Cytogenetic Effect of Cadmium chloride on Swiss albino Mice

Somia M. Allamushi* and Nasser M. Salama*

* Zoology Department , Faculty of Science Universityn Al- Fateh, P.O.Box 13227, Tripoli, Libya,

S.P.L.A.J.E-mail: Allamushi99@yahoo.com

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الملخص

يعتبر كلوريد الكادميوم مادة واسعة الإنتشارملوث للبيئة وسام للجينات ومسرطن للحيوانات. أوضحت الدراسة التـــى إجريت على هذه الفئران أن هناك علاقة تناسبية بين كلوريد الكادميوم والتغيرات الممرضة (الغيـــر طبيعيـــة) فـــي النويات وكذلك تكرار إضطراب الكروموسومات وشكل الحيونات المنوية. دلت النتائج المتحصل عليها بأن الجرعـــات العالية (3 and 5 mg / Kg body weigh) لهذا المركب سببت زيادة تشوهات الصبغيات ونويات كريات الدم الحمـــراء الموجودة في نخاع العظم وكذلك شكل النطف.

إن تأثير كلوريد الكادميوم يعود الى طفرة جينية وكذلك التعبير الجيني الذي قد سبب في إحداث إضطراب فى نويات كرات الدم الحمراء والشذوذ الصبغي وشكل النطف.

Cadmium chloride is a well known genotoxic and teratogenic effects in animals and a wide spread environmental pollutant. The genotoxic effect of cadmium chloride was evaluated in laboratory animals (Swiss albino mice). The frequencies of micronuclei and chromosomal aberrations were analysed in bone marrow in the context of heavy metal (Cadmium chloride).

Significant correlations were obtained between cadmium chloride and frequency of micronuclei, chromosomal aberrations and sperm morphology. The results showed that high doses of CdCl2 caused significant increase in chromosomal aberrations, polychromatic micronuclei and sperm abnormality. The present studies reveal the substantiate role of gene mutation and gene expression as causative of micronuclei, chromosomal aberrations and sperm abnormality.

Introduction

Cadmium chloride (CdCl2) is a white crystalline compound of cadmium and chlorine. Hygroscopic solid is highly soluble in water and slightly in alcohol (Green wood and Earnshaw, 1997). Cadmium waste streams from the industries end up in soil and may also enter the air through household waste combustion and burning of fossils fuels. Human uptake of cadmium takes place during the exposure to hazardous waste sites and through food stuffs that are rich in cadmium. Cadmium has been recognized as a major health and possesses a potential threat to the general population (Nayak et al., 1989).

Cadmium has been shown to have toxic effects on human neuroplastoma cells, porcine, rat kidney cells and human prostate epithelial cells. It has been also shown to attribute to carcinogenicity by enhancing DNA mutation rates and to express some potential that control cellular proliferation (Mukherjee et al., 1994).

CdCl2 has been shown to cause an increase in chromosomal damage as well as DNA strand breaks when human cells were treated invitro with CdCl2 (DEFRA, 2002).

No complete information and experimental studies have been done to evaluate the effectiveness of cadmium chloride on male reproduction through abnormal sperm morphology, from this point it is necessary to evaluate the influence of CdCl2 on human reproduction in germ cell.

For evaluating both mutagenic and teratogenic potential of CdCl2, we have investigated in mice the cytogenetic changes (chromosomal aberration, pathological changes in erythrocytes "micronuclei" and sperm abnormality) and to formulate an applicable approach for handling the problem of CdCl2 as public health problem.

Materials and Methods

Strain and Care

Swiss albino mice were inbred in animal house of the Zoology Department, Faculty of Science, AL-Fateh University. They were kept until they reached an age of 8-12 weeks before used. Mice were housed in plastic cages containing wooden flakes in air conditioned room. Day length was 12 hours and temperature approximately 23ºC, mice were fed and watered adlibitum .

Treatments

The experiments were performed on adult male swiss albino mice of 8-12 weeks of age. The animals were divided into 4 groups (five mice/cage). Experimental groups and control groups were injected intraperitoneally at 3 different dose levels (1, 3, 5 mg/kg body weight) with total dose 3,9,15 mg/kg respectively of cadmium chloride dissolved in 0.9% NaCl solution at 48 hours intervals.

Control mice were similarly injected with solvent. Mice were weighed before first, second and third injections and before killing. The animals were observed carefully for signs of toxicity and their body weights were recorded.

Fours hrs before killing all the mice were injected IP with 4mg/kg colchicine and were killed by cervical dislocation 48 hrs after last injections.

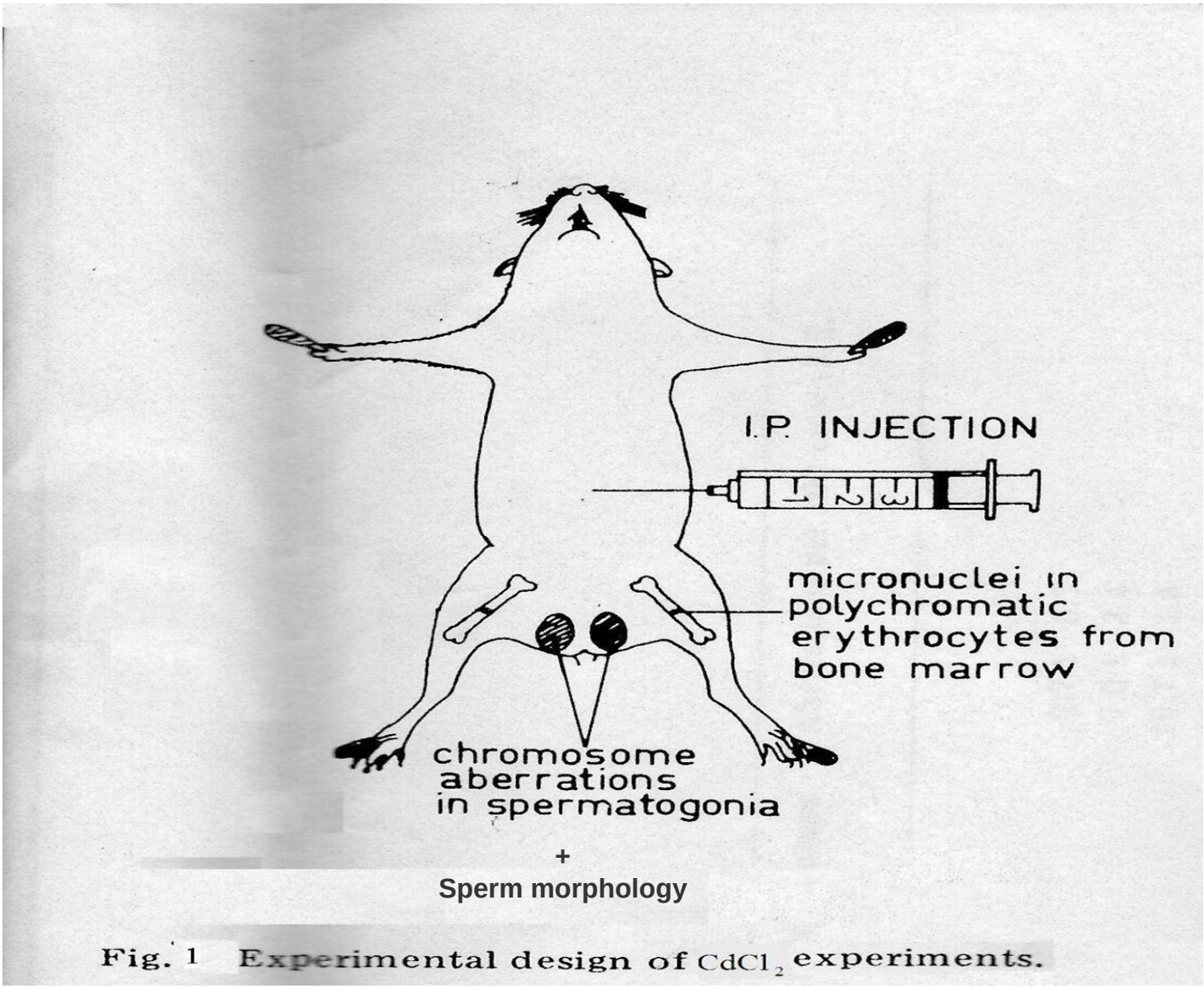

Fig. 1 Experimental design of $CdCl_2$ experiments.

Cytogenetic studies

a) Micronuclei tests.

1-The bone marrow cells were flushed from both femurs using 1 ml disposable Plastic syringe attached with 21 G1 needle in a petri dish containing 3 ml Of human AB serum.

2- Cells were aspirated by repeated number of flushing until a fine suspension of Cells were formed.

3- The cell suspension was centrifuged at 1000 rpm for 10 minutes. The Supernatant was removed and to the sediment half a drop of serum was Added.

4- A small drop of viscous suspension was placed on well cleaned slide and Smears were prepared.

5- Slides were stained for 3 minutes in May Gruen Wold stain.

6- A total of 2000 cells were screened for the presence of micronuclei in Polychromatic erythrocytes and normochromatic erythrocytes.

b) Chromosomal analysis

Chromosomal analysis was performed on animal testes of control and treated groups. All chromosome preparations were made by dropping fixed cell suspension on cold wet slide. Well-spread metaphases per individual were examined using buffered Giemsa stain (Dev and Tantravahi, 1982). Numerical and gross morphological changes in chromosomes were recorded.

Determination of sperm abnormality

Groups of untreated and treated male mice were killed by cervical dislocation 5weeks after treatment. Sperm from vasa deferentia of each mouse were freed by stripping into 1ml of 32 C^{o} sperm Ringer solution (Ficsor et al., 1981).

For sperm morphology test a drop of sperm suspension was transferred to a clean slides, smears were made and allowed to dry in air. Smears were then stained with 1% Eosin stain and examined of 400 magnifications for morphological abnormalities.

Statistical analysis

The test of significance between the data of the experimental and control series was determined by student's t-test and the analysis of variance (two way ANOVA) (Fisher and Yates, 1986).

Results and Discussion

Experiments were designed to assess the mutagenic effects of cadmium chloride for incidence of micronuclei in bone marrow erythrocytes of Swiss albino mice. The results on the frequency of micronuclei in bone marrow erythrocytes of cadmium chloride treated animals are presented in tables (1,2) and illustrated graphically in figure (1,2). There was a gradual increase in the frequency of micronuclei in polychromatic erythrocytes at all doses when compared with control values.

The frequency of micronuclei in polychromatic erythrocytes was 0.25 in control animals and it has increased to 0.46, 0.68 and 0.97 after the administration of 1, 3 and 5 mg/kg CdCl2 respectively.

The frequency of micronuclei in normochromatic cells was 0.12 in control animals and it has increased to 0.22, 0.33 and 0.45 at 1, 3, and 5 mg/kg CdCl2 treated animals (table 1. Fig. 1). The P/N ratio was 0.98, 0.97 and 0.94 in 1, 3 and 5mg/kg CdCl2 treated animals as against the control 0.99.

The t-values for difference in the frequencies of micronuclei in polychromatic erythrocytes between control and CdCl2 treated groups were found to be statistically significant. The differences in between the treated groups were also found to be significant ($P<0.01$.Table 2).

During the development of erythrocytes the erythroblast a few hours after the completion of last mitosis expels their nucleus for unknown reason and the micronuclei as obstructive elements in the cytoplasm of young erythrocytes.

CdCl2 in invivo assays induced chromosomal damage as well as disturbances in hemopoiesis which exhibited mutagenic effect in the bone marrow erythrocytes in mice this in accordance with those of (Chorvatovicova et al .,1991) who showed the mutagenic effect in both rats and guinea pigs using the bone marrow micronucleus. Our results are also in accordance with those of (Dhir et al., 1990).

The Chromosomal damage caused in the germ cells by CdCl2 was evaluated using the methods of (Dev and Tantravahi, 1982). The results obtained from these studies are illustrated graphically in figures (3, 4 and 5).

Different types of chromosomal aberrations such as (**a)** changes in chromosomal number and (**b)** changes in chromosomal structure were observed in treated groups of animals.

The data indicate that there were a gradual increase in the frequency of various types of chromosomal aberrations at all dose levels.

The analysis of chromosomal damage in germ cells, diakinesis metaphase I of meiosis II regarded as suitable stage. Since at the diakinesis first metaphase stage of meiosis, the characteristic meiotic configuration of spermatocyte chromosomes are suitable for studying chromosomal aberrations .

Spermatogonia constitute the most important germ cell stages as they represent a permanent cell population of cells induction of mutations in spermatogonia by toxicant of

mutagen is hamrful. In meiotic tissues the polyploid cells are believed to arise by either fusion of nuclei of two or more spermatocytes or by irregular division of spermatogonia by toxicant or mutagen (Leonard,1973).

The mean value and percentage of abnormal shaped sperm in treated mice were significantly ($P<0.01$) high in comparison with values of untreated animals (Table 3 and Fig. 6,7). Abnormally shaped sperm heads were the most common forms of aberration in treated mouse. thus pre-leptotene, late spermatogonium and spermatogonial stem cells were effected .

Abnormally shaped sperm may be a result of induced germ line mutations or a large number of the alleles are involved in the differentiation of spermatozoa, Interference with DNA\RNA\Protein transcription, mutation may be expressed as defects in sperm head shape (Salama and EL –Ansari,1995).

From examinations of micronuclei, chromosomal aberrations and sperm abnormality from cadmium chloride treated bone marrow and the testes the authors conclude the following:

1.Assessment of mammalian micronuclei,chromosomal aberrations and sperm abnormality is adaptable to different species dosage regimes, sampling time and regime routes of exposure. This versatility makes it a useful model for human exposure.

2. These assays are rapid inexpensive and generally qualitative. Large samples can be obtained for analysis.

Table 1. Frequency of micronuclei in bone marrow erythrocytes of male mice treated with various doses of cadmium chloride.

Treatment mg/kg	Micronuclei in polychromatic cells "P"	Micronuclei in normochromatic cells "N"	Micronuclei in total cells "P+N"+SE	P/N ration
Control	37/14562=(0.25)	18/14642=(0.12)	55/29204=0.19	14562/14642=0.99
1mg/kg	66/14426=(0.46)	32/14622=(0.22)	98/29048=0.33	14426/14622=0.98
3mg/kg	98/14382=0.68*	49/14782=(0.33)	147/29164=0.50	14382/14782=0.97
5mg/kg	139/14366=0.97*	69/15222=(0.45)	208/29588=0.70	14366/15222=0.94

The values in parentheses are percentage ($p<0.01$).

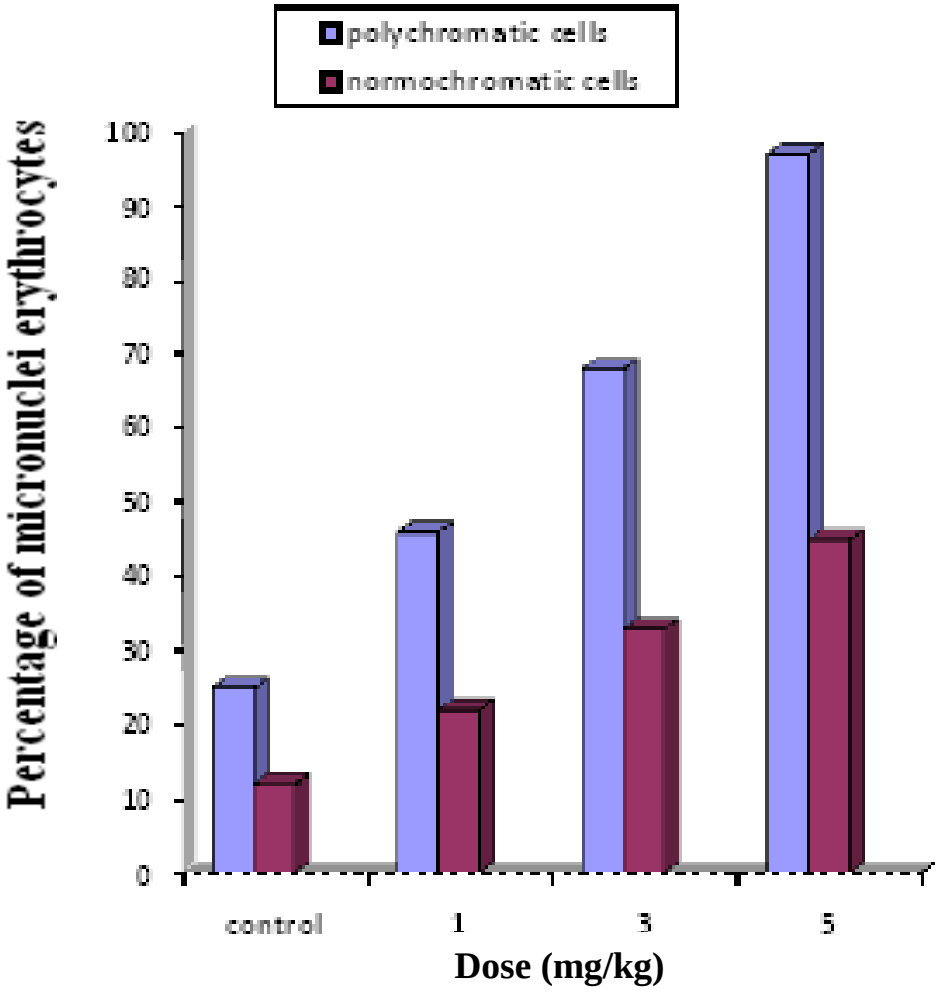

Fig.1. Incidence of micronuclei in bone marrow Erythrocytes treated mice

Table 2. 't'-values for the incidence of micronuclei in polychromatic Erythrocytes of cadmium chloride treated mice.

Treatment	't'-values
Control Vs 1mg/kg	7.7 *
Control Vs 3mg/kg	14.*
Control Vs 5mg/kg	21.2 *
1mg/kg Vs 3mg/kg	6.9 *
1mg/kg Vs 5mg/kg	19.4 *
3mg/kg Vs 5mg/kg	7.4 *

***P<0.01**

Table 3.Effect of CdCl2 on testes weight and sperm morphology

Treatment $CdCl_2$mg/Kg	Mean of testes weight(mg)	Total sperm count	Percentage sperm Normality	Percentage sperm abnormality *
control	230	555	67.42	32.57
1mg/Kg	190	466	67.38	32.62
3mg/Kg	160*	39	34.23	65.77 *
5mg/Kg	140*	18	16.67	83.33 *

*p< 0.05

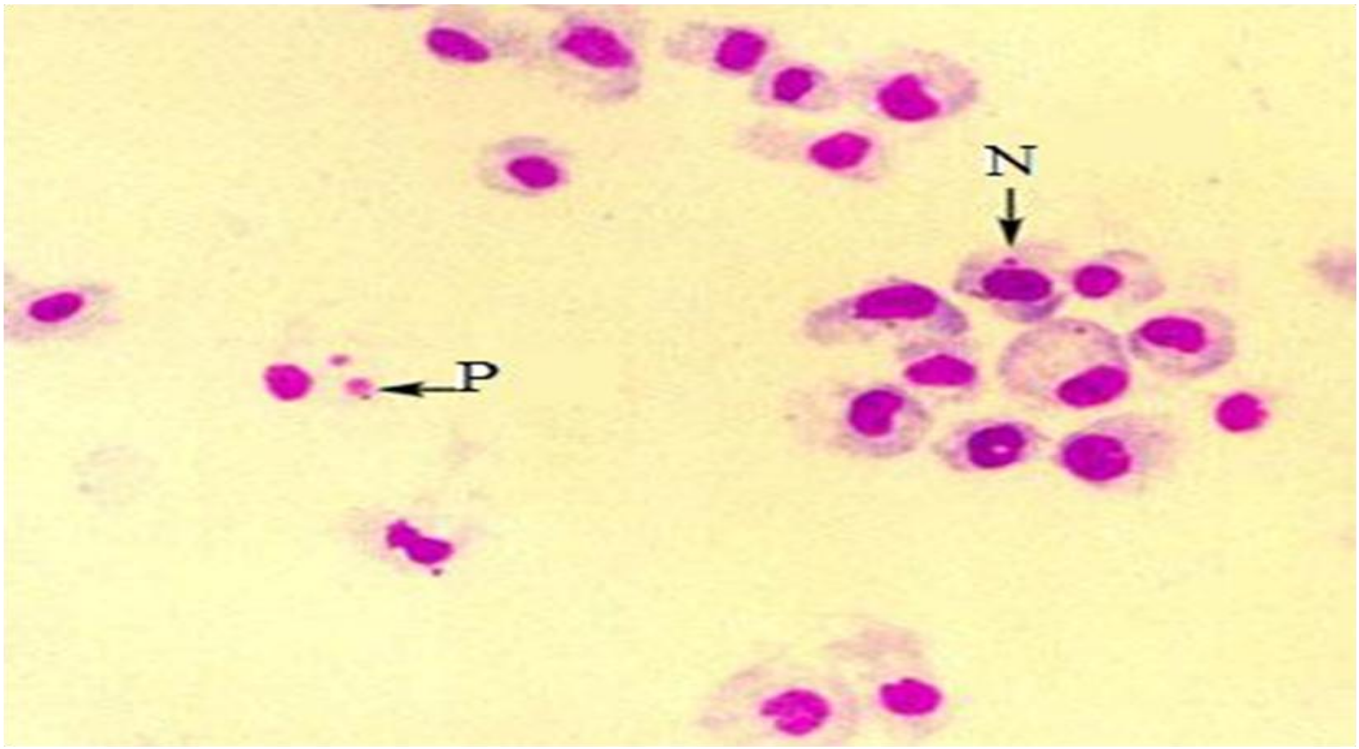

Fig. 2. Polychromatic erythrocyte with micronucleus.

(P= polychromatic cells, N=normochromatic cells).

(X400).

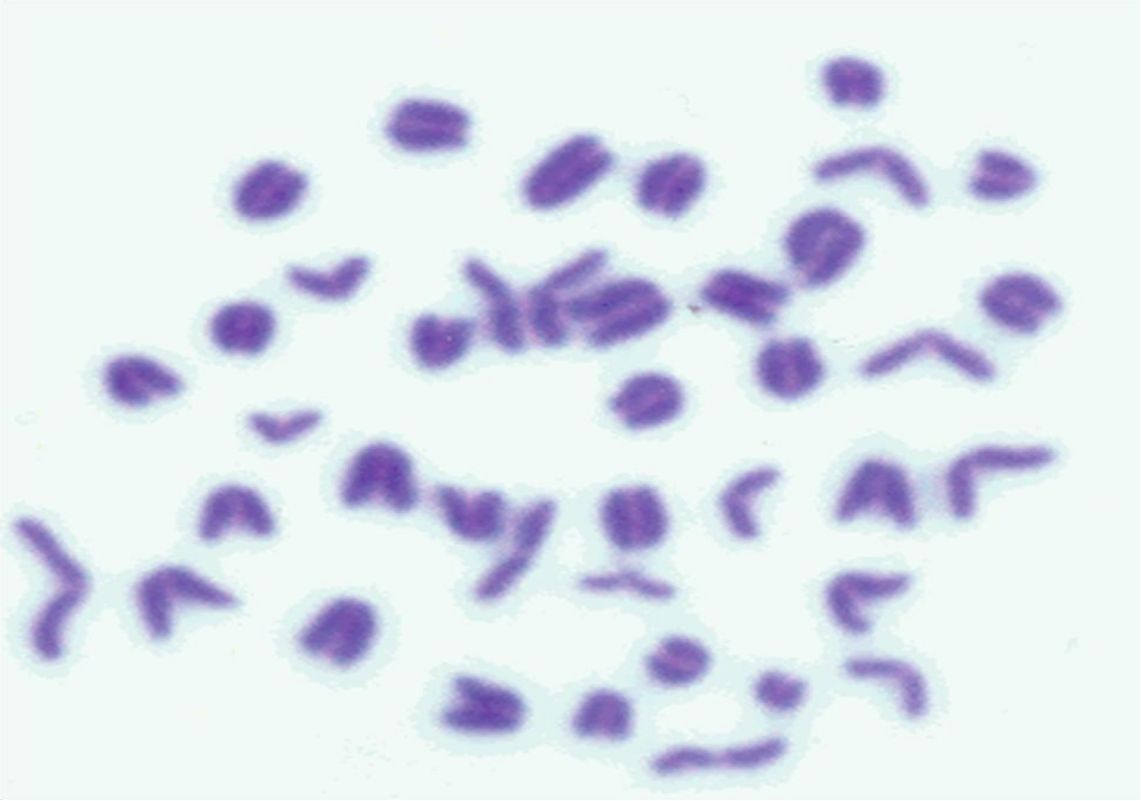

Fig. 4 a. meiotic metaphase from control animals.

(Normal chromosomes=40).

(x1000).

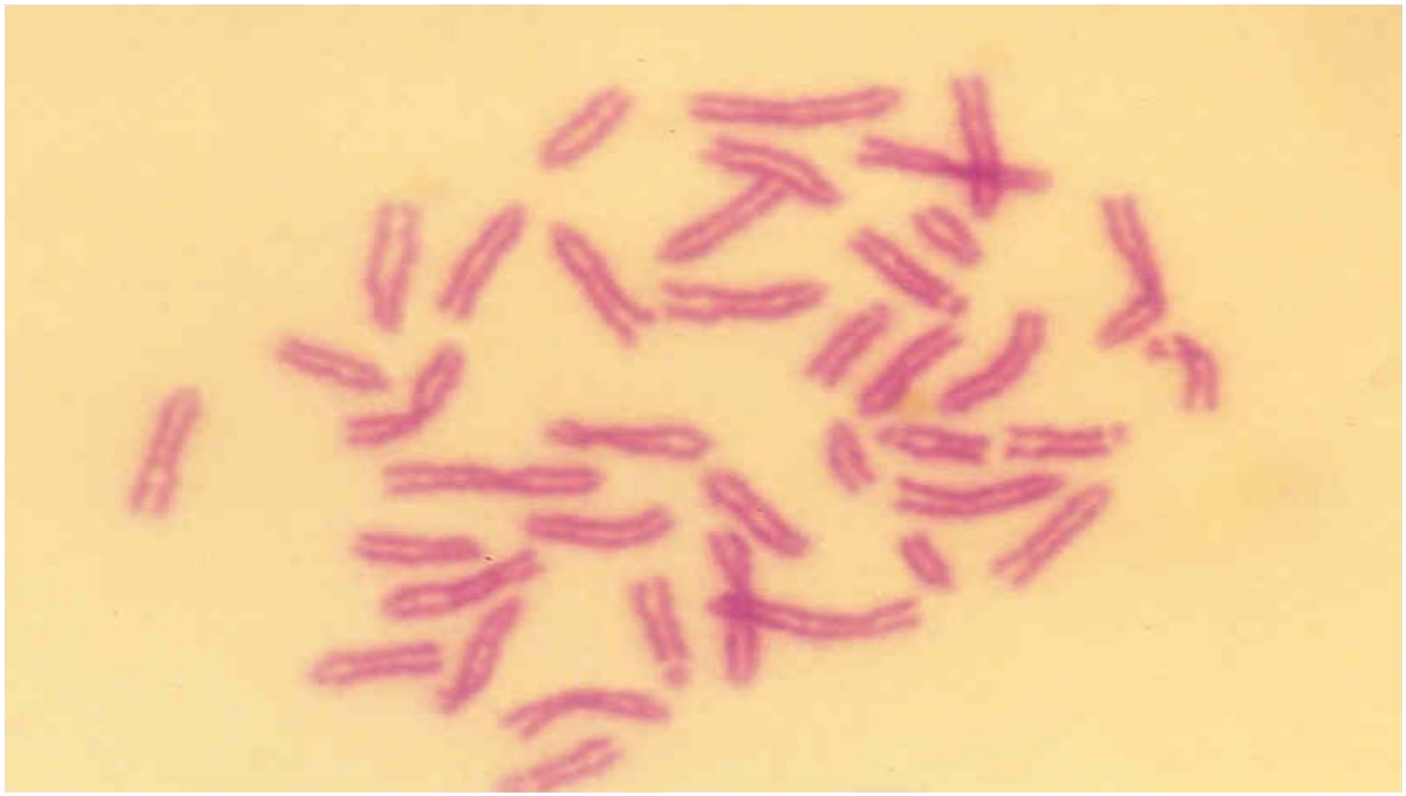

Fig.3. meiotic metaphase from control animals

(x1000).

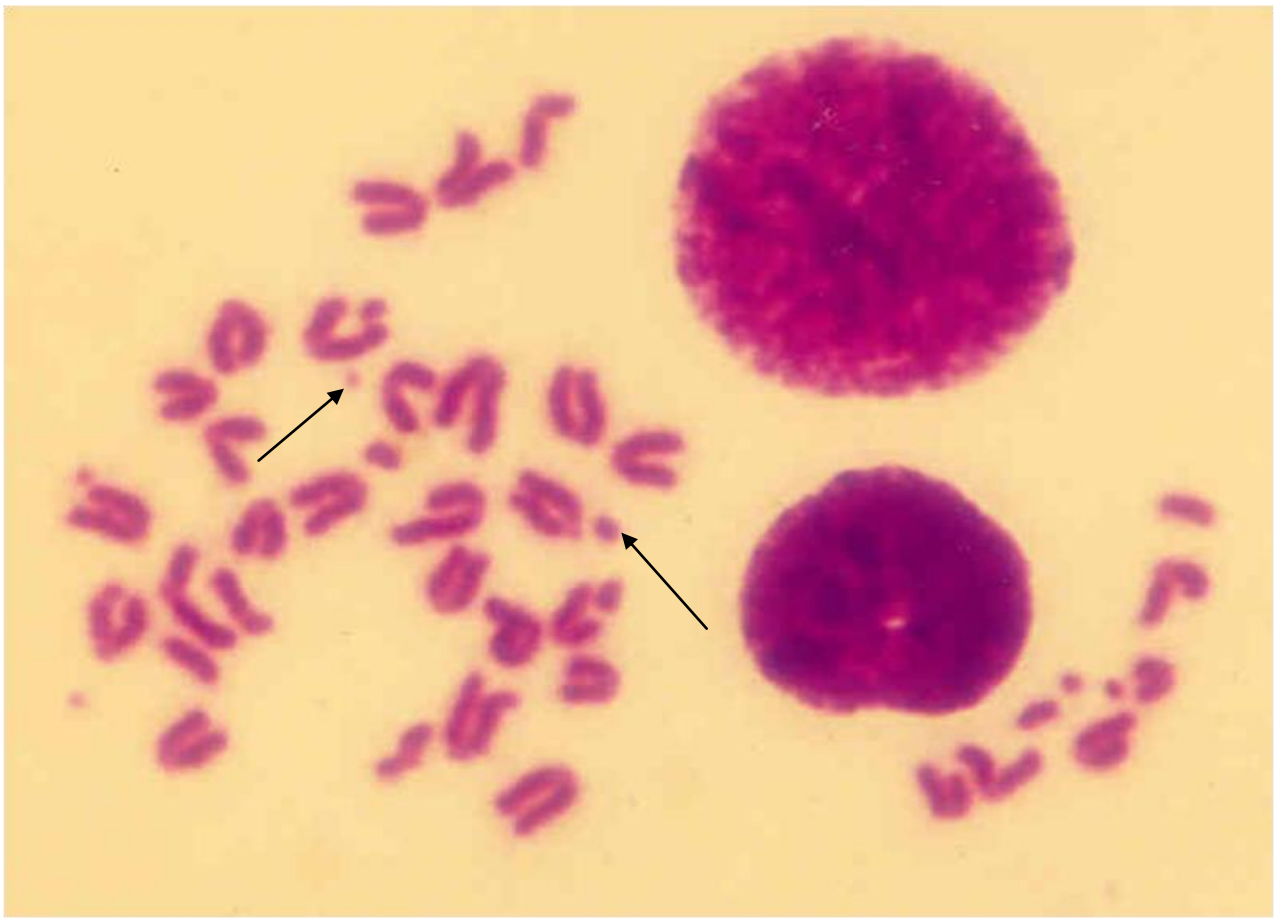

Fig.4. Injected with 3 mg CdCl2 /Kg

Metaphase with fragments (arrows).

(x1000).

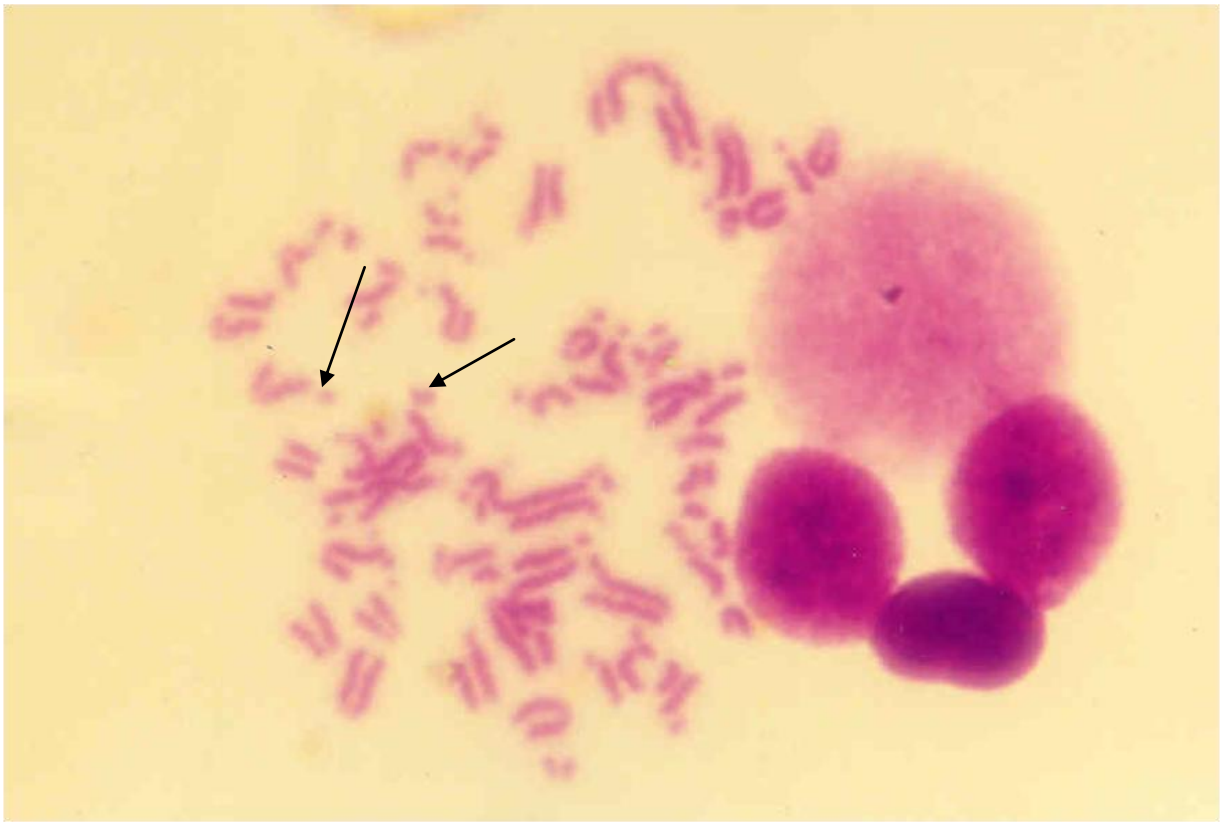

Fig.5. Injected with 5 mg Cdcl2/kg.

Metaphase with approx. 100 fragments (arrows).

(x1000).

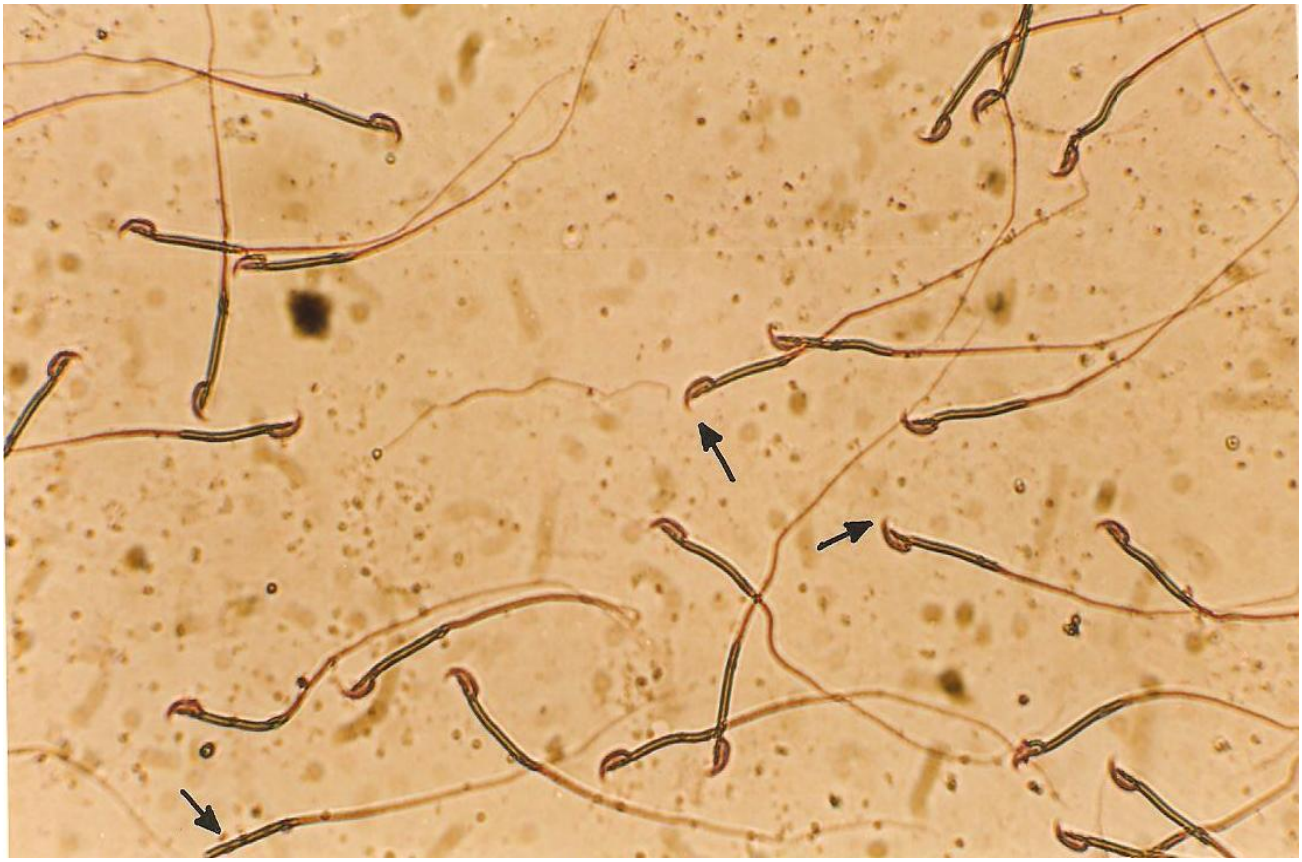

Fig.6. Photomicrograph of normal sperm. Sperms were prepared as described in the material and methods .
Note: Presence of the hook. Eosin Y (H2O) stains, (312X).

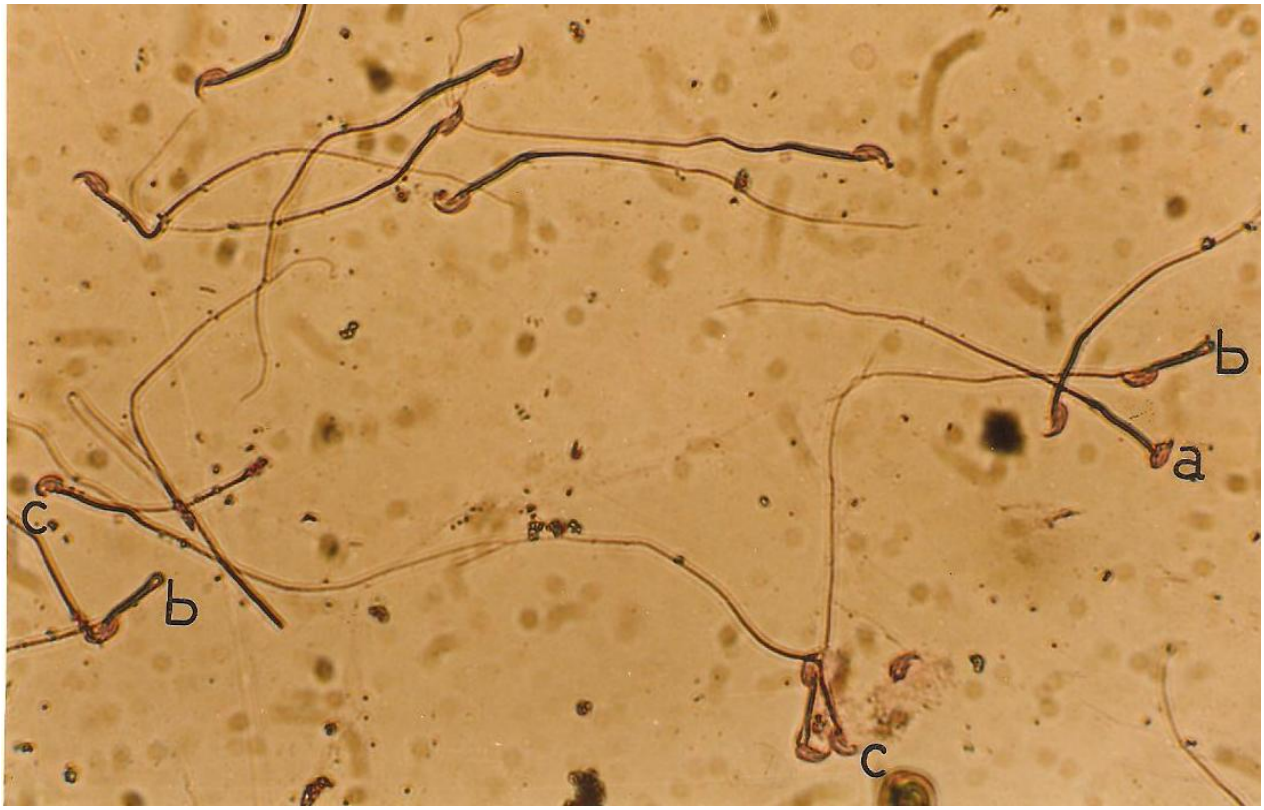

Fig.7: Photomicrograph of sperm morphology of treated mouse with 5mg / Kg $CdCl_2$

After 5 weeks shows some sperm abnormality: a) Fused head b) Tight tail

c) Sperm without hook d) Banana shape. Eosin Y (H2O) stain, (312X).

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