; 2, 3,4, treated by 15,10,5 mg\kg respectively.

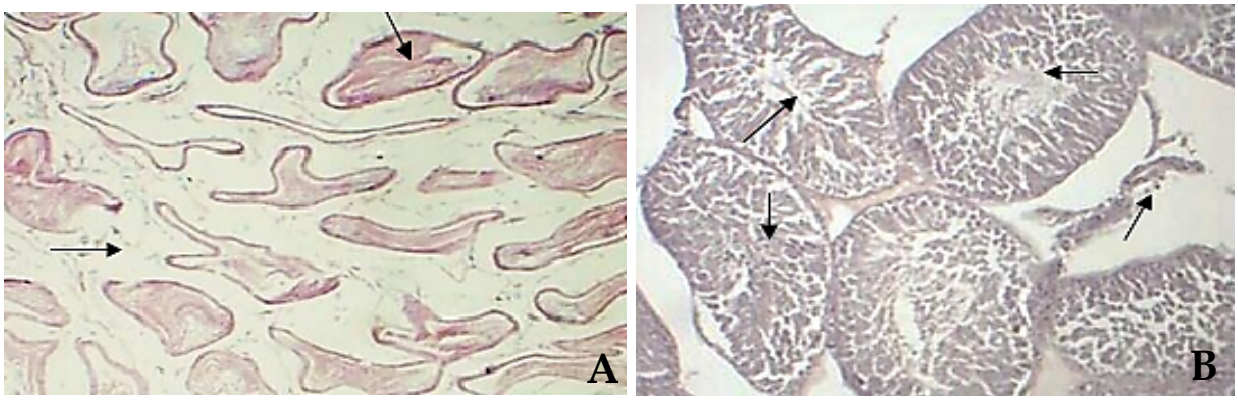

Figure A: Cross section in epididymis of rats treated by cyclophosphamide show interlobular space and present edematous fluid in some tubules. **Figure B**: Cross section of testes of rats show stop in spermatogenesis, necrosis, disappear in leydig cells and interlobular space.

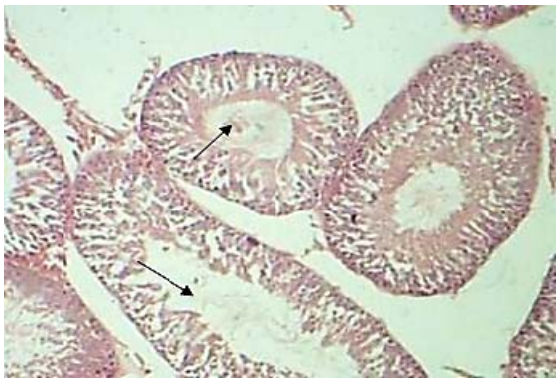

Figure C: Cross section in testes show reduction in sperm number, edematous fluid and interlobular space.

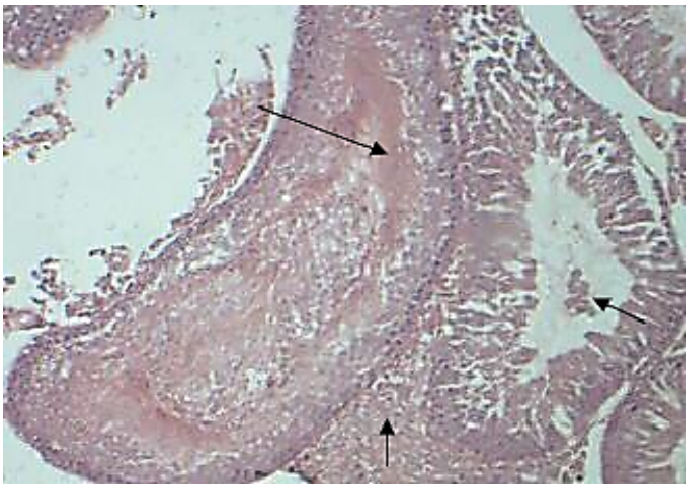

Figure D: Cross section in testes shows present residual bodies and other material in lumen and between tubules, present edematous fluid.

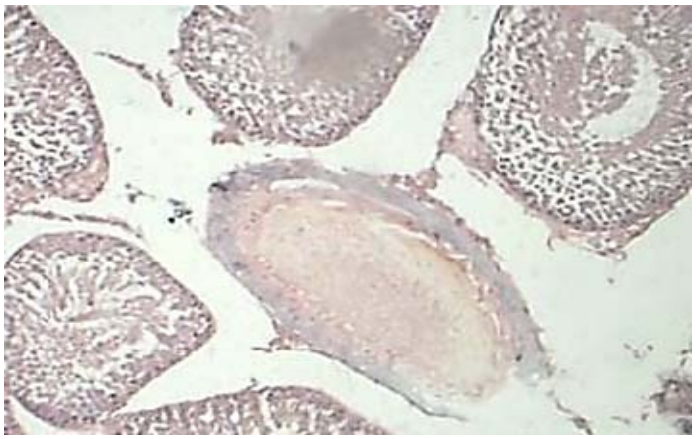

Figure E: Cross section of testes shows intercellular space and present edematous fluid.

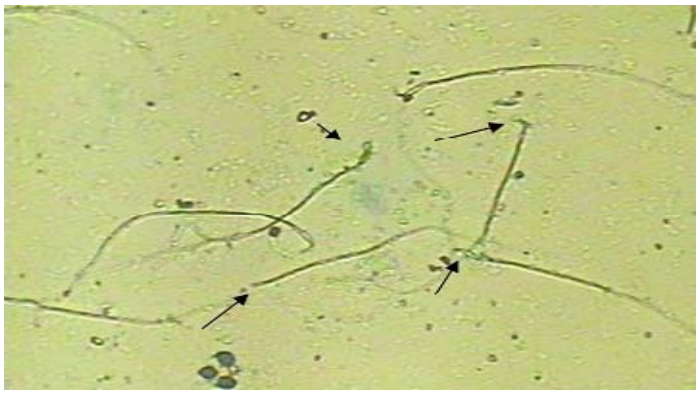

Figure F: Some type of sperm head abnormalities show missed head, lobule head, globular head .

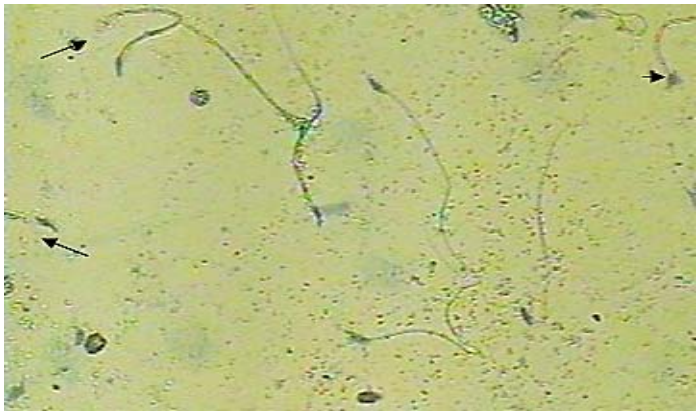

Figure G: Some type of sperm head abnormalities show head only, tail only, lobular head.

Discussion

The results of this study showed that treatment with cyclophosphamide induce the teratospermia (sperm abnormalities) in both testes and epididymis. Losing the normal shape of sperm is related to genetically or physiologically change in these sperms (Mohammed, 2009).
The increase in percentage of abnormal sperms gave clear evidence about the effects of the carcinogen agent in different stages of spermatogenesis and these abnormalities are due to DNA and chromatin damages in testes cells (Biswas, 2007).

Jha, et al, 2009 had explained these changes as the destruction of genes that responsible for spermatogenesis or the introduction of point mutation or the loss of a fragment of the genome which can all leads to abnormality in spermatogonia cells.
CP is an alkaline compound that causes DNA structural changes in cells also induce chromosomal abnormalities in germ cell line (Natarajan, 2005) as a result sperm head abnormalities increased dramatically. Varqhese and his colleges 2009 found that there is a strong correlation between DNA fragmentation and the physical shape of sperms, as well as the head shape of sperms.

The treatment with Etopsid caused inhibition in topoisomerase enzyme by making a 3 dimensional structure between the drug, the enzyme, and the DNA, this effect could lead to aneuploidy in germ cells. Cisplatin and Bleomycin has the same effect in patients that receiving chemotherapy (Philip et al, 2001). The physiological changes are caused by the modification of some enzymes and hormones activity, the treatment with CP caused decreased in HSD (Testicular – β – hydroxyl steroid dehydrogenase), 17 – β - hydroxyl steroid dehydrogenase enzyme activity, and testosterone hormone (Ray et al, 2005). Also the treatment with CP could cause oxidation stress because the metabolisms of CP produce ROS (Reactive oxygen species) (Stankiewic et al, 2002).

The mean of sperms abnormalities has changed throw the different stages of spermatogenesis, the percentage of these abnormalities has increase in proportional way with the time. The dose 5mg/kg show the highest percentage of sperm abnormalities even it's a low dose when compared to other doses used in this study and this could be explained to the long period of treatment (35 days) which can affect all the stages of spermatogenesis, and the spermatiogonia has received the CP from the time of formation and this step is a crucial step because of the high mitotic activity of these spermatogonial cells (Natrarjan and Tates, 1976).
The two other doses (10, 15 mg/kg) caused a similar effect on percentage of sperm abnormalities, a (14.75) and (13.19) respectively, and this is because CP effect was on the

primary spermatocytes stage and on the secondary spermatocytes, even with high concentration of the two used doses when compared to the 5mg/kg and the explanation of that is CP doesn't interfere with the last stages of spermatogenesis and the spermatid in these stages is almost mature and doesn't effected by mutagen compounds (Zdzienicka et al 1982).

Our results were compatible with the result of Codrington and his colleges 2007, they find that exposure to CP induce a great impact on DNA and spermatogenesis, CP caused DNA breakages in different stages of spermatogenesis and this could lead to mRNA and protein synthesis defect, especially capping protein and this effect will lead to loss fertility (infertility). The results obtained from this study also agree with the results of Cohen and his colleges 1992, they mention that CP caused hemorrhage in bladder and hyperplasia in the rats treated with it. Also CP caused hyperplasia and degeneration in epithelial cells of rat's embryo (Lucia and Azoubel 2005), Acrolyn is one of the metabolite compounds of CP and it's considered as a toxic compound for the cells both in vivo and in vitro and caused hemorrhage in bladder of patients treated with CP for long period (Sakata et al 1989).

The treatment with CP dose 100mg/kg caused complete destruction of ovarian follicles and suspends the ovulation process (Ray and Potu 2010), also the oxidative stress caused histopathological changes because of free radicals that attack the cellular components and lead to changes in structure and function of these components. Previous studies proved that CP induce oxidative stress and generate ROS (Salvia et al 1999).

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