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SPERM HEAD MORPHOLOGICAL DEFECTS AFTER CYCLOPHOSPHAMIDE TREATMENT

Health Science

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ABSTRACT

Cyclophosphamide (CP) is a class of medications called alkylating agents used against many cancers. It works by slowing or stopping the growth of cancer cells in the body. CP has genotoxic effect on normal cells of reproductive system. Present investigation is carried out to find the effect of Cyclophosphamide on sperm head morphology. Results showed that Cyclophosphamide treated rats showed a significant decrease in epididymal sperm count, testosterone level and sperm head abnormalities. This study indicates anticancer drug Cyclophosphamide caused the germ cell toxicity in male rats which ultimately affect the reproductive behavior of organism.

KEYWORDS

Cyclophosphamide, alkylating agent, spermatogenesis

INTRODUCTION

Cyclophosphamide (CP) is one of the most prevalent cytotoxic alkylating agents often used in cancer treatment (Fernandes et al, 2020). It is known to disturb spermatogenesis and induce testicular toxicity (Wtwt et al, 2012 and Namoju et al, 2021). CP is used for the treatment of cancer diseases such as Lymphoma, Myloma, Leukemia, Mycosis, Neuroblastoma Adenocarcenoma, Retinoblastoma, and Breast carcinoma (Shanafelt et al, 2007 and Cronin et al, 2018).

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). Its cytotoxic effects are the result of chemically reactive metabolite that creates DNA adducts, DNA-DNA and DNA-protein cross links, sister chromatid exchanges, chromosomal aberration and DNA strand breaks in many cell types, including germ cells (Condrington et al, 2007). One of the many consequences of Cyclophosphamide treatment is a negative effect on male fertility (Vaisheva et al, 2007 and Fernandes et al, 2020) by disturbing spermatogenesis, sperm parameters, and increase in oxidative stress. Present study was emphasis on the effect of CP on sperm head morphology and count.

MATERIALS AND METHODS

A. Drug

The anticancer drug Cyclophosphamide (Endoxan-N, CAS no. 50-18-0), with the chemical formula $C_7H_{15}Cl_2N_2O_2P$ and molecular weight, 261.086 g/mol. manufactured by Candila Healthcare Limited, Goa was used for the present experiments.

B. Animals

Male Wistar rat, *Rattus norvegicus* weighing between 250-300g obtained from Shree Farma, Bhandara (MS) were used. Animals were maintained in the laboratory under an absolute hygienic condition as per the recommended ethical standards. They were fed *ad libitum* with standard pellet diet and had free access to water, kept on a 12-h light-dark cycle.

C. Treatments

Animals were allowed 3 to 5 days acclimation period before being treated. They were randomly selected and divided into four groups with six animals in each group. For the chronic study, rats were treated with 5mg, 7mg and 10mg/Kg on body weight basis for six days a week for two weeks by intraperitoneal injections of Cyclophosphamide whereas control group received same amount of normal saline for two weeks.

D. Sperm Count

Animals were sacrificed by using chloroform 24 hours after the last day of each experiment. The cauda epididymis was removed and placed in a normal saline. The epididymis was minced into small pieces to allow the sperms to swim out. The sperm suspension thus obtained was centrifuged at1000rpm for 5min. After centrifugation, 1ml of the supernatant was taken and the epididymal sperm count was determined using Neubauer's hemocytometer. Data were expressed as number of sperms per mg weight of epididymis.

E. Sperm Head Abnormality

The cauda epididymis was removed and placed in normal saline solution. The epididymis was minced into small pieces to allow the sperms to swim out. The sperm suspension thus obtained was stained with 2% eosin solution and kept undisturbed for 1 hour. Smears were prepared using the above solution, air dried and fixed with absolute methanol for 5 min. The sperms from control and CP treated rats were examined for sperm head morphological abnormalities at 1000X magnification. Sperm head morphology was scored under the category of normal, amorphous head, headless sperm, deformed head, extremely curved head, reduced curvature of head, flattened or banana shaped head.

F. Statistical analysis:

Statistical analysis was reported in terms of mean ±SEM. Difference between the groups was statistically determined by Student 't' test (Dalgaard, 2008). The average data generated for the group of rats treated with Cyclophosphamide were compared with data on Vehicle control group of rats. A significant level of $P<0.05$ was accepted.

RESULTS

Cauda epididymal sperm count

Cyclophosphamide exposed experimental groups (5mg, 7mg and 10mg/kg) were differ statistically from the vehicle treated control group ($p< 0.01$) in total cauda epididymal sperm counts (Table 1).

Table 1: Cauda epididymal sperm count

Sr. No.	CP Treatments mg/KgBW for 2weeks	Sperm Count (x106/ml)
1	Control	82.833±0.543
2	5mg	65.000±0.577*
3	7mg	42.333± 0.760*
4	10mg	29.500±0.764*
Values are mean ± SEM, *Significant at P < 0.05		

Sperm head abnormality

The sperm from vehicle-treated control rat showed sickle shaped head (Fig. 1) while the CP treated sperm head abnormalities were classified into amorphous head, headless sperm, deformed head, extremely curved head, reduced curvature of head, flattened or banana shaped head (Figs.2-7). The head abnormalities were increased in a dose depended manner in CP received groups of rats (5mg, 7mg and 10mg/kg).

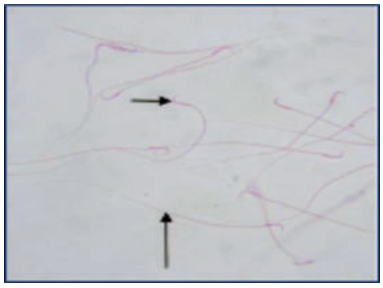
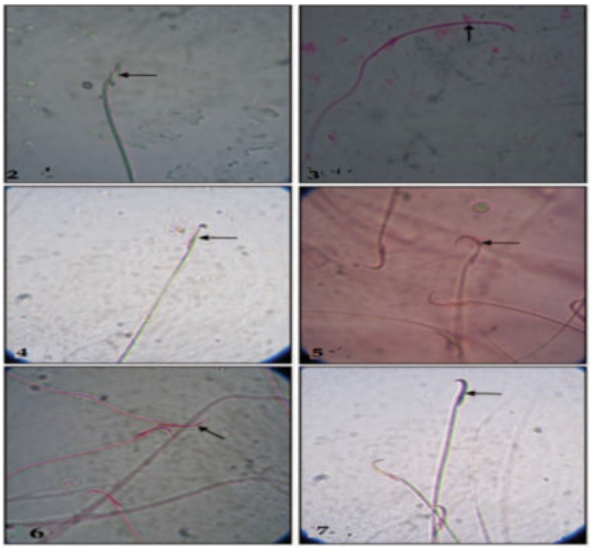


Fig.1: Normal Sperm



CP treated sperm head abnormalities- Fig 2: Amorphous head (arrow) X 1000; Fig.3: Headless sperm/decapitated/detached head (arrow) X 1000; Fig.4: Deformed head (arrow) X 1000; Fig. 5: Extremely curved head (arrow) X 1000; Fig.6: Reduced curvature of head (arrow) X 1000; Fig.7: Flattened or banana shaped head (arrow) X 1000.

DISCUSSION

Cyclophosphamide is a commonly used anticancer and immuno suppressive drug which is used primarily in cancer chemotherapy. CP interfere with Nucleic acids transcription and translation, it also affects the cell division because of the cumulative damages of DNA (Davidson et al, 2003).

Morphologically abnormal sperm are an indicator that a disruption in spermatogenesis, maturation, or semen handling has occurred. When an increased number of sperm in an ejaculate exhibit abnormal morphology, the vitality of the sperm that appears "normal" comes into question because both the normal and abnormal sperm present in a given ejaculate undergo spermatogenesis, maturation, and handling at approximately the same time. Sperm morphology estimation, therefore, should be used primarily as a quality control parameter for overall quality of the ejaculate (Cooper, 2011). The precise sperm morphology characteristic forms a first and obligatory step for critical evaluation of fertility. Similarly, animals and human studies have shown that sperm anomalies can be used as indicators and in certain cases, dosimeters of induced spermatogenic effects. Sperm head morphology assay in animals is in vivo cytogenetic assay which is important in the detection of those agents which cause mutations in germ cells.

The hook-shaped form of the rodent sperm head has been implicated in formation of sperm clusters that aid sperm motion (Moore et al, 2002). The morphological defects of sperm head after CP treatment resulted in drastic change in the shape like amorphous, compressed, smallness, loss of acrosome, acrosome thrown into bulb-like vacuolated acrosome, lateral displacement of head, loss of DNA due to vacuolation, extreme elongation of nucleus or stippling in the nucleus etc. Present study showed increased frequencies of abnormally shaped sperm or uncondensed spermatids are the results of mutational events. Thus, the spermatocytes treated with CP must have been blocked or delayed in their elongation and condensation during spermiogenesis. Also, the differentiated spermatogonia are most sensitive cells, due to their short mitotic cycle involving DNA synthesis and cell division. Moreover, the depletion or depopulation of the sperms and germinal epithelium was by the leaky or disrupted "blood-testis barrier". Lateral displacement of head has been recorded by Kaneto et al,1999. Decondensation of sperm nuclei or damage to DNA in the nucleus, nuclear swelling and nuclear elongation, nuclear area, curvature and length of spermatozoa significantly smaller than control has been described by Higuchi et al, 2001; Aguilar-Mahecha et al, 2002 and Barton et al, 2003 above results are in concordance to the present observation.

CONCLUSION

Thus, from the present it can be inferred that CP treatment has altered epididymal function resulting in inhibition of sperm maturation by decreasing dihydrotestosterone (DHT) at all doses and thus increased the number of spermatozoa head morphological defects. Also, it was clearly demonstrated that CP induced germ cell toxicity in rat by decrease in sperm count and increasing sperm head abnormalities and these changes were dose and duration dependent.

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