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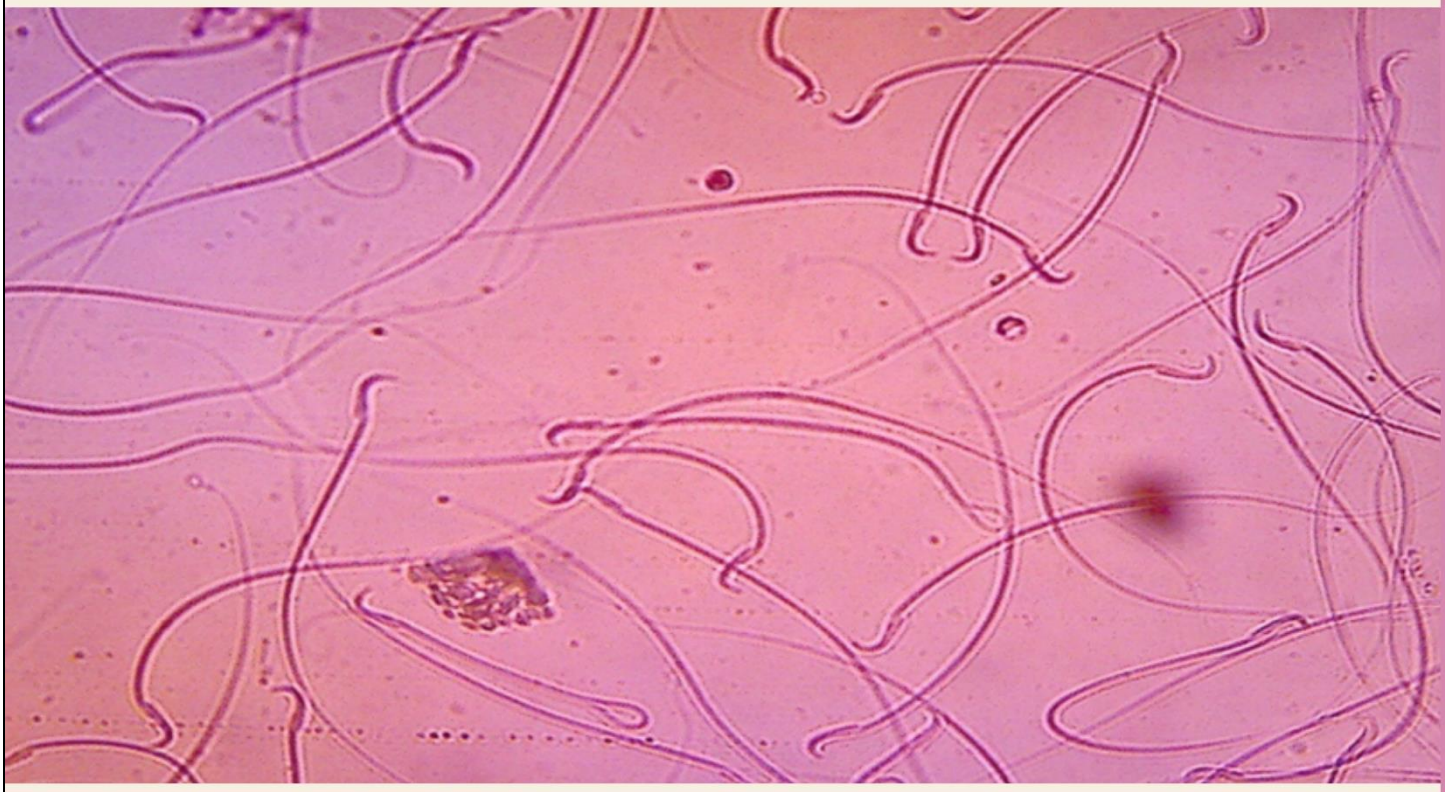
FINAL TECHNICAL REPORT OF

UGC MINOR RESEARCH PROJECT

# Effect of Vinca alkaloid Vincristine on sperm study in albino rat (*Rattus rattus*)

Dr. Rajeshwar M. Chaudhari



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# Effect of Vinca alkaloid Vincristine on sperm study in albino rat (*Rattus rattus*)

**(ISBN: 978-93-48620-10-1)**

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## *PREFACE*

*It is our pleasure and privilege to present this book on "Effect of Vinca alkaloid Vincristine on sperm study in albino rat (Rattus rattus)" in the hand of research student those who are doing research in anticancer drugs, reproductive physiology and andrology. Andrology is one of the recent branches in Life science and is not much more explore. It was difficult task for us to write compressive book on this topic. In this book all abbreviation and tables are explain. This book is written in easy and simple language so student will prefer it. This book fulfills all the requisites of the research scholars and stakeholders. Suggestions and corrections if any will be accepted from the students, teachers and stakeholders.*

*I am grateful to the University Grants Commission (UGC), Western Regional Office (WRO), Pune for providing the financial assistance for the implementation and completion of this research project. We are very much tankful to Bhumi Publications, Kolhapur for publishing this book in a very short time. We are also thankful all the staff of Bhumi Publishing, Kolhapur for their cooperation to printing and publishing this book.*

**- Dr. Rajeshwar M. Chaudhari**
(Principal investigator)

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*I am grateful to the University Grants Commission (UGC), Western Regional Office (WRO), Pune for providing the financial assistance for the implementation and completion of this research project. I am extremely thankful to Mr. S.B. Patil (Principal, Shri. S.I. Patil, Arts, G. B. Patel Science &STSKVS Commerce College, Shahada), Hon'. Bapusaheb Deepak P. Patil, (President, Pujya Sane Guruji Vidhya Prasarak Mandal, Shahada), Dr. A.S. Patil, Director of P.G Shri. S.I. Patil Arts, G. B. Patel Science & STSKVS Commerce College, Shahada) for their moral thrust and support.*

*I would like to thanks all the teaching staff of Department of Zoology, Mr. M.S. Manik (Ex. Laboratory Assistant), Mr. S.V. Patil (Ex. Laboratory Attendant) and Mr. Gopal Sonar (Clerk) for helping me in many ways during the tenure of project.*

*I am also thankful to my dear students Mr. Ganesh Sonwane and Pitambar Padvi for helping me for handling and transporting the experimental animals.*

*Lastly, I am grateful to my wife Mrs. Anjali Chaudhari and my son master Yash, without whose support I could not have completed this project.*

**- Dr. Rajeshwar M. Chaudhari**

(Principal investigator)

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## GENERAL INTRODUCTION

## VINCRISTINE

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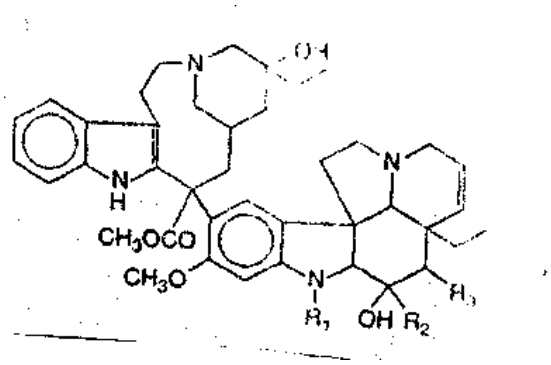
|| || ||

– CH – C – $OCH_3$ – O – C – $CH_3$

**Molecular formula**



**Structural formula of Vincristine**

Vincristine ($C_{46}$ $H_{54}$ $N_4$ $O_{10}$) is one of the most widely used effective curative agents for cancer. It is an indole-indolin alkaloid extracted from periwinkle plant *Vinca rosea*. It is an important clinical agent for treatment of leukemia's, lymphomas, and testicular cancer (Jorden *et al.,* 1985). The biological activities of this drug can be explained by their ability to bind specifically to tubulin and to block the ability of the protein to polymerize into microtubules. The cell division is arrested at metaphase is mainly due to disruption of the microtubules of the mitotic apparatus. However, 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. Thus, the chromosomes lose its ability to segregate correctly during mitosis presumably leads to cell death due to chromosomal mutation.

This drug is also known to arrest both mitosis and meiosis of male germ line results in the death of cells. Indeed, these cells die through apoptosis and are exfoliated into the lumen of seminiferous tubules (Averal *et al.,* 1995). Administration of vincristine to adult male rat results in the arrest of spermatogenesis that lead it to azoospermia. It is reported that in the lumen of disorganized seminiferous tubules there are giant cells of different sizes are present. Similarly, the cells appear to originate from the spermatogonia due to

endomitosis (Stanley and Akbarsha 1992a). Thus, vincristine affects the mitosis and meiosis that results in impairment of spermatogenesis. However, the cause of apoptosis may be directly corelated with sperm count, sperm morphology and its motility. Another cause for this reason can be due to the deprivation of blood testosterone level and hence the changes in sperm might be due to anti-androgenic and anti-spermatogenic effects of vincristine.

Vinca leaf extract affects by relative decrease in percentage of motile sperm and introduce the abnormality in the sperm including the categories like double-tailed, detached head, detached tail, mid-piece bending, irregular head and mid-piece loop formation. It is also known to decrease significant relative percentage of live sperm. However, sperm count is considered to be one of the important parameters which affect fertility.

*Vinca rosea* extract that generally lead to decrease in sperm count has opened up a promising avenue for anti-fertility methods and therefore toxic in effects. According to them sperm have two principal attributes, namely motility and fertilizing ability. Motility is an important pre-requisite for fertilization in the case of internally fertilizing organisms. Thus, any negative impact on motility would seriously affect fertilizing ability. *Vinca rosea* appears to exhibit the deleterious effect by increasing the relative percentage of abnormal sperms and decreasing live sperm of cauda epididymis (Murugavel *et al.,* 1989).

Present work is a little piece of effort in terms of effect of vincristine on sperm count, sperm morphology and sperm motility. It is point out that very little work has been done in this direction except few works of Gobello and Corrada, 2002 and Sarastis *et al.,* 2000, but the detail study related to sperm is not available till now.

**HISTORICAL REVIEWS**

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. The beneficial properties of the Madagascar periwinkle plant, *CatharanThus, roseus* (formerly called *Vinca rosea*), a species of myrtle, have been described in medicinal folklore in various parts of the world. While exploring claims that extracts of the periwinkle might have beneficial effects in diabetes mellitus, Noble and coworkers (1958) observed granulocytopenia and bone marrow suppression in rats, effects that led to purification of an active alkaloid. Other investigations, by Johnson and associates (1963) demonstrated activity of certain alkaloidal fractions against an acute lymphocytic neoplasm in mice. Fractionation of these extracts yielded four active dimeric alkaloids; vinblastine, Vincristine, vinleurosine, and vinrosidine. Two of these, vinblastine and Vincristine, are important clinical agents for treatment of leukemias, lymphomas, and testicular cancer. Another agent, vinorelbine, has important activity against lung cancer and breast cancer (Jorden *et al.,* 1985).

The vinca alkaloids are cell-cycle specific agents and, in common with other drugs such as colchicine, podophyllotoxin, and taxanes, block cells mitosis (George *et al.,* 1965; Bensch and Malawista, 1968; Dustin, 1984). 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. When cells are incubated with Vincristine, dissolution of the microtubules occurs, and highly regular crystals are formed that contain 1 mol. of Vincristine per mol of tubulin. 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. Both normal and malignant cells exposed to vinca alkaloids undergo changes characteristic of apoptosis (Smets, 1994). The Vinca alkaloids are also known to arrest the cell cycle at the stage of synthetic phase and to suppress nucleic acid synthesis (Creasy and Markiw; 1964; Cook *et al.,* 1978).

Bensch and Malawista (1968) stated that the precise mechanism of action of vincristine in arresting cancer is not fully known. Still, it can be suggested that tubulin left unpolymerised or produced due to microtubule disassembly is known to be acted upon by Vinca alkaloids and known as Vinca crystals.

Joshi and Ambaye (1968) administered a crude alkaloidal extract of Vinca to male rats and noticed marked changes in the testis. The testis appeared flaccid and seminiferous tubules showed reduction in size, the spermatogenesis was suppressed and possessed up to primary spermatocyte and autolysis of spermatogenic elements.

De Krester *et al.* (1972) stated that in adults and in children basal and / or stimulated FSH levels are frequently used to assess reproductive capacity. Azoospermia was documented confirming the close relationship between elevated FSH levels and germ cell damage.

Sherins and Vita (1973) observed marked atrophic changes in the testis with most seminiferous tubules consisting chiefly of Sertoli cells after vinca alkaloids administration Thus, showing azoospermic condition.

Sherins *et al.* (1978) reported gynaecomastia and germinal aplasia in the Hodgkins disease patient after vincristine treatment as well as lower testosterone levels.

Brooks (1981) investigated than the regressive and degenerative changes in the seminiferous tubules reflecting the antiandrogenic action because it has been reported that most stages of spermatogenesis, particularly meiosis.

Chapman *et al.* (1981) described 80-90% incidence of azoospermia with vincristine treatment, lower testosterone values but high LH levels indicative of compensated Leydig cell failure and poor sperm quality.

De Vita (1981) reported germ cell depletion in the testis with antitumor drug, vincristine and therefore the incidence of high rate (80 to 90%) of azoospermia. Also observed normal ranges of testosterone. According to them Leydig cell damage also occurs but the injury appears to be less extensive.

Hoffer *et al.* (1981) noticed a change in the location of the cytoplasmic droplet from the proximal to distal end of mid-piece in an increasing percentage of spermatozoa during their epididymal transit in pig tail monkey, *Macaca nemestrina*.

Mann and Lutwak Mann (1981) stated that suppression and degeneration of Leydig cell point to antiandrogenic action. The regressive and degenerative changes in seminifeorus tubules reflect the anti-androgenic action because it has been reported that androgen is essential for most stages of spermatogenesis, particularly for meiosis.

Russell *et al.* (1981) studied that (VCR) and (VLB) both these drugs are known to cause disruption of spermatogenesis by disrupting microtubule leading to pathological changes.

Wyllie (1981) opined that extensive vacuolation in the principle cells and apical cells reflect a direct action of VCR in causing mitochondrial swelling and the consequent hypoxia.

Wilson *et al.* (1982) demonstrated that vincristine causes depolymerization of the existing microtubules.

Chinoy and Geetha Ranga (1983) observed that *Vinca rosea* leaf extract (1 mg/0.2 ml/day/rat) treatment manifested antiandrogenic and antifertility effects in intact male albino rats. According to them the antifertility effects were attributed to reduction in sperm density, percent motility and alteration in morphology of cauda spermatozoa after treatment for 7 and 15 days. The sperms were found to be sluggishly motile and were unable to fertilize the normal cycling fertile females. They further noticed that Vinca alkaloid treatment manifested a strong anti-androgenic effect, thereby causing reduction of most of the androgenic parameters in androgen dependent target organs namely the organ weight, activities of acid phosphatase, levels of fructose in seminal vesicle besides bringing about a significant decrease in the body weight of the treated rats elucidating an androgen deprivation effect to the target organ. The authors also stated that the antiandrogenic effect of Vinca alkaloids are coupled with its anti-anabolic action, as is evident from the decrease in body weight and those of the reproductive organs as well as marked histological alteration in testis, caput and cauda epididymides. According to them mechanism of action of Vinca rosea leaf extract seems to be via causing androgen deprivation to the target organs which results in alteration in their histo-physiology.

Jordan *et al.* (1985) examined vincristine and its derivatives for their abilities to inhibit net tubulin addition at the assembly ends of bovine brain microtubules at steady state. Vincristine caused 25% inhibition.

According to Chakraborti and Mukherji (1988) vinca rosea (Linn) (*CatharauThus, roseus*) of the family Apocynaeceae, with proven efficacy is anticarcinogenic, from which vincristine has been extracted which is antispermatogenic and antiandrogenic.

Rao *et al.* (1988) described gonadal dysfunction with vincristine.

Murugavel *et al.* (1989) stated that vinca leaf extract did not affect body weight, sperm count decreased significantly to 67%, relative percentage of motile sperm decreased by 44% on treatment, relative percentage of abnormal sperm, categories like double-tailed, detached head, detached tail, mid-piece bending, irregular head and mid-piece loop formation were predominant, relative percentage of live sperm decreased significantly.

Sperm count is considered to be one of the important parameters which affect fertility. Decrease in sperm count of *Vinca rosea* extract opens up a promising avenue for anti-fertility methods and therefore toxic effects. According to them sperm have two principal attributes, namely motility and fertilizing ability. Motility is an important pre-requisite for fertilization in the case of internally fertilizing organisms. Thus, any negative impact on motility would seriously affect fertilizing ability. In increasing the relative percentage of abnormal sperms and decreasing the relative percentage of live sperm vinca rosea appears to be deleterious at the level of cauda epididymis.

Hansen *et al.* (1990) with vincristine and cisplatin treatment observed azoospermia. Regarding the Leydig cells, only compensatory functional insufficiency was noted.

Russell and Russell (1991) are of the opinion that there is general agreement that various anti-neoplastic drugs exert a prejudicial effect on spermatogonia provoking the death of these cells including Cisplatin, Vincristine etc. According to them microtubules constitute one of the important aspects of the cytoskeleton of the Sertoli cell, and play a critical role in determining the shape of the cell which are vulnerable to VCR treatment and thereby causes early exfoliation of germ cell.

According to Stanley and Akbarsha (1992) vincristine is one of the most widely used effective curatives for cancer. At present it is one of the drugs used in combination chemotherapy regimens. It is an indole – indolin alkaloid obtained from the periwinkle, *Vinca rosea* or prepared from vinblastine another Vinca alkaloid. It is proposed that this drug prevents metastatic growth by preventing the formation of spindle fiber, thereby arresting mitosis, without affecting replication of DNA. They emphasized that when used as anticancer, this drug is known to cause several toxic side effects, yet its toxic effects on the male reproductive system have been only poorly studied.

Stanley and Akbarsha (1992a) stated that treatment of vincristine sulphate to adult male albino rats' results in arrest of spermatogenesis and in azoospermia, the highly disorganized seminiferous tubules contained giant cells of different sizes in the lumen, the cells appear to originate from the spermatogonia due to endomitosis.

Stanley and Akbarsha (1992b) reported the fate of giant cells formed in the seminiferous tubule lumen in the testis of albino rats after vincristine treatment, by probably affecting spermatogenic mitosis. These giant cells in the caput epididymis were spherical and ranged in diameter from 10 to 40μm. Each cell contained a single nucleus, the size of which differed among the cells. The chromatin appeared as clumps, dispersed inside

the nucleus. These cells showed no trace of cytolysis or phagocytosis. These giant cells densely pack the cauda epididymal lumen and appeared fragmented due to cytolysis and phagocytosis by phagocytes as it happens in the case of dead sperm. The authors suggested that since these giant cells remain intact in the caput, these giant cells can be isolated free from contamination by flushing of the caput epididymal tubule, for studying the nucleo-cytoplasmic ratio, ploidy, DNA content, tubulin etc. these can be cultured for nuclear transplantation experiments involving polyploid nuclei, in elucidating nucleocytoplasmic interactions in spermatogenic cells. The giant spermatogonial cells would be of two advantages in this regard, namely (i) the large size, overcoming the technical difficulty of smallness of the nuclei of renal adenocarcinoma cells which are used to achieve successful transplantation, (ii) the giant polyploid cells are germinal rather than somatic. Giant Hela cells used in elucidating nucleo-cytoplasmic interactions are produced in vitro by subjecting Hela cells to X-irradiation, which treatment invariably results in the formation of nuclear fragments therefore these giant cells produced in vivo would overcome this problem.

Akbarsha *et al.* (1995) tested the effect of vincristine (VCR), currently in use as a mitotic spindle poison in combination chemotherapeutic regimens for cancer, on the Leydig cell and the accessory reproductive organs in the light of the reports that it affects spermatogenesis. They administered VCR to Wistar strain male albino rats at a daily dose of 20μg for 15 days. According to them the seminal vesicle and ventral prostate were regressed; lumen of the caput epididymis lacked sperm but contained giant cells, in the cauda, giant cells appeared disintegrating. Secretory acini / follicles of the seminal vesicle / ventral prostate exhibited decreased secretory activity. Fructose content of the seminal vesicle also decreased. Cytoplasm of Leydig cell of treated rats appeared highly vacuolated and the nuclear chromatin-depleted. The authors interpreted that the regression and other derangements in the accessory reproductive organs appear to be manifestation of the toxic effect of the drug on Leydig cell.

Averal *et al.* (1996) administrated Wistar strain male albino rats with Vincristine (VCR) sulphate (10μg/day for 15 days); epithelial cell types of the caput (zone II) and cauda (zone V) were studied light microscopically adopting semi thin sectioning. VCR caused conspicuous pathological changes in the principal and apical cells of the caput and the clear cells of the cauda. According to them the study points to toxic effect of VCR on these cell types, suggesting impairment of epididymal function, particularly concerning

sperm maturation and endo-cytotic removal of the contents of the cytoplasmic droplets and dead sperm.

Bokemeyer *et al.* (1996) described azoospermia with vincristine treatment.

Robbins *et al.* (1997) studied the effects of chemotherapy in children suffering from Hodgkins's disease. They concluded that the patients receiving chemotherapy induced disomies and diploidies due to chromosomal aneuploidy.

Foresta *et al.* (2000) stated that chemotherapy reflects the alteration of testicular structure and resulted in to severe oligozoospermia. The sex chromosome abnormality passes to the children.

Saratsis *et al.* (2000) administered vincristine intravenously at dose level 0.6 mg in dog and evaluated semen parameters like semen volume, sperm concentration, total spermatozoa per ejaculate, percentage of progressive motility, percentage of dead spermatozoa and percentage of abnormal spermatozoa. He reported that semen quality transiently deteriorated during treatment.

Saba *et al.* (2009) injected ethanolic extract of the whole fruits of *Lagenaria breviflora* to the Wistar rats. The extract caused morphological alteration of sperm cells and resulted secondary abnormality in sperms. He further stated that the abnormality induced in sperm were bent mid piece, curved midpiece, bent tail, curved tail, normal tail without head, normal head without tail, looped tail and coiled tail. It was resulted due to interference with maturation stage of spermatogenesis. He further noted that the sperm count was lowered in comparison with control group.

Avadhani and Arunachalam (2014) administered Vincristine sulphate at different dose level to Swiss albino mice. They reported abnormal sperms from 0.74 to 11.16% in treated mice. The sperm shape reported were amorphous type predominantly, followed by hookless banana, folded and double headed or tail. In high dose drug induced nearly azoospermia.

Hasim *et al.* (2014) demonstrated that the natural incidence of abnormal spermatozoa was 13% after exposure to vincristine for 2 weeks, increased the number of morphological abnormal spermatozoa was four folds. It was occurred due to the damage of DNA and was detected by using FISH molecular test.

## MATERIALS AND METHODS

### About the drug

Vincristine is also known as vincristin sulphate and obtained as cytocristin as its brand name. It is manufacture by Cipla Ltd. It is purchased from market and available in aqueous form as one mg one ml**.** Doses were decided from the previously carried out experiments.

### Treatment of animals

In present study animals were collected from the animal house of R.C. Patel College of Pharmacy, Shirpur, Dist Dhule (M.S.). Animals brought with special care to the College departmental laboratory and acclimatized for a week. Even though the order rodentia is highly exploited for the experimental purposes are easily available and can undergo all tests. The six adult male rats weighing between 284 to 360 gms were selected for the laboratory tests without any difficulty, hence is suitable for experimental work.

### Handling of animals

A special care was taken while injecting the drug. The animal was hold fast out of the cage by its leg and drug was injected intravenously with disposable insulin syringe.

### Experimental protocol

In all three sets of experiments using high and low-doses of Vincristine (0.06 and 0.12 mg/kgBW/day) were performed for the present study for the duration of 10 days (Tables 1). Animals were sacrificed 24 hours after the last day of each experiment by exposing to chloroform. Immediately the organs were excised. Both the cauda epididymis was utilized 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.

**Table 1: Experimental Design for Vincristine Treatment**

| No of animals and sex | Treatment | Dose (mg/Kg BW) | Route | Duration |
|---|---|---|---|---|
| 6 males (Experimental) | Vincristine | 0.06 mg daily | I.V. | 10 days |
| 6 males (Experimental) | Vincristine | 0.12 mg daily | I.V. | 10 days |
| 6 males (Control) | Saline | E.V. | I.V. | 10 days |

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

**Assessment of sperm motility**

Motility of sperms in the experimental and control animals were determined by putting a drop of semen on the cavity slide covered with a thin glass slip. Cavity slide was used since depths less than 20µm constrains the rotational movement of spermatozoa. The freshly made wet preparation was left to stabilize for approximately one minute.

The motility of spermatozoa from treated groups was observed as follows.

a. Rapid progressive motility
b. Slow or sluggish progressive motility
c. Non-progressive motility
d. Immobility
e. Rotational motility

**Sperm count**

Sperm count was done by using Neubauer's haemocytometer. The sperms were counted in five Thomas ruled chambers after charging the haemocytometer with the above solution and calculated by using the formula 50,000 n x d where 'n' is the number of sperms and 'd' is dilution which was 1ml.

**Assessment of sperm morphology**

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

**Classification of sperm morphology**

**Normal spermatozoa:**

| | | |
|---|---|---|
| Head Region | **:** | Curved |
| Acrosomal Region | **:** | Well defined, occupying 40-70% of the head area |
| Mid Piece Region | **:** | Slender, about one and half times the length of the head and attached axially to the head |
| Tail Region | **:** | Straight, uniform, thinner than the middle piece, uncoiled |

**Abnormal spermatozoa**

**Head defects**

Namely large, hookeless, banana shaped, flattened, pin head, amorphous, vacuolated head (>20% of the head area occupied by unstained vacuolar areas) heads with small acrosomal area (<40% of head area) and headless.

**Neck and midpiece defects**

Namely 'bent' neck (the neck and tail form an angle greater than 90% to the long axis of the head) asymmetrical (the neck may be absent or bifurcated or swollen or insertion of the midpiece into the head, thick or irregular mid piece, bent mid-piece, abnormally thin midpiece (i.e. no mitochondrial sheath) or any combination of these.

**Tail defects**

Namely short, multiple, hairpin, broken tails, bent tails (>90%) tails of irregular width, coiled tails, looped tail, double tails, curve tail, rudimentary or absent or any combination of these.

**OBSERVATIONS**

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:

**1. Vehicle Treated Control**

**Sperm Count**

Sperms from cauda epididymis were counted in Neubaure's slide. As the animal was in breeding stage the epididymis of vehicle treated group showed swarms of spermatozoa ($11.8 \times 10^6$) and hence a condition of normospermia existed.

**Sperm motility**

We have recorded sperm motility by capturing the video but poor quality of it could not succeed to keep the record. However, rapidly fast-moving sperms were observed. The types of motilities were rapid progressive motility, slow or sluggish progressive motility, non-progressive motility, immobility and rotational motility. The numbers of sperms showing sluggish progressive motility, non-progressive motility, Immobility and Rotational motility were few in quantity

**Sperm morphology**

The head was hooked; acrosomal region was well defined, occupying 40-70% of the head area. Midpiece region was slender, about one and half times the length of the head and attached axially to the head. Some sperms were bent and had curved mid-pice (figs. 8&9). The tail region was straight, uniform, thinner than the midpiece and uncoiled. Very few sperms were had bent tail, curve tail, coiled tail, and looped tail (figs.11, 12, 13, 14&15).

**2. Low Dose Treatment**

**Sperm count**

Sperms from cauda epididymis were counted. Reduction in the number of sperms was observed in the aqueous saline solution (Table-2 &bar diagram) Thus, a significant condition of oligospermia was observed ($P < 0.001$).

**Sperm motility**

Some sperms showed 'progressive movement', However, most were immotile, some with 'non-progressive movement' and largely remaining sperms were showing "rotational movement". We were not able to keep a record of motility because of poor video quality.

**Sperm morphology**

Several observations of cauda epididymis with measured quantity of saline in the cavity slide showed following types of sperm abnormalities:

**I. Head defects:**

i. Hook less head (fig.3).
ii. Banana shape head (fig.4).
iii. Amorphous head (fig.5).
iv. Pin head (fig.6a, b).
v. Tailless head (fig.7).
vi. Headless tail (fig.10).

**II. Mid-piece defect:**

i. Bent mid- piece (fig.8).
ii. Curved mid-piece (fig.9).

**III. Tail defects:**

i. Headless tail (fig. 10).
ii. Bent tail (fig. 11)
iii. Curved tail (fig.12)
iv. Coiled tail (fig.13)
v. Looped tail (fig.14, 15)

**3. High Dose Treatment**

**Sperm count**

Sperms from cauda epididymis were counted showed reduction in their number of (Table-2 &bar diagram). Thus, a significant condition of oligospermia was observed ($P < 0.001$).

**Sperm motility**

Some sperms showed “progressive movement”, However, most were immotile, and some were showing “non-progressive movement”. At the same time largely, some remaining sperms were showing “rotational movement”. Of course, due to poor video quality it was not possible to keep the record.

**Sperm morphology**

Several observations pertaining to cauda epididymis showed following sperms abnormalities:

**a) Head defects:**

i. Amorphous head (figs. 5).
ii. Hookless head (figs. 3).
iii. Headless tail (fig. 10)
iv. Banana shape head (figs. 4).

**b) Mid-piece defect:**

i. Bent mid- piece (fig. 8).
ii. Curved mid-piece (figs. 9).

**c) Tail defects:**

i. Headless tail (figs. 10).
ii. Bent tail (fig. 11)
iii. Curved tail (fig. 12)
iv. Coiled tail (fig. 13)
v. Looped tail (fig. 14, 15)

**DISCUSSION**

The sperm count, sperm motility and sperm morphology were 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 (Man & Man, 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 distrupt ciliary action and chelate motility of spermatozoa.

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.

One of the most prominent effects of vincristine instead of tubulin inhibitor was to damage the DNA of germinal epithelium and it was detected by FISH molecular test (Hasim *et al.,* 2014). The chromosomal abnormality resulted due to vincristine induced sperm abnormalities and the possible caused was hyper-ploidy (Brandrift *et al.,* 1994). The abnormality of sperm was affected by the administration of vincristine. This drug mostly acts as a chemical mutagen and affect on sperm head abnormality (Topham, 1980). It was also noticed that the head parameters found main in the cytotoxicity of vincristine (Ettin *et al.,* 1984).

Administration of cytotoxic drug in male rat resulted the different types of sperm abnormality includes bent mid piece, curved mid piece, bent tail, curved tail, normal tail without head and normal head without tail. All these abnormalities came under secondary types (Saba *et al.,* 2009). Our results were accordance to it but we reported primary abnormality includes rudimentary tail in high dose treatment. Vincristine also resulted into induced deterioration of semen parameters like semen volume, sperm concentration, total spermatozoa per ejaculate, percentage of progressive motility and percentage of dead spermatozoa (Saratsis *et al.,* 2000). Our results were also accordance to it and decrease sperm count, sperm motility and also resulted into different types of head, mid piece and tail abnormalities.

In present study we have reported that, the motility of sperm decreased in low dose and high dose as compare to control. The motility observed were of progressive type, very few sperms showed rotational motion. Our results were accordance to Soratsis *et al.,*

(2000). We have also reported dead sperms and it was due to impairment of epididymal function (Averal *et al.,* 1996).

Abnormalities induced due to Vincristine in present study included primary and secondary abnormalities. I got both the types, primary types reported in our results was rudimentary tail and not reported by previous workers. Secondary types of abnormality obtained in present study were hookless head, banana shape head, amorphous head, tailless head, headless tail, bent tail, curve tail, bent mid piece, coiled tail, looped tail and curved midpiece. Our results were in accordance to Saba *et al.,* 2009. But I got some additional types of abnormalities like pin head and the sperm with two heads and two tails with single mid piece. In these both head and tail jointed by common mid piece.

Mine result of the sperm counts was reduced in low dose and high dose as compared to the control. But in the present study I have reported the oligospermia in low dose and high dose. These observations agreed with Choudhary *et al.,* (2002) and Dobzynska *et al.,* (2005).

Thus, in the present study Vincristine at dose level 0.06 mg and 0.12 mg resulted in decrease in sperm count and motile sperms with abnormal morphology. The results were more predominant in high dose as compared to control and low dose treatments. In high dose treatment the sperm count was very low but it did not show the severeness of morphological defects. It might be due to the early death of germ cell during the process of spermatogenesis. It is suggested that the vincristine treatment induce structural chromosomal aberration in spermatocytes, this attributed to interfering with DNA replication by preventing the cell from entering $G_1$ phase cause an arrest of mitotic and meiotic division to metaphase followed by cell death, so result in sperm defect or no sperm formation (Diab *et al.,*2011)

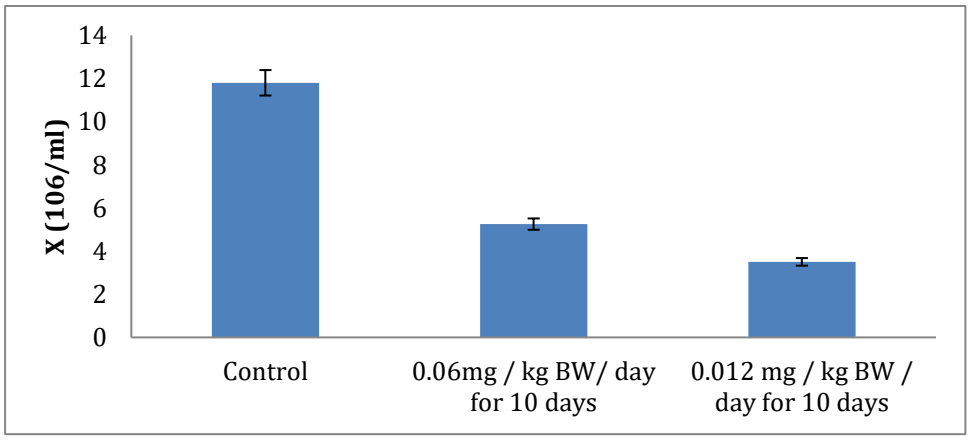
The effect of vincristine on sperm abnormalities and their causative mechanism are elaborated here. Vincristine is an anti-proliferative, radiomimetic, anti-carcinogenic drug. This drug arrests cell growth through their effect on cyto-skeletal elements and tubulin formation. As a result, there is no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

**Figure 1: Illustrating how the animal was hold fast by its leg for injecting drug intravenously on the thigh with disposable insulin syringe**

**Table 2: Effect of 0.06 mg and 0.12 mg VCR / day for 10 days on Cauda epididymal sperm count. The number of sperm was observed in 5 different microscopic fields (values are mean ± SE)**

| Parameter | Control | 0.06mg / kg BW/ day for 10 days | 0.012 mg / kg BW / day for 10 days |
|---|---|---|---|
| Sperm count ($10^6$/ml) | 11.80 ± 0.170 | 5.25 ± 0.047 | 3.50 ± 0.095 |

P value - P < 0.001

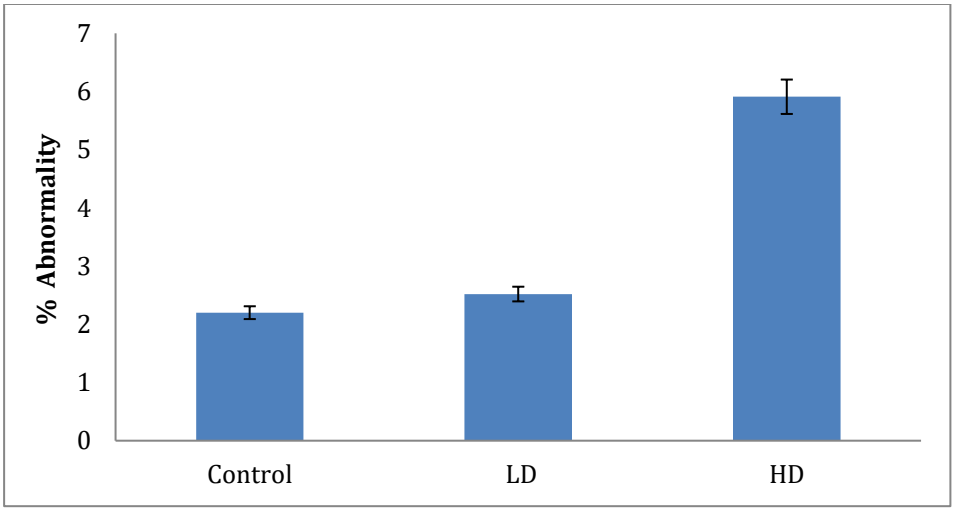



**Graph 1: Sperm count (($10^6$/ml)**

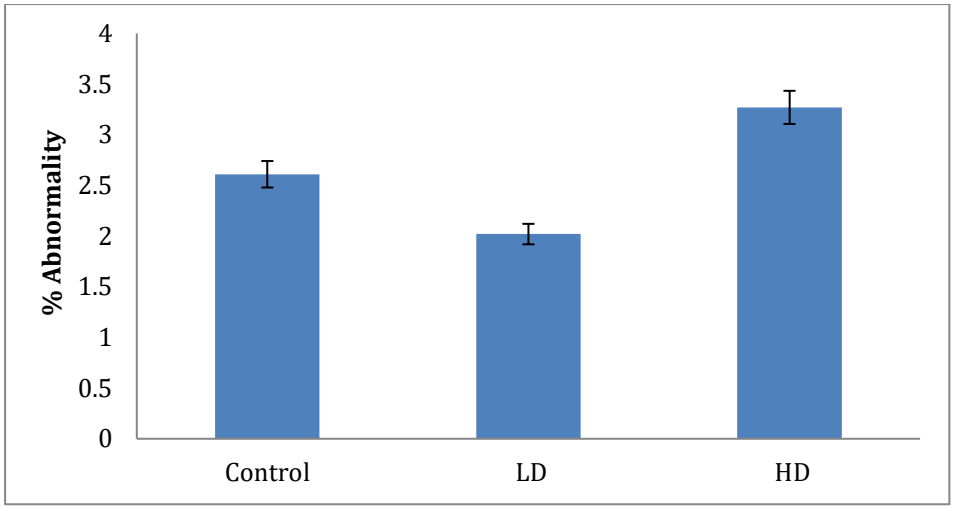
**Table 3: Effect of 0.06 mg and 0.12 mg VCR / day for 10 days on sperm morphology and percentage occurred of different sperm abnormalities (values are mean ± SE)**

| 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**### |

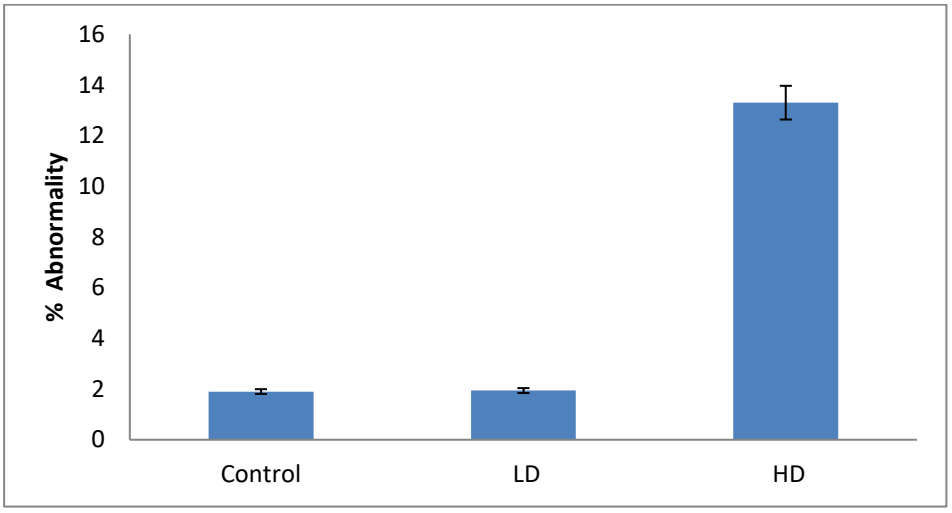
#p<0.5, ##p<0.01, ###p<0.001 and *Insignificant



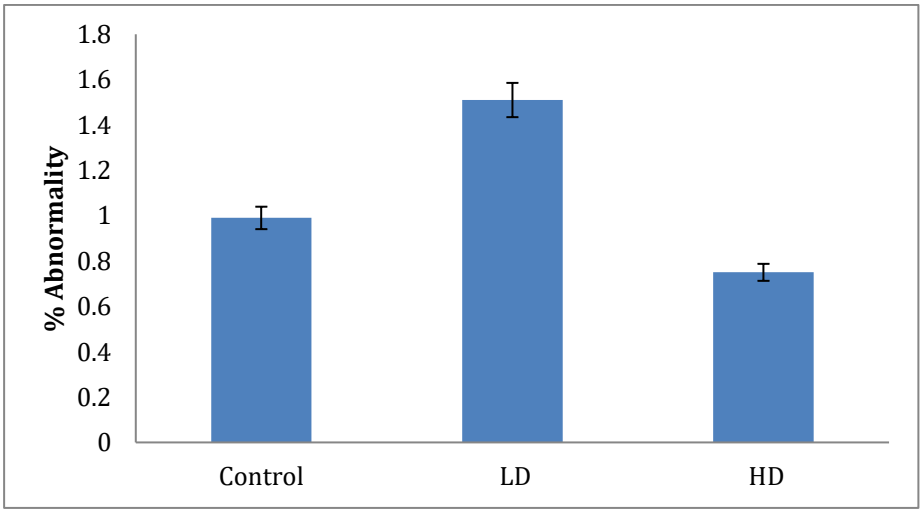

**Graph 2: Hookless head**



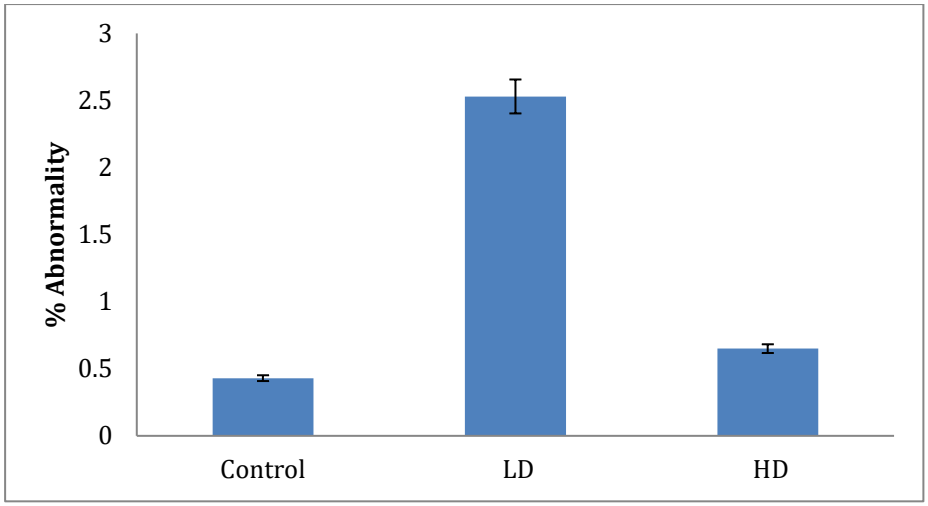

**Graph 3: Banana shaped head**



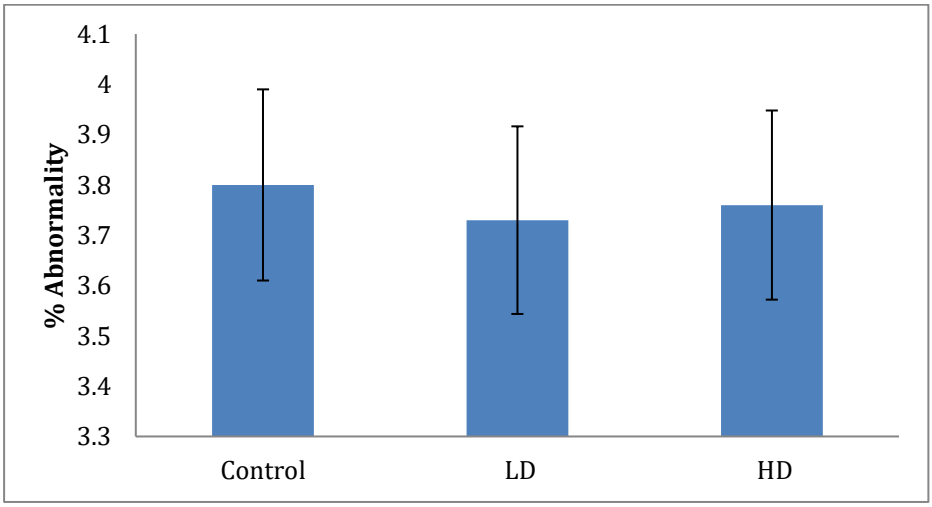

**Graph 4: Amorphous head**



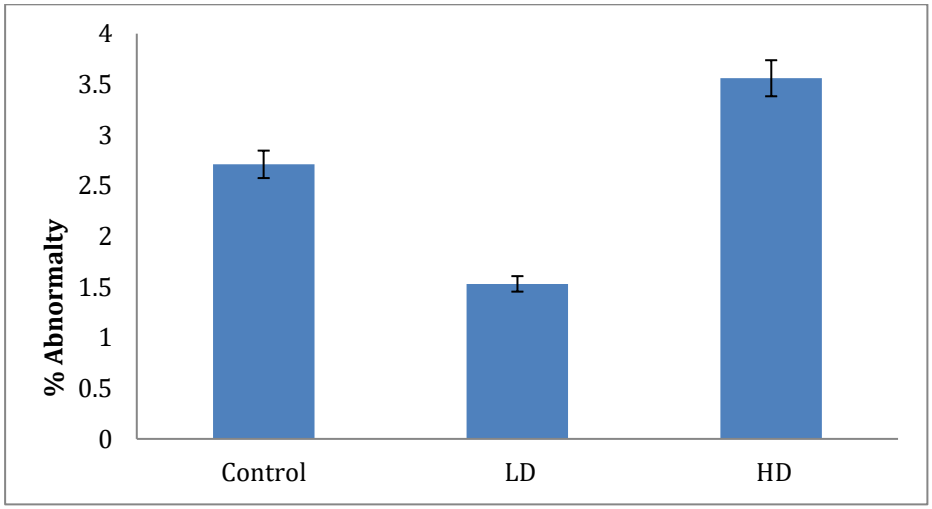

**Graph 5: Pin head**



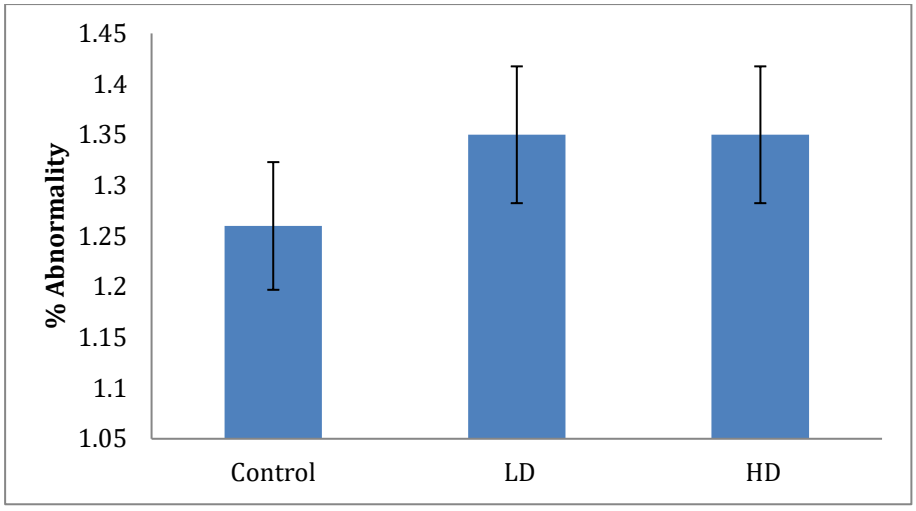

**Graph 6: Tailless head**



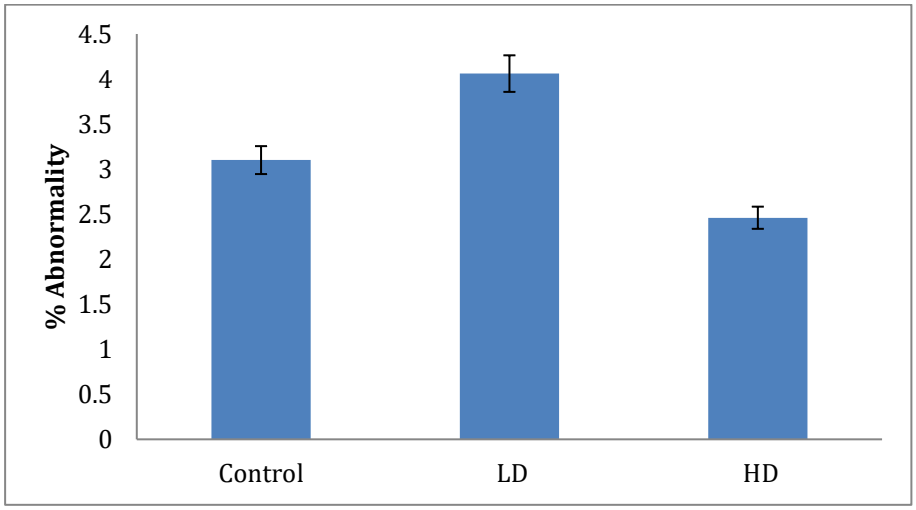

**Graph 7: Bent mid piece**



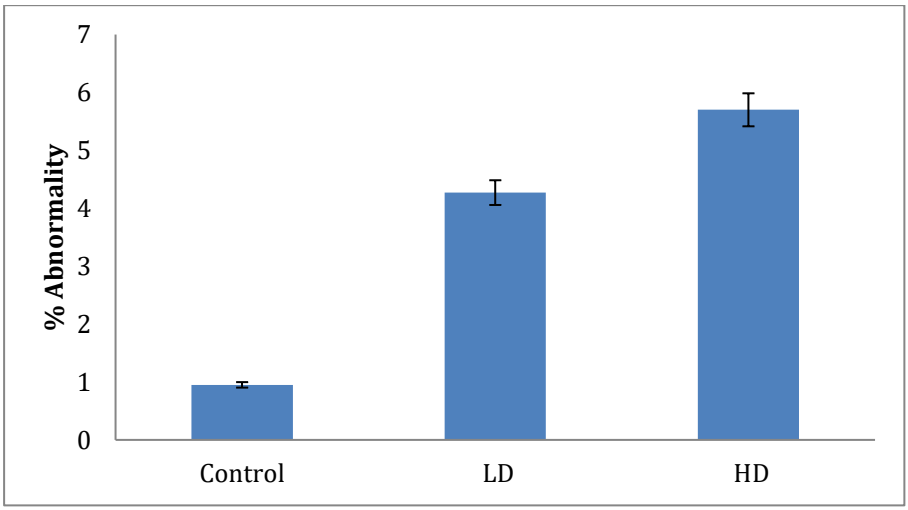

**Graph 8: Curved mid piece**



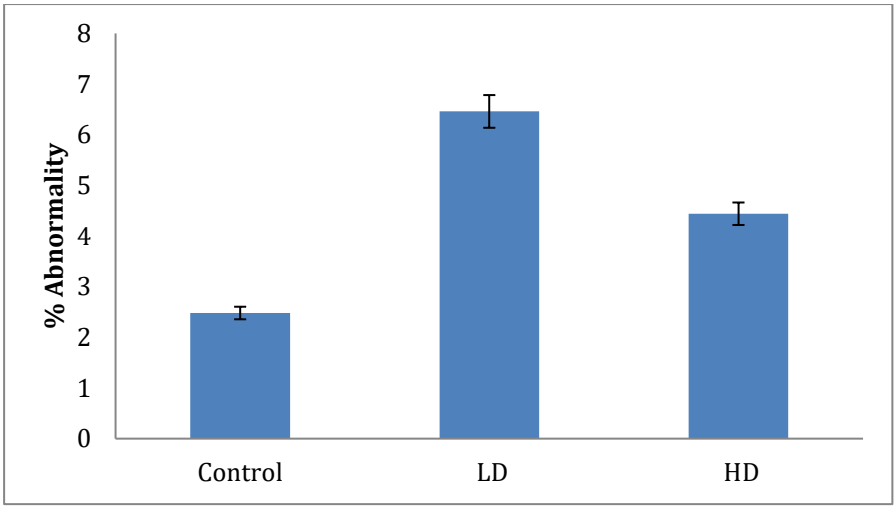

**Graph 9: Headless tail**



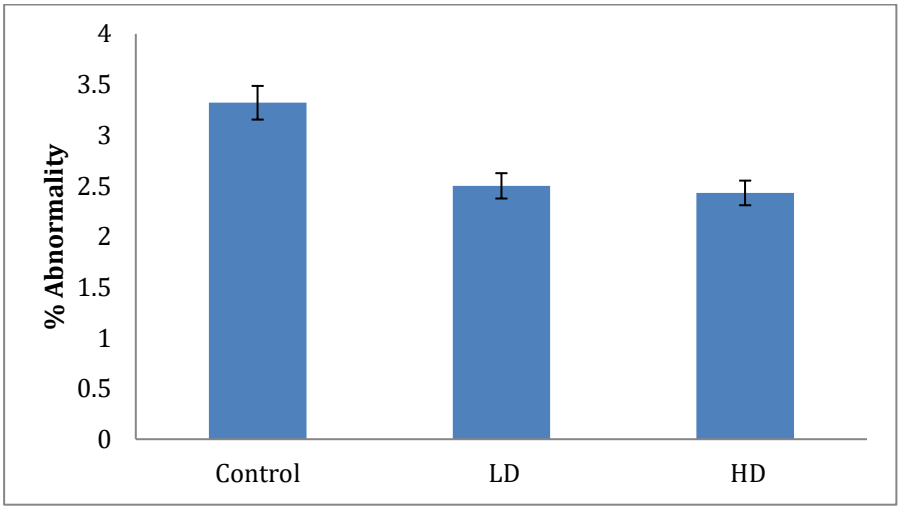

**Graph 10: Bent tail**




**Graph 11: Curved tail**




**Graph 12: Coiled tail**




**Graph 13: Looped tail**

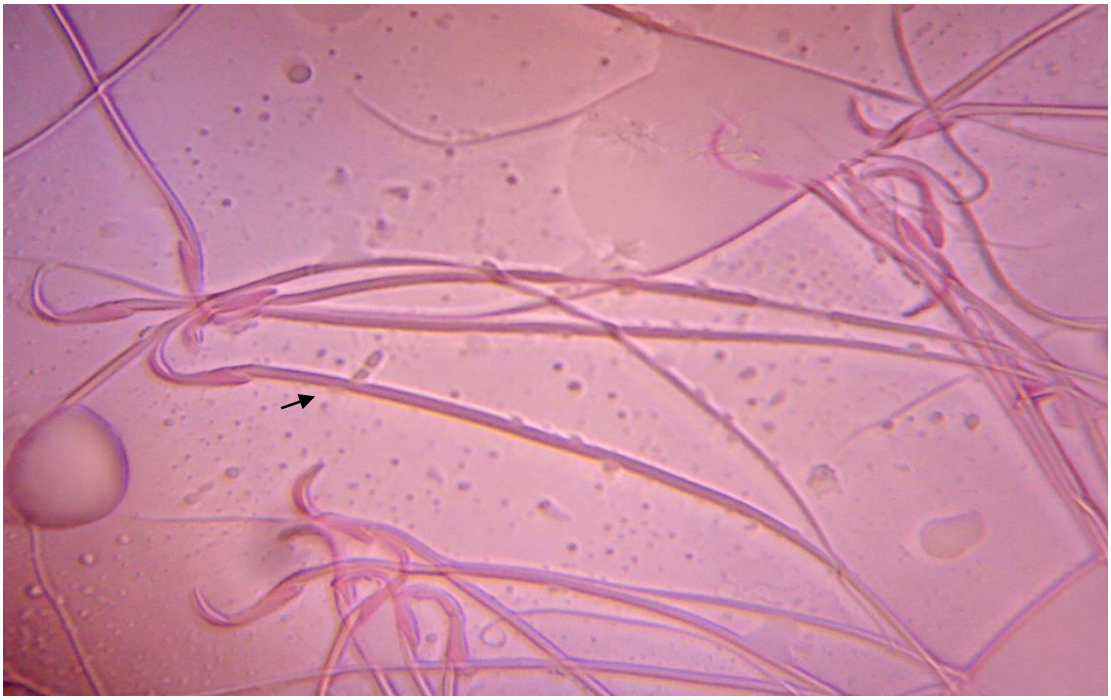


**Figure 2a: Microphotograph showing swarms of normal sperms (arrow) X 1000.**

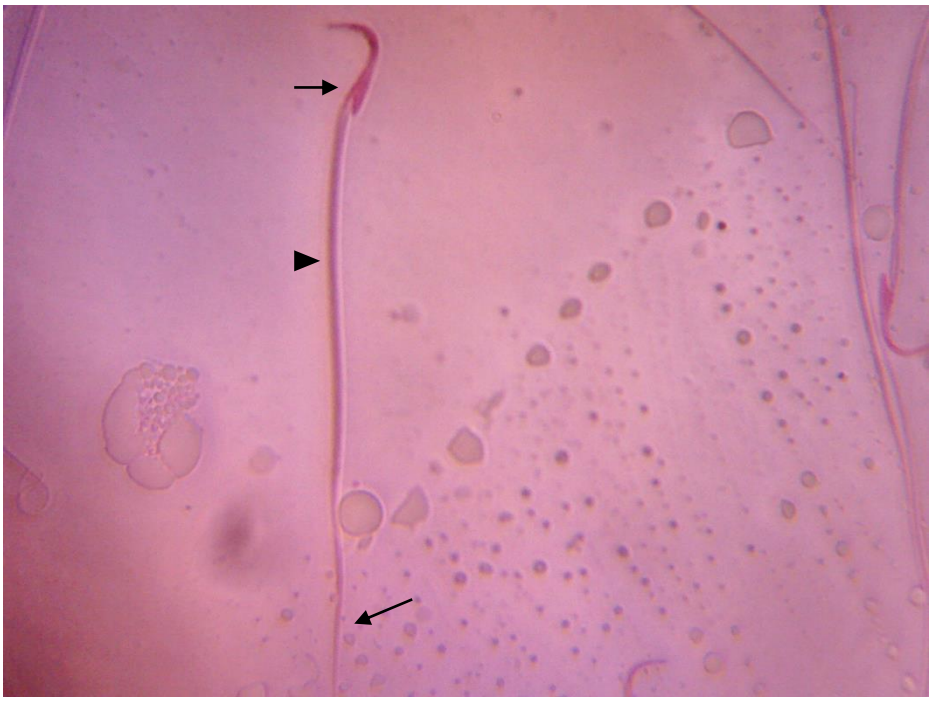


**Figure 2b: Photograph showing single sperm with normal head (arrow), mid piece (arrow head) and tail (long arrow) X 1000.**

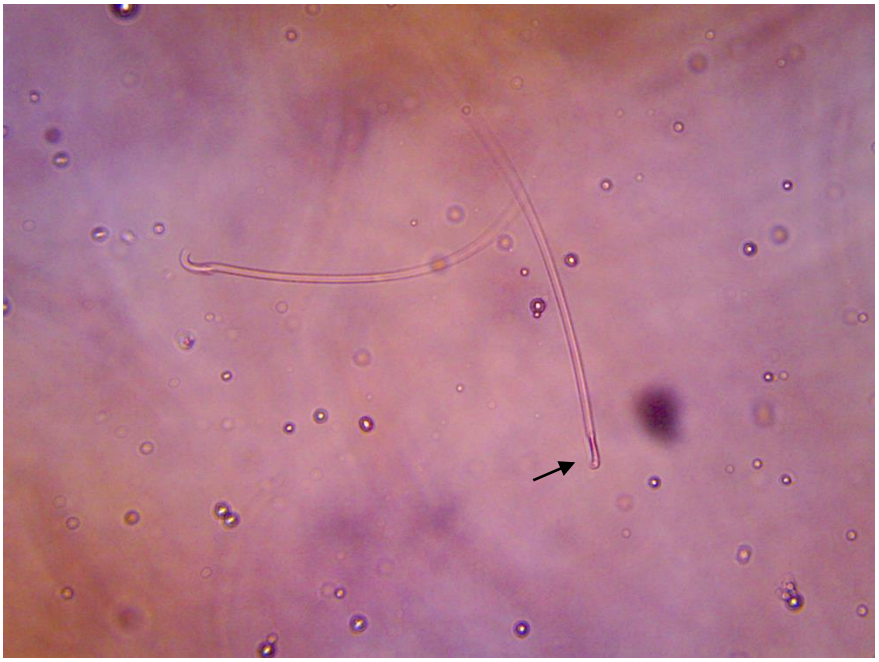


**Figure 3: Microphotograph of sperm with hook less head (arrow) X 400**

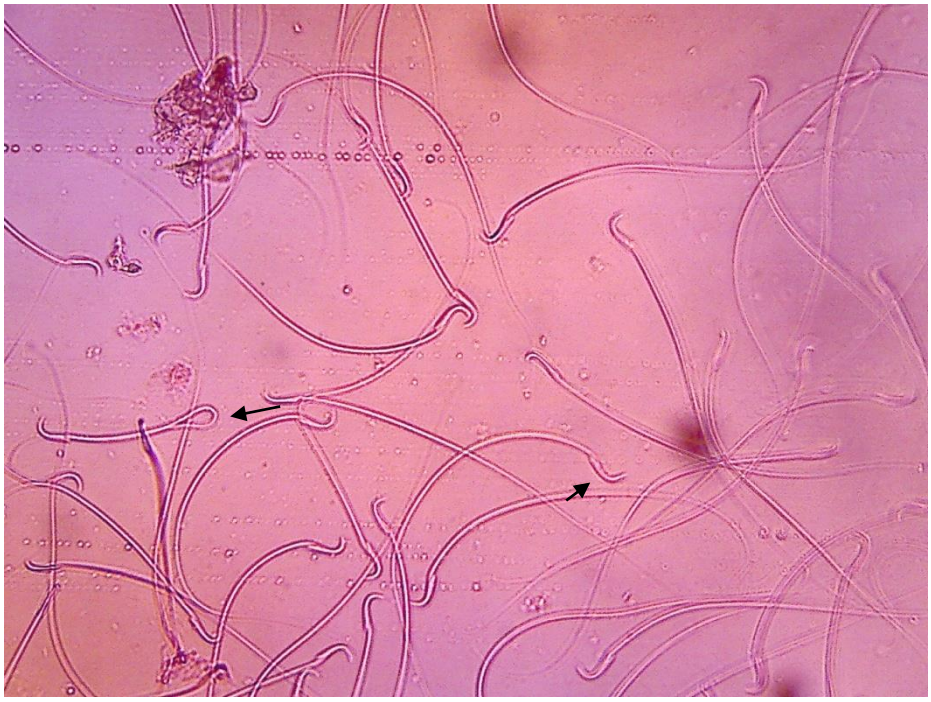


**Figure 4: Photograph of few sperms showing banana shape head (arrow) and looped tail (arrow head) X400.**

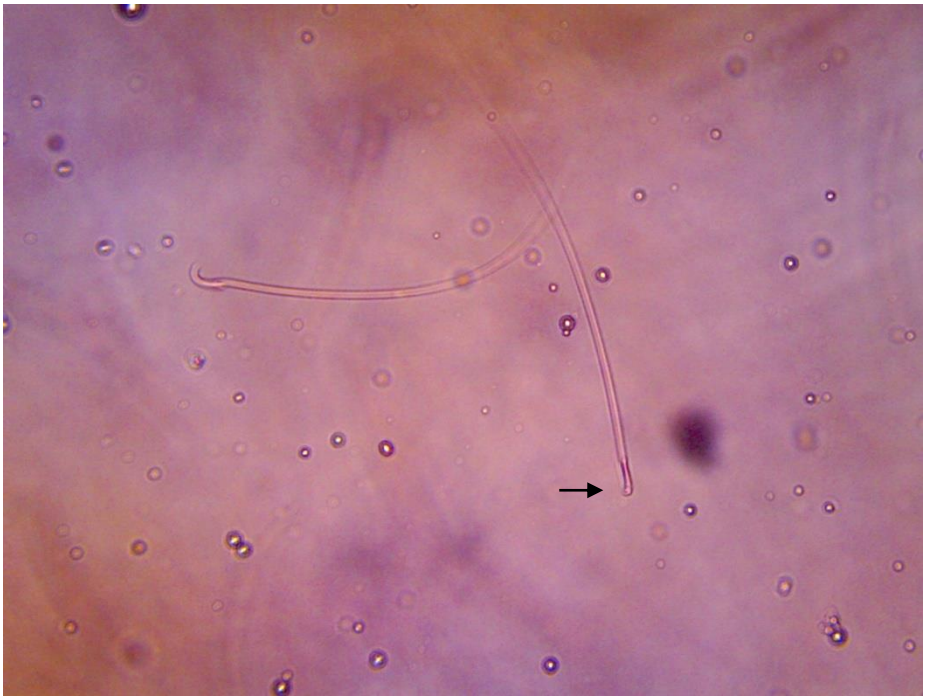


**Figure 5: Photograph of sperm showing amorphous head (arrow) X 400**

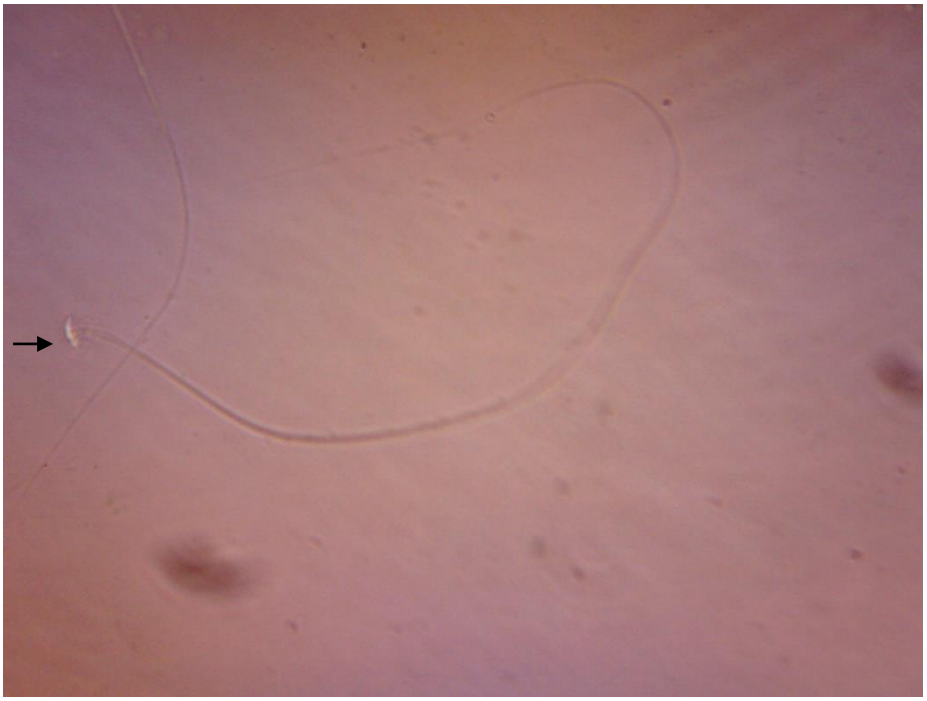


**Figure 6a: Photograph with pin headed (arrow) X 400**

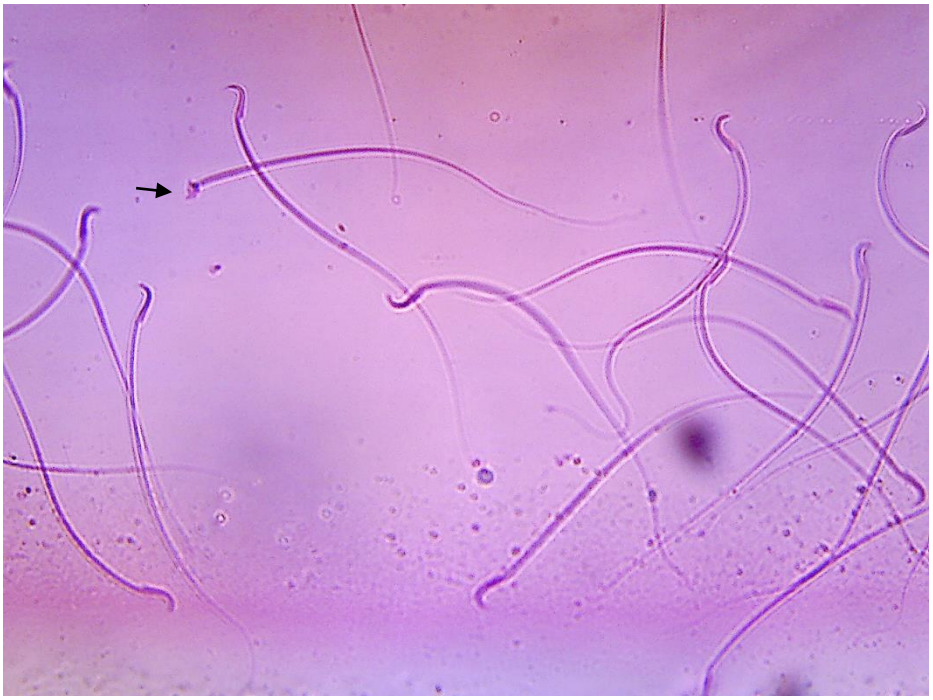


**Figure 6b: Photograph of sperm with pinheaded (arrow) X 400**

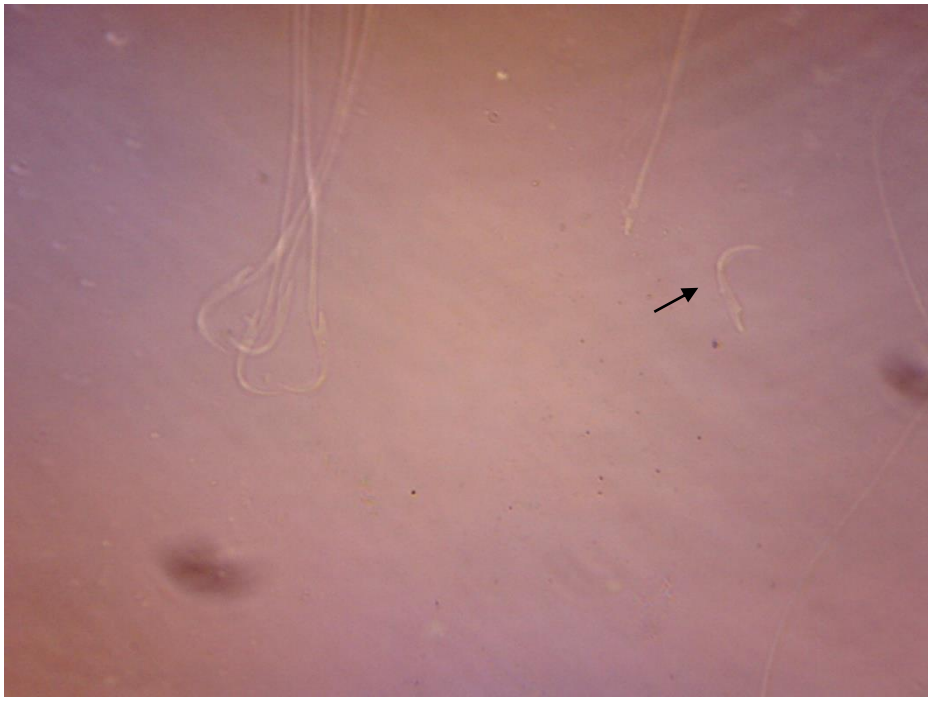


**Figure 7: Photograph of sperm showing tailless head (arrow) X 400**

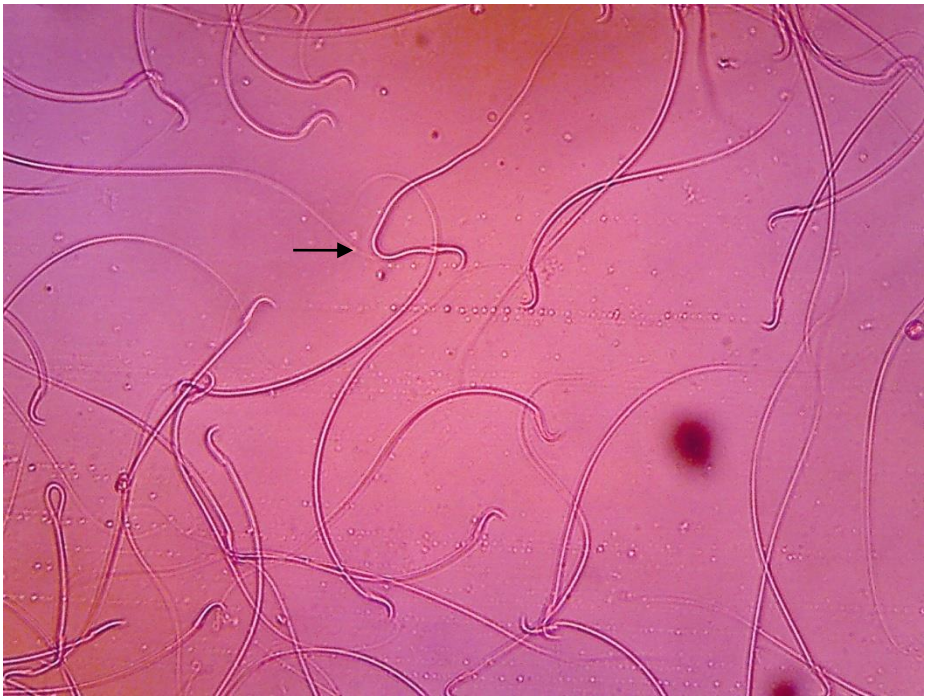


**Figure 8: Microphotograph showing sperm with Bent mid piece (arrow) X 400**

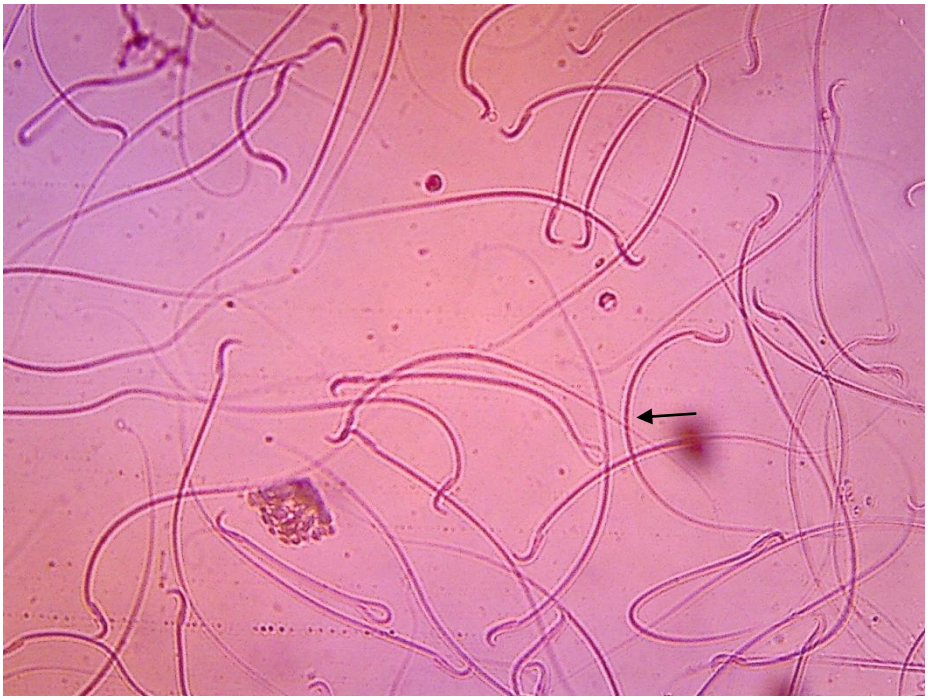


**Figure 9: Photograph of sperm with curved midpiece (arrow) X 400**

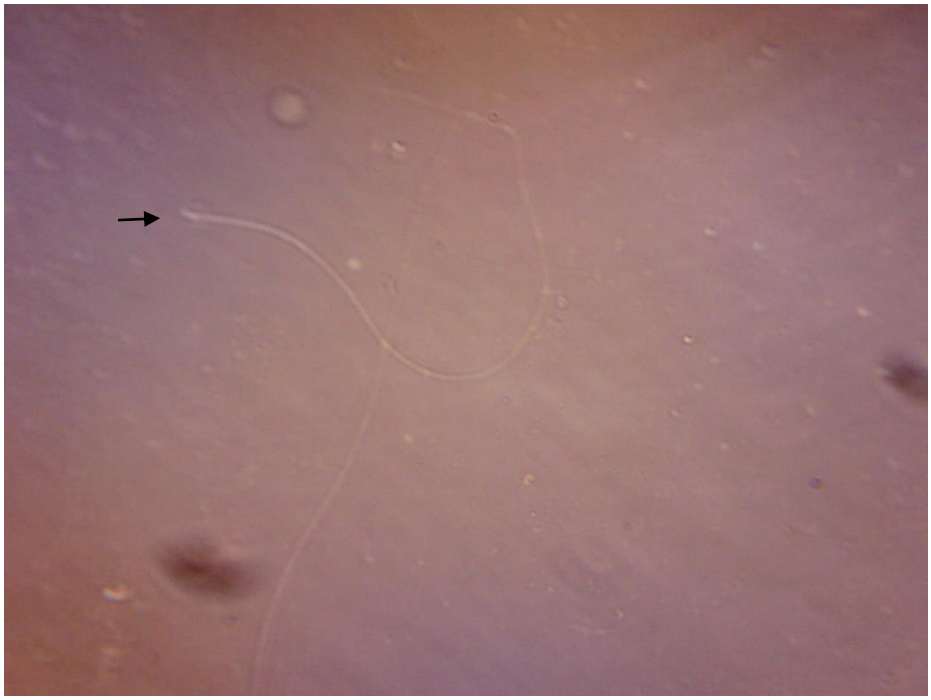


**Figure 10: Photograph of sperm with headless tail (arrow) X 400**

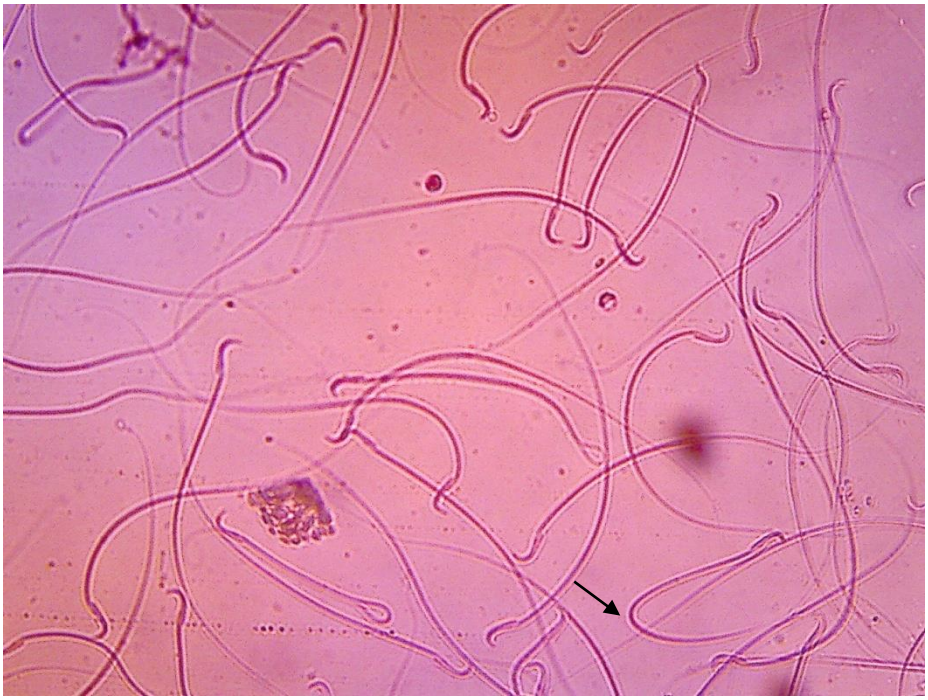


**Figure 11: Photograph of sperm with bent tail (arrow) X 400**

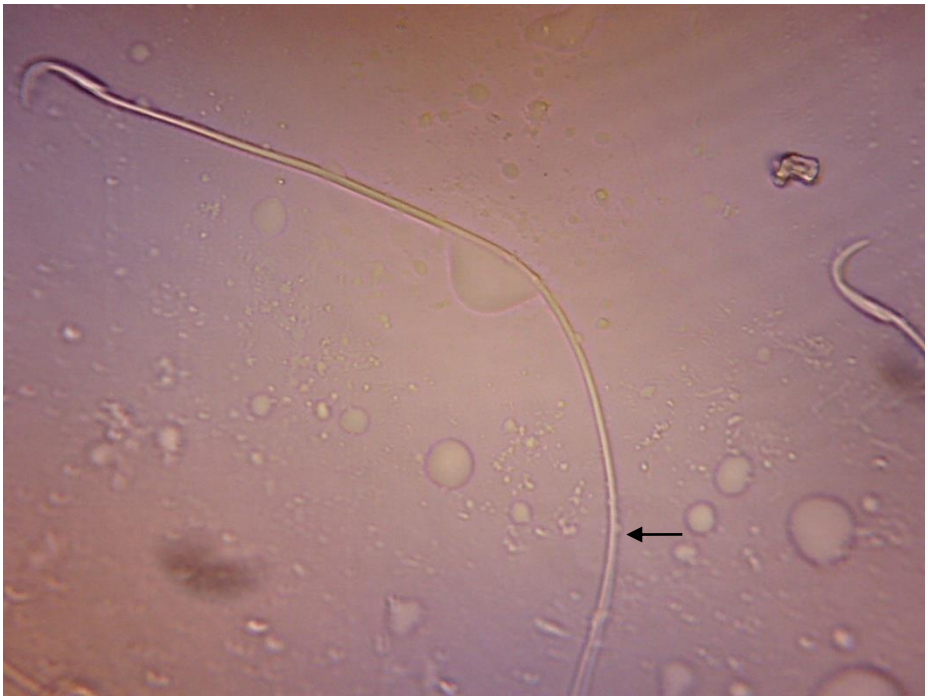


**Figure 12: Photograph of sperm with curved tail (arrow) X 1000**

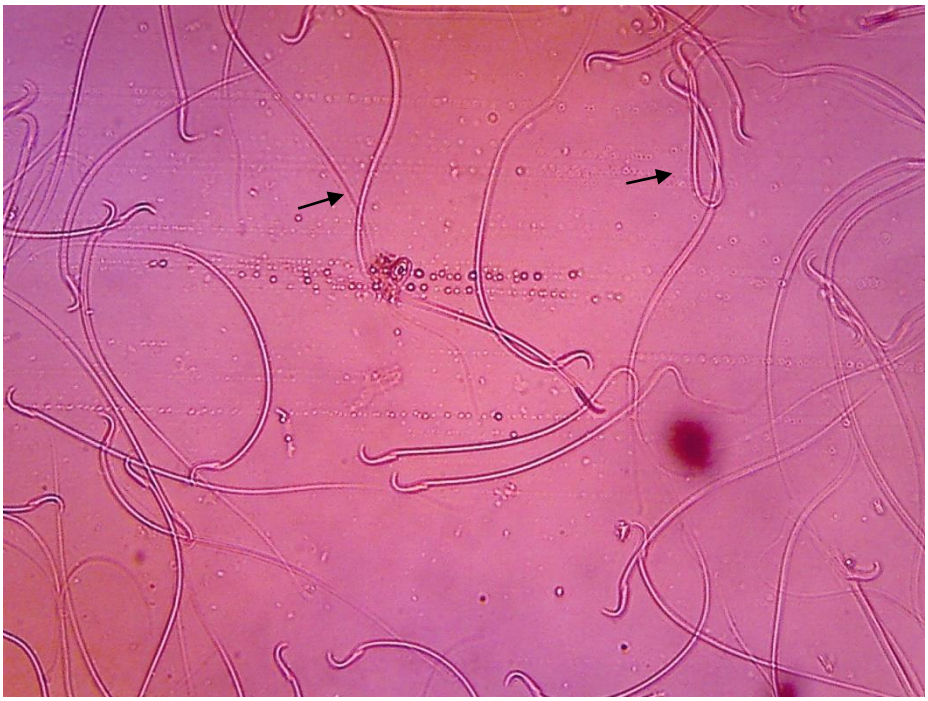


**Figure 13: Microphotograph showing sperm with coiled tail (arrow) X 400**

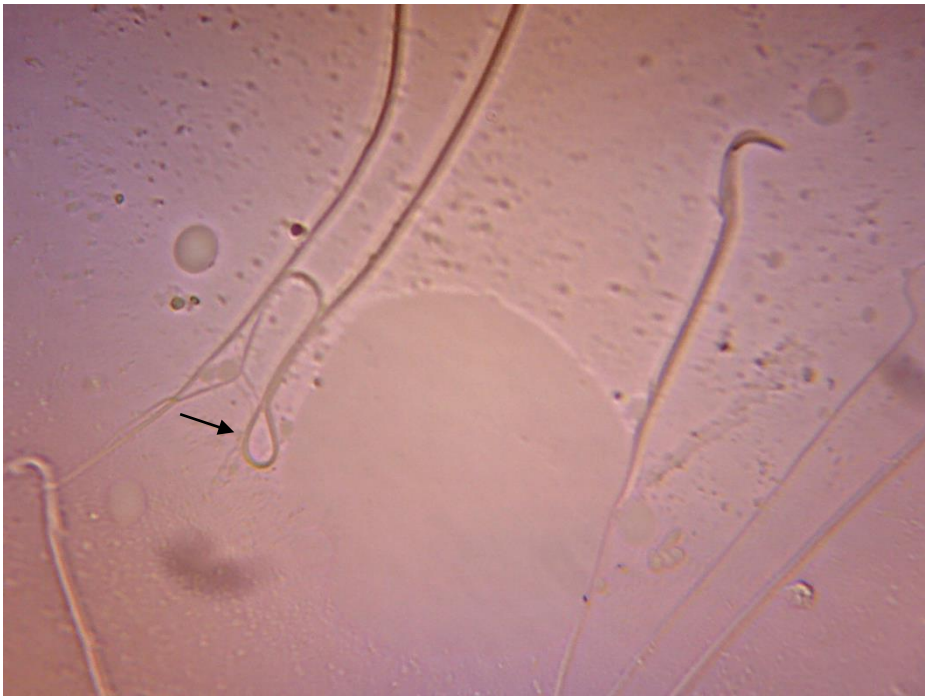


**Figure 14a: Photograph of sperm with looped tail (arrow) X 1000**

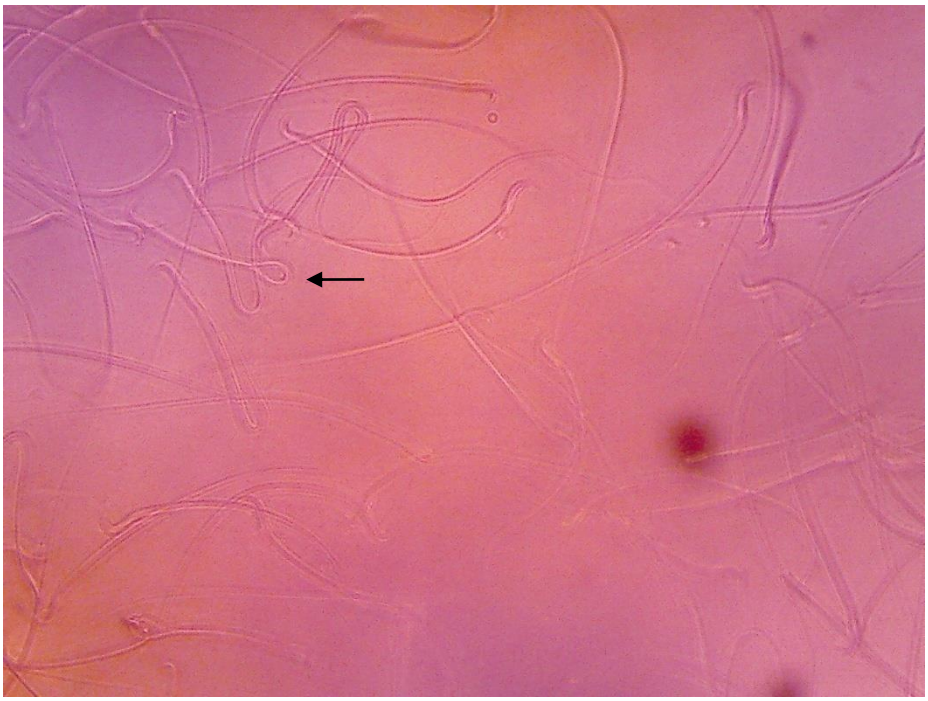


**Figure 14b: Photograph of sperm with looped tail (arrow) X 400**

**SUMMARY AND CONCLUSION**

In present work Vincristine induced toxicities in sperm maturation and also affect on count and motility. Vincristine is a powerful alkaloid used in chemotherapy to cure the cancer. The mechanism of action of vincristine discussed by different workers proved that it inhibits the spindle formation by inhibition of tubulin synthesis. It results chromosomal mutation due to non disjunction.

Thus, from the present work it is concluded that, administration of Vincristine in low dose and high dose regiment significantly decreased in sperm count. But it never resulted azoospermia, in low dose it is oligospermia and in high dose oligozoospermia.

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Dr. R. M. Chaudhari is a distinguished academician and researcher with over two decades of expertise in the field of Zoology. He holds an M.Sc., Ph.D., and B.Ed., and currently serves as the Associate Professor and Head of the Department of Zoology at Shri. S.I. Patil Arts, G.B. Patel Science, and S.T.S.K.V.S. Commerce College, Shahada, Dist. Nandurbar (M.S.). With 25 years of teaching experience at the undergraduate level and 19 years at the postgraduate level, Dr. Chaudhari has mentored countless students and guided numerous research projects. A prolific researcher, Dr. Chaudhari has 20 years of research experience, with a primary focus on Mammalian Reproduction, Andrology, and Reproductive Toxicology. His contributions to the scientific community include 22 publications, 10 authored books, and active participation in 40 conferences, seminars, and workshops, including 12 oral presentations. Recognized for his academic excellence, he has been honored as a Postgraduate Teacher and Research Guide by KBC NMU, Jalgaon. Additionally, he has successfully completed a UGC-funded minor research project, further solidifying his reputation as a dedicated scholar. Dr. Chaudhari's work reflects his passion for advancing knowledge in reproductive biology and his commitment to fostering academic growth. His extensive experience, coupled with his research achievements, makes him a respected figure in the field of Zoology.

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