RESEARCH ARTICLE

Effect of anticancer drug Vincristine on sperm morphology in Albino rat

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ABSTRACT

Vincristine (C_{46} H_{56} N_4 O_{10}) is one of the most widely used effective curative for cancer. It is an indole-indolin alkaloid from periwinkle plant. The drug was administered intravenously to six adult male rats at dose levels of 0.06 and 0.12 mg/KgBW/day respectively. The object of the present work is to study the Vincristine effect on the reproductive accessory gland and testes. This drug prevents metastatic growth by preventing the formation of spindle fibers, thereby arresting mitosis without affecting replication of DNA. Vincristine affect on sperm morphology and resulting into primary and secondary sperm abnormality. The different sperms abnormalities were found to be in head, mid-piece and tail. The higher percentages of abnormalities are amorphous head, coiled tail and hook less head. The percentages of sperm abnormalities are more significant in low dose and high dose treatment as compare to vehicle treated control. From the foregoing mono-therapeutic study, it is concluded that this chemotherapy schedule belongs to the category of anti--androgenic.

Keywords: Vincristine, Sperm study, Albino rat

INTRODUCTION

Vincristine (C_{46} H_{56} N_4 O_{10}) is one of the most widely used effective curatives for cancer. It is an indole-indolin alkaloid from periwinkle plant. Vincristine isan important clinical agent for treatment of leukemia's, lymphomas, and testicular cancer (Jorden *et al*., 1985). The biological activities of these drugs can be explained by their ability to bind specifically to tubulin and to block the ability of the protein to polymerize into microtubules. Through disruption of the microtubules of the mitotic apparatus, cell division is arrested in metaphase. In the absence of an intact mitotic spindle, the chromosomes may disperse throughout the cytoplasm (exploded mitosis)or may clump in unusual groupings, such as balls or stars. The inability to segregate chromosomes correctly during mitosis presumably leads to cell death due to chromosomal mutation.

MATERIALS AND METHODS

Animals and Treatment: Adult male albino rats of 200 to 250gms were obtained from the animal house facility of R.C.Patel Pharmacy College Shirpur

Shirpur. After a week of acclimatization to laboratory conditions Vincristine (C_{46} H_{56} N_4 O_{10}) dissolved in saline was administered intravenously. The control animals received same amount of saline (Table-1).The animals were sacrificed using chloroform 24 hours after the last day of each experiment. Immediately the organs were excised. Both the cauda epididymis wasutilized for sperm analysis.

The spermatozoa present in the cauda epididymis were collected after mincing/slicing the tissue in a cavity block containing 1ml of physiological saline and centrifuged at 600 rpm for 1 minute with a drop of 5% aqueous eosin (WHO, 1999). Coslab digital microscope with Phase contrast adjustment was used to observe the sperms. All evaluations were done at 25X, 45X and 100X.

Assessment of sperm morphology

The saline solution of cauda epididymis prepared for studying the sperm concentration was directly observed several times for assessing the sperm morphology.

OBSERVATIONS AND RESULTS

In the present study the Vincristine (VCR, Cytocristin) drug was used to find out the changes in the sperm of albino rat *Rattus rattus*. They include:

Sperm morphology

1 Head defects (Table-2)

Hook less head: The population of spermatozoa with hook less head sperm abnormality fig.3)was significantly increases in low dose ($p<0.01$) and high dose ($p<0.001$).

Banana shape head: The population of sperms with banana shape head abnormality (fig.4) was significantly decreased in low dose ($p<0.05$) and increased in high dose ($p<0.01$).

Amorphous head: The population of spermatozoa with amorphous head abnormality (fig.5) was significantly increased in low dose and high dose as compare to vehicle treated control ($p<0.001$).

Table 1: Experimental Design for Vincristine Treatment

Number of animals and sex	Treatment	Dose (mg/Kg BW)	Route	Duration
6 males (Experimental)	Vincristine	0.06 mg daily	I.V.	15 days
6 males (Experimental)	Vincristine	0.12 mg daily	I.V.	15 days
6 males (Control)	Saline	E.V.	I.V.	15 days

Abbreviations:- E.V. = Equal Volume, I.V. = Intra muscular, BW=Body weight.

Table 2: Effect of 0.06 mg and 0.12 mg VCR / day for 15 days on sperm morphology and percentage occurred of different sperm abnormalities (values are mean ± SE).

Sr. No.	Mean Sperms Abnormality(%)	Control	0.06mg / kg BW/ day for 10 days	0.012 mg / kg BW / day for 10 days
1	Hook less head	2.20±0.27	2.52±0.16 #	5.91±0.15###
2	Banana shape head	2.61±0.29	2.02±0.18 #	3.27±0.16##
3	Amorphous head	1.90±0.28	1.94±0.21#	13.30±1.44###
4	Pin head	0.99±0.11	1.51 ±0.11###	0.75 ±0.13#
5	Tailless head	0.43±0.19	2.53±0.17##	0.65±0.07#
6	Bent mid piece	3.80±0.17	3.73±0.23*	3.76±0.22*
7	Curved mid piece	2.71±0.23	1.53±0.26##	3.56±0.13##
8	Headless tail	1.26±0.21	1.35±0.25*	1.35±0.08*
9	Bent tail	3.10±0.12	4.06±0.72#	2.46±0.34#
10	Curved tail	0.95±0.10	4.27±0.18###	5.70±0.31###
11	Coiled tail	2.48±0.35	6.46±0.35##	4.44±0.16##
12	Looped tail	3.32±0.19	2.50±0.30#	2.43±0.14##
	Total mean sperm abnormality (%)	**25.75±0.35**	**34.42±0.36**###	**47.58±0.62**###

#p<0.05, ##p<0.01, ###p<0.001 and *Insignificant

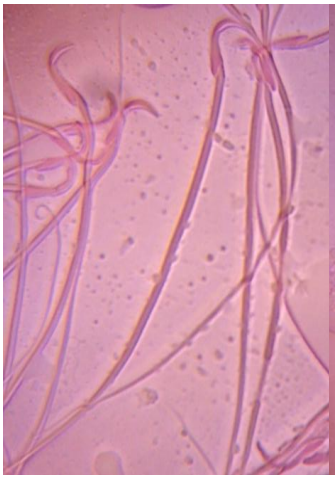

Fig.1a: Microphotograph showing swarms of normal sperms (arrow) X 1000.

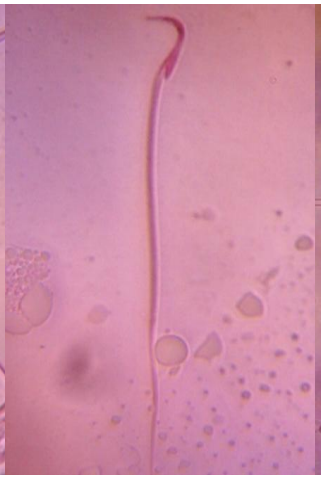

Fig. 1b: showing single sperm with normal head(arrow), mid piece (arrow head) and tail(long arrow)X 1000.

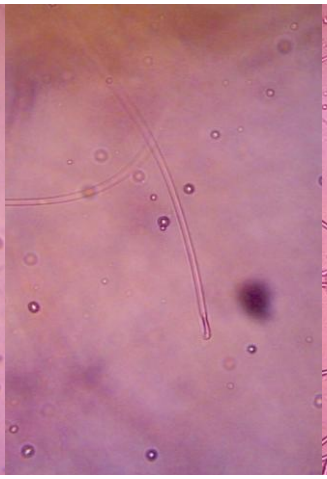

Fig. 3: Microphotograph of sperm with hook less head (arrow) X 400.

Fig. 4: Photograph of few sperms showing banana shape head (arrow) X400

Fig. 5: Photograph of sperm showing amorphous head (arrow) X 400.

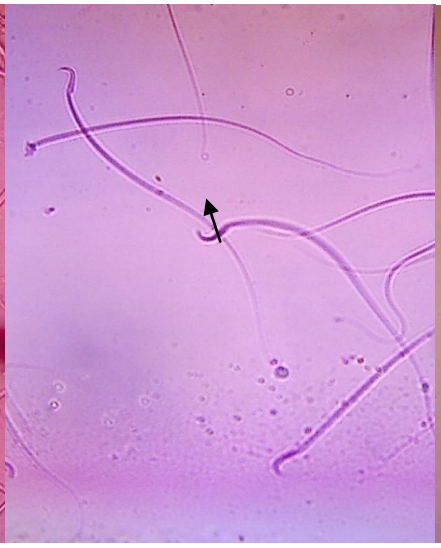

Fig. 6: Photograph with pin headed sperm (arrow) X 400.

Fig.7: Photograph of sperm showing tailless head (arrow) X 400.

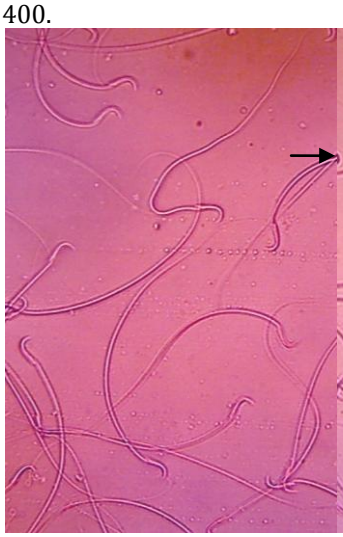

Fig. 8: Microphotograph showing sperm with bent mid piece (arrow) X 400.

Fig.9 : Photograph of sperm with curved midpiece (arrow) X 400.

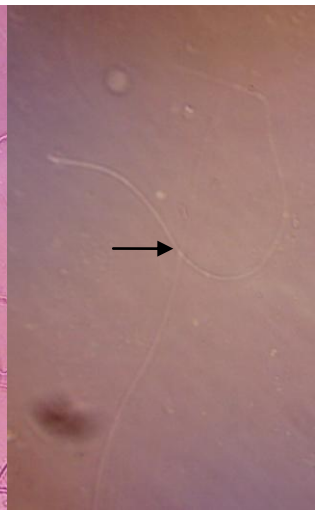

Fig.10: Photograph of sperm with headless tail (arrow head) X 400.

Fig.11: Photograph of sperm with bent tail (arrow) X 400.

Fig.12: Photograph of sperm with curved tail (arrow) X 1000.

Fig. 13: Microphotograph showing sperm with coiled tail (arrow) X 400.

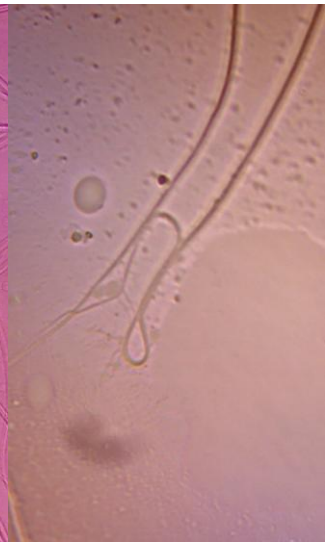

Fig.14: Photograph of sperm with looped tail (arrow) X 1000.

Pin head*:* Occurrence of pin head sperm abnormality (fig.6) was significantly ($p<0.001$) higher in low and high dose treatment as compare to control.

Tailless head*:* Sperm abnormality with tailless head (fig.7) had lesser in control group and it was significantly increased ($p<0.01$) in low dose and high dose treatment.

2 Mid-piece defect (Table 2)

Bent mid- piece: The spermatozoa of rats in control group show more sperms withbent mid piece(fig.8). The differences of means were not significant in low dose and high dose treatment.

Curved mid-piece: Occurrence of Curved mid-piece abnormality of sperms (fig.9) were significantly decreased ($p<0.01$) in low dose and further increased in high dose.

3. Tail defects:

Headless tail*:* Occurrence of headless tail sperm abnormality(fig.10) was not significant in low dose and high dose treatment.

Bent tail*:* The spermatozoa with bent tail abnormality (fig.11) was significantly increased (**$p<0.01$**) in low dose and decreased in high dose.

Curved tail *:* The sperm abnormality with curved tail (fig.12) was significantly increased (**$p<0.01$**) in low dose and high dose treatment.

Coiled tail*:* The population of spermatozoa with coiled tail (fig.13) was significant (**$p<0.01$**) in low and high dose regimen.

Looped tail*:* Occurrence of looped tail (fig.14) abnormality of sperms in control was significant ($p<0.01$) in low and high dose treatment.

DISCUSSION

The sperm morphology was used in this study to evaluate the effects of Vincristine by using albino rat model. The drug can affect on reproductive system resulting in the sperm production. Abnormal forms of spermatozoa occurred in all mammals (Mann & Mann, 1981). Vinca alkaloid is an anti-neoplastic and anti-carcinogenic drug. The drug arrest cell growth through its effects on cytoskeletal elements and inhibits spindle formation essential for normal cell division. Vincristine acts as a cytotoxic agent to differencing spermatogonia (Lu & Meistrich, 1979). Vincristine works as ancolytic agent, preferentially kill cells of specific stages of the spermatogonic pathway at doses with clinical range for human.

Abnormalities induced due to Vincristine in present study included primary and secondary abnormalities. In present study observed both the types, primary types reported in our results were hook less head, headless tail and tailless head. Secondary types of abnormality obtained in present study were, banana shape head, amorphous head, bent tail, curve tail, bent mid piece, coiled tail, looped tail and curved mid piece. Our results were in accordance to Saba *et al.,* 2009. In recent study I got one more additional types of abnormality includes pin head. Thus in the

present study Vincristine at dose level 0.06 mg and 0.12 mg resulted in abnormal morphology. The results were more predominant in high dose as compared to control and low dose treatments.

Vincristine is an anti-proliferative, radiomimetic, anti-carcinogenic drug. This drug arrests cell growth through their effect on cyto-skeletal elements and tubulin formation. As a result there is no formation of spindle which is essential for normal cell division. Acrosomal head shape sperms is disrupted from the normal by affecting the tubulin polymerization in the microtubule and by inhibiting axoplasmic flow (Avadhani and Kumar, 1994). Vincristine induced all the wide range of abnormalities depending upon dose level, amorphous head followed by bent mid piece and bent tail.

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