



Effect of cisplatin on chromosomes of male mice (*Mus musculus*)

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Abstract

Cisplatin is an anticancer drug. It is used to treatment of cancer. The present work studies the effect of cisplatin on chromosomes of male mice. 35 healthy adult male Swiss albino mice (*Mus musculus*) were divided into 7 groups, composed of 5 animals each.

The first group was normal (control), the second, third and fourth groups were administered single doses of 5, 10 & 15 mg/kg of cisplatin I.P, respectively. Mice were then anesthetized and killed after one day of cisplatin administration. The fifth, sixth and seventh groups were injected the same doses, but mice were anesthetized and killed after 5 days of cisplatin injection. Bone marrow of mice femurs were prepared the examining of chromosomal aberrations and mitotic index.

various chromosomal aberrations were recorded as structural aberrations (deletion, fragmentation, centric fusion, ring, end to end, break and gaps) and as numerical aberration (monosomy and trisomy). Also, the result of this study revealed that cisplatin caused reduction in the mitotic index at various doses. It was found that cisplatin had adverse effect on chromosomes and mitotic index.

Keywords: Cisplatin, I.P (Intraperitoneally)

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1. Introduction

Chemotherapy is defined as the drugs synthesized or procured from natural or synthetic sources for cancer inhibition and cure and these drugs are commonly called as chemotherapeutics. One of these chemotherapeutic drugs is Cisplatin.

Cis-Dichlorodiammineplatinum-II, commonly named as cisplatin which has proved to be effective against a wide variety of experimental malignant tumors [1-3] and in the treatment of many human cancers as well [4,5].

Many of the biological properties and effects of cisplatin have been well documented [6,7] but, therapeutic efficacy of cisplatin has been limited because of the development of drug resistance [8] and dose-limiting side effects, mainly nephrotoxicity [9].

Furthermore, the mutagenic potency of cisplatin reported in bacteria [10] and also in mammalian system [11] raises the concern which its use in cancer chemotherapy may have carcinogenic risk with the development of secondary malignancies [12].

Many searches have been published describing the intercalation of cisplatin with DNA [13-15] which discussed that the cellular DNA could be the primary target for cisplatin in its cytotoxicity.

It has been revealed that cisplatin functions as a bifunctional alkylating agent, producing DNA-protein crosslinks and DNA-intrastrand crosslinks [15].

In addition to its interaction with cellular DNA, the changes in various biochemical enzymatic parameters, immune response, cell surface etc. have been noted as well that led to propose the involvement of multistep and

multilevel effects of cisplatin in the tumor cell/host during cisplatin mediated chemotherapy against cancers [16].

Cisplatin can cause chromosomal aberrations and mutations in cultured mammalian cells [11,17and18].

2. Materials and Methods

Experimental animals:

Healthy 35 adult male mice *Mus musculus* weighting approximately (25 – 30) gm and aging approximately (8 - 9) weeks were obtained from national research center (N.A.C) in Dokki, Cairo.

The animals were housed in suitable rodent cages and maintained at standard conditions of temperature and humidity with an alternating light cycle (12hr light/dark). Animals were fed standard rodent diet which was obtained from Egyptian company of oils and soup kafr-Elzayat Egypt that manufactured especially for laboratory purpose. The diet is composed of 20% casein, 15%corn oil, and 55% corn starch 5% salt mixture and 5% vitaminized starch, in addition to water. The animals acclimatized to laboratory condition one week before using them.

- Drug:

1mg cisplatin/1ml vial was obtained from Hospira INI-VK.

- Grouping of animals:

Group 1: Control (normal) composed of 5 male mice.

Group 2: Composed of 5 male mice and each mouse was injected intraperitoneally with single dose of 5 mg/kg cisplatin and anesthetized then killed after one day.

Group 3: Composed of 5 male mice and each mouse was injected intraperitoneally with single dose of 10 mg/kg cisplatin and anesthetized then killed after one day.

Group 4: Composed of 5 male mice and each mouse was injected intraperitoneally with single dose of 15mg/kg cisplatin and anesthetized then killed after one day.

Group 5: Composed of 5 male mice and each mouse was injected intraperitoneally with single dose of 5mg/kg cisplatin and anesthetized then killed after 5 days.

Group 6: Composed of 5 male mice and each mouse was injected intraperitoneally with single dose of 10mg/kg cisplatin and anesthetized then killed after 5 days.

Group 7: Composed of 5 male mice and each mouse was injected intraperitoneally with single dose of 15mg/kg cisplatin and anesthetized then killed after 5 days.

Metaphase spreads were prepared according to [19, 20]. Fifty well were examined/each animal. Recording and photographing the type and frequency of chromosomal aberrations were done. Also calculating the mitotic activity of the cells was occurred as the number of dividing cells including prophase and metaphase per 1000 cells.

Statistical analysis:

The statistical analysis was carried out by using SPSS software, Ver .22 (IBM Corp. Released 2013). One-way ANOVA was done to determine the significance among groups. According to [21]. Data were treated as a complete randomization design. Multiple comparisons between groups were carried out applying Duncan test. The significance level was set at p value < 0.05.

3. Results

Monosomy and trisomy as a numerical chromosomal aberration are evaluated in table (1) and figure (11) as a result of treatment with cisplatin at different doses and different periods. Significant increase in chromosomal aberrations was noticed in the male mice. These aberrations were numerical that includes monosomy and trisomy.

Also, significant increase of structural aberrations (deletion, fragmentation, centric fusion, ring, end to end, gaps and breaks) are showed in table (2) and figure (12) at the same groups. It is observed that the total number of structural and numerical chromosomal aberrations increased by increasing the dose of cisplatin. The mean values of all chromosomal aberrations (numerical – structural) in all doses (5mg/kg – 10mg/kg – 15mg/kg) after one day treatment were more significant than the mean values of all chromosomal aberrations in all doses after 5 days treatment.

This means that total chromosomal aberrations (numerical - structural) were noticed to be maximum values after 24h treatment and decreased during other periods.

The presented data in table (3) and figure (13) showed that the mean value of mitotic index decreased by increasing the dose of cisplatin. The mean values of mitotic index of mice left 5 days after injection were significantly higher than the mean values of mitotic index of those left only one day.

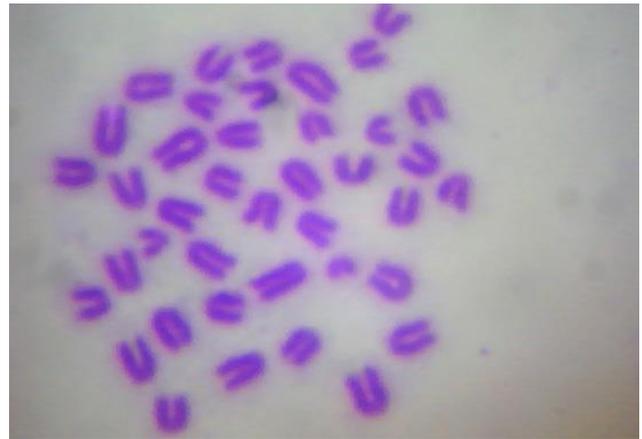


Fig (1): Normal metaphase spread in bone marrow cells of (*Mus musculus*).
(Giemsa stain x 1000)



Fig(2): metaphase spread showing deletion (D) in bone marrow cells of (*Mus musculus*).
(Giemsa stain x 1000)

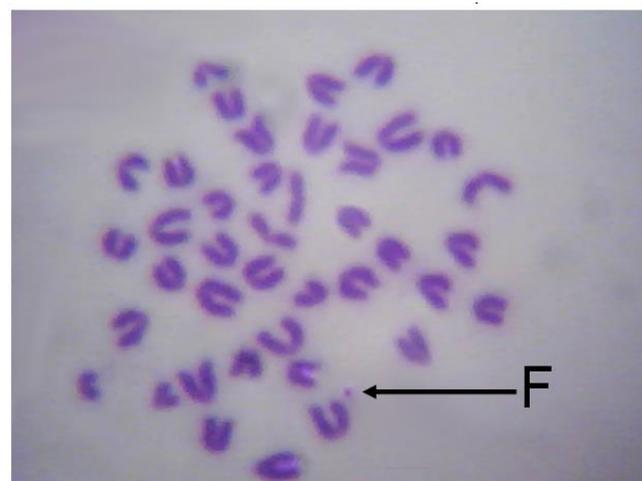


Fig (3): Metaphase spread showing fragmentation (F) in bone marrow cells of (*Mus musculus*).
(Giemsa stain x 1000)

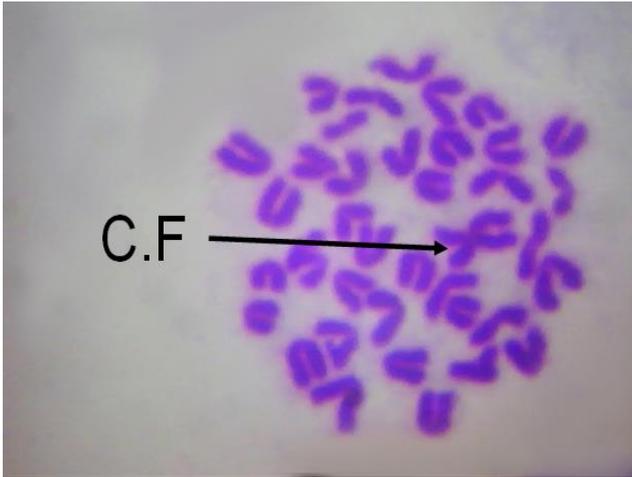
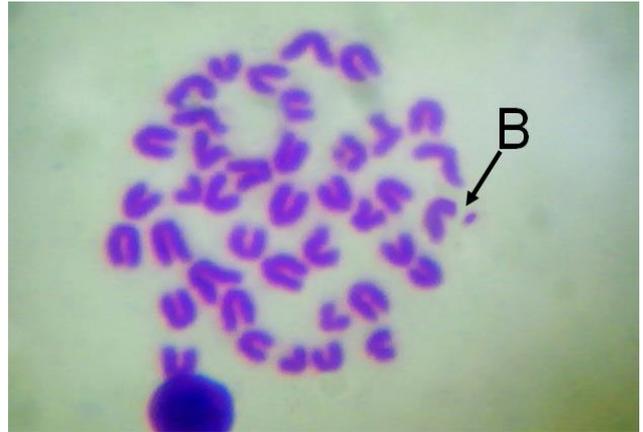


Fig (4): Metaphase spread showing centric fusion (CF) in bone marrow cells of (*Mus musculus*) (Giemsa stain x 1000)



Fig(7): Metaphase spread with break (B) in bone marrow cells of (*Mus musculus*). (Giemsa stain x 1000)



Fig (5): Metaphase spread showing ring chromosome (R) in bone marrow cells of (*Mus musculus*). (Giemsa stain x 1000)

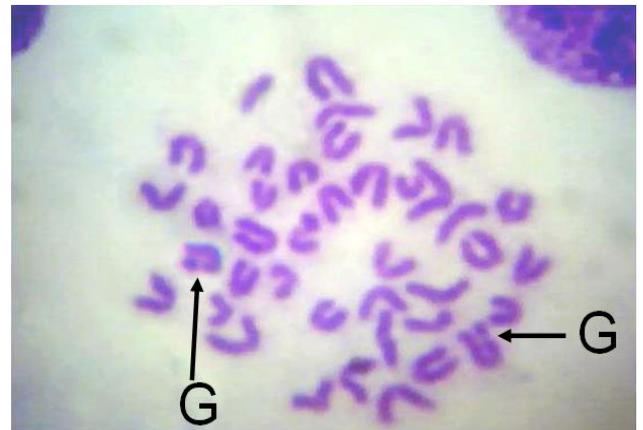


Fig (8): Metaphase spread with gap (G) in bone marrow cells of (*Mus musculus*). (Giemsa stain x 1000)



Fig (6): Metaphase spread with End to End association (E to E) in bone marrow cells of (*Mus musculus*). (Giemsa stain x 1000)



Fig (9): Metaphase spread of monosomy in bone marrow cells of (*Mus musculus*). (Giemsa stain x 1000)

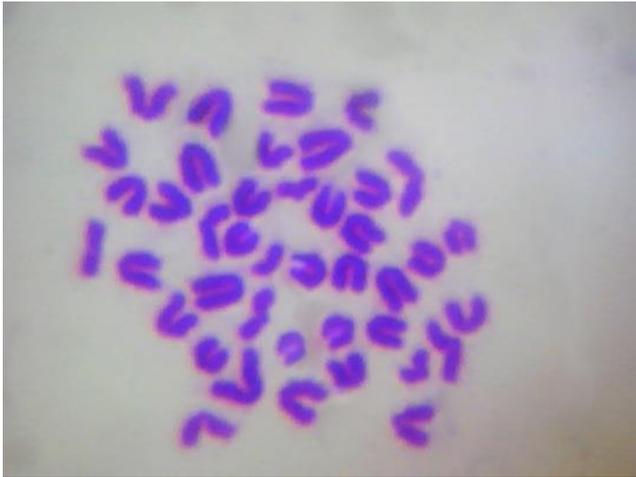


Fig (10): Metaphase spread of trisomy in bone marrow cells of (*Mus musculus*). (Giemsa stain x 1000)

Table (1): The mean values of numerical chromosomal aberrations

Treatments	Period (days)	Monosomy	Trisomy	Total
Normal	0	0.6±0.40 ^d	0±0 ^d	0.6±0.4
5 mg	1	3.2±0.66 ^{bc}	1.2±0.58 ^{abc}	4.4±1.24
	5	1.60±0.51 ^{cd}	0.2±0.20 ^{bc}	1.8±0.71
10 mg	1	4.0±0.84 ^{ab}	1.6±0.51 ^{ab}	5.6±1.35
	5	2.0±0.71 ^{cd}	0.4±0.24 ^{bc}	2.4±0.95
15 mg	1	5.6±0.51 ^a	2.4±0.68 ^a	8.0±1.19
	5	3.0±0.71 ^{bc}	1.00±0.45 ^{bc}	4.0±1.16

a, b & c: There is no significant difference ($P>0.05$) between any two means, within the same row have the same superscript letter.

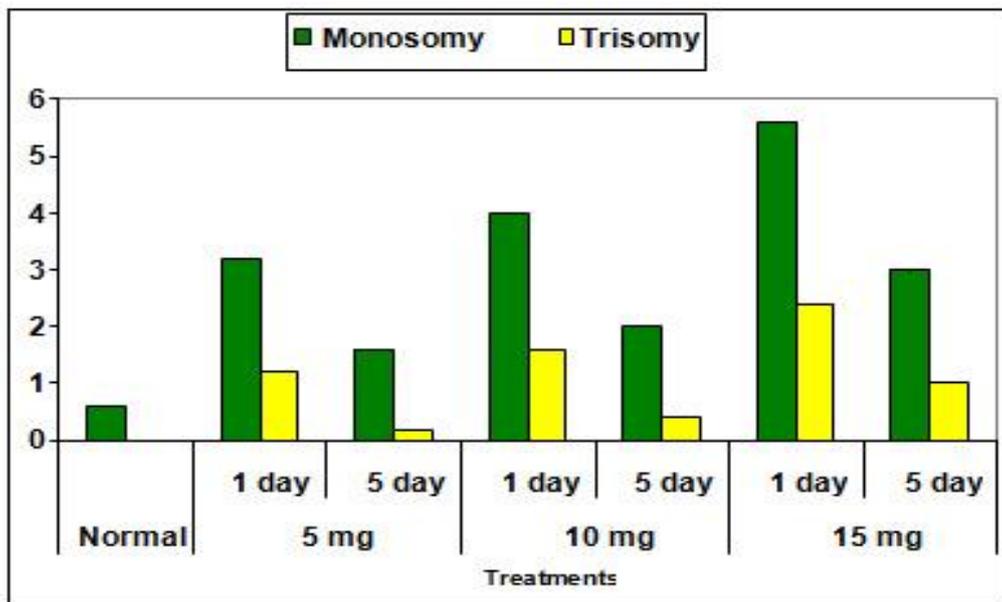


Fig (11): The mean values of numerical chromosomal aberrations

Table (2): The mean values of structural chromosomal aberrations

Treatments	Deletion	Fragmentation	Centric fusion	Ring	End to End	Breaks	Gaps	Total
Normal	7.00 ±0.71 ^d	0±0 ^c	0±0 ^b	5.80 ±0.58 ^e	0.60 ±0.40 ^b	0±0 ^c	0±0 ^c	13.40 ±1.69
5 mg 1 day	15.40 ±0.68 ^{bc}	1.20 ±0.58 ^{bc}	0.80 ±0.37 ^{ab}	10.00 ±0.71 ^{bc}	1.40 ±0.51 ^{ab}	1.40 ±0.51 ^{bc}	1.40 ±0.51 ^{bc}	31.60 ±3.87
5 mg 5 day	7.80 ±0.73 ^d	0.20 ±0.20 ^c	0.20 ±0.2 ^b	7.20 ±1.02 ^{de}	0.60 ±0.40 ^b	0±0 ^c	0.20 ±0.20 ^c	16.20 ±2.75
10 mg 1 day	16.4 ±0.87 ^{ab}	2.00 ±0.71 ^{ab}	1.00 ±0.45 ^{ab}	11.00 ±0.71 ^{ab}	2.00 ±0.71 ^{ab}	2.40 ±0.81 ^b	2.60 ±0.81 ^{ab}	37.40 ±5.07
10 mg 5 day	13.00 ±0.71 ^c	0.60 ±0.40 ^{bc}	0.40 ±0.24 ^b	8.40 ±0.51 ^{cd}	0.80 ±0.37 ^b	0±0 ^c	0.40 ±0.24 ^c	23.60 ±2.47
15 mg 1 day	18.4 ±1.12 ^a	2.80 ±0.66 ^a	2.00 ±0.71 ^a	13.00 ±0.71 ^a	3.00 ±0.71 ^a	3.80 ±0.66 ^a	4.00 ±0.71 ^a	47.00 ±5.28
15 mg 5 day	15.20 ±0.86 ^{bc}	1.00 ±0.45 ^{bc}	0.60 ±0.40 ^b	9.20 ±0.58 ^{bcd}	1.20 ±0.58 ^b	0.20 ±0.20 ^c	0.60 ±0.40 ^c	28.00 ±3.47

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

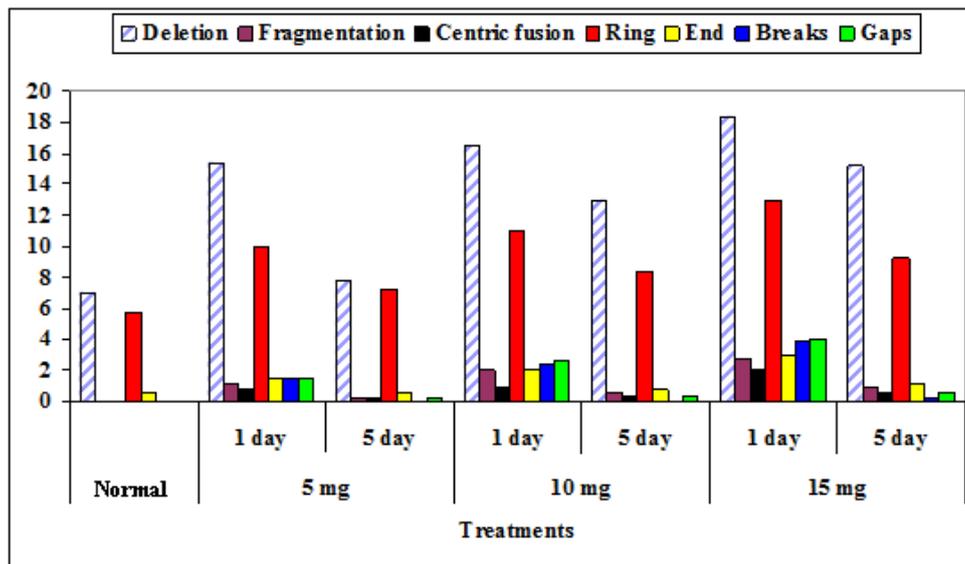


Fig (12): The mean values of structural chromosomal aberrations

Table (3): The mean value of Mitotic Index of chromosomes in bone marrow cells of male mice treated with cisplatin at different doses and different periods

Treatments	Mitotic Index
Normal	848±5.15 ^a
5 mg 1 day	810±3.63 ^c
5 mg 5 day	830±4.23 ^b
10 mg 1 day	759±4.81 ^e
10 mg 5 day	774±4.30 ^d
15 mg 1 day	700±3.54 ^f
15 mg 5 day	754±5.79 ^e

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

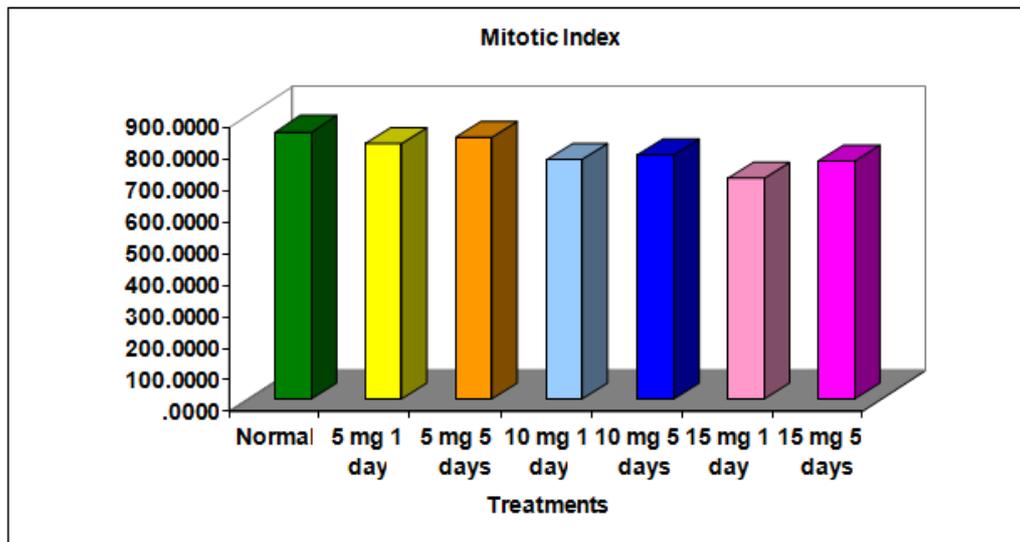


Fig (13): The mean value of Mitotic Index of chromosomes in bone marrow cells of male mice treated with cisplatin at different doses and different periods

4. Discussion

Although the very wide use of chemotherapeutic drugs in the treatment of cancer for improvement of the quantity of life of cancer patients and even for the cure of disease, treatment with some of the most effective anti-cancer drugs causes a number of symptoms of direct toxicity. Also, many anticancer drugs have been shown to be mutagenic, teratogenic and carcinogenic in experimental systems [22].

There are many chemicals in use, such as ionizing radiation and cancer therapeutic agents which have been proven to be DNA reactive and that cause a distinct spectrum of base pair substitution mutations and structural chromosome changes [23].

Currently, mice were injected with 5mg/kg 1day - 10mg/kg 1day - 15mg/kg 1day - 5mg/kg 5day - 10mg/kg 5day - 15mg/kg 5day) of cisplatin. The increase in chromosomal aberrations was noticed in the male mice of these groups. These aberrations are structural (deletion, fragmentation, centric fusion, ring, end to end, gaps and breaks) and numerical aberrations include monosomy and trisomy. Mitotic index was also affected after treatment with cisplatin. Many authors studied the mechanism of the aberrations of the chromosomes [24-26]. Others considered that chromosomal aberrations are the consequence of repair processes that operate on damage caused by different factors on chromosomal DNA. When deoxyribonuclease released in the cell nucleus at an appropriate stage of mitotic cycle, as well, chromosomal aberrations damage may happen [27].

Although cisplatin is an effective antitumor drug, it has serious side effects on non-tumor cells, including free radical generation [28]. Cisplatin can cause damage DNA by elevating level of reactive oxygen species (ROS) [29] such as superoxide anions and hydroxyl radicals can damage DNA and form strand breaks of DNA [30].

Many studies on the genotoxicity of cisplatin that react with nucleic acid component indicate that the cellular DNA could be the primary target in its anticancer activity [15,31]. Platinum compounds of cisplatin reacted with the nitrogen atoms of nucleic acid bases guanine, adenine and cytosine [13]. It was suggested that those at N-7 positions of adenine and guanine were the most likely sites to be involved in reaction with DNA [15,32]. Development of chromosomal aberrations in the cells have been commonly used as the mutagenic bioassays of a drug [33,34].

In a previous study, [35] found that single dose of cisplatin (5mg/kg) cause an increase in the number of mitotic metaphase chromosomal aberrations and mitotic index didn't change at 24h which suggested that cisplatin is non-mitotoxic at given dose. Also [36] have reported that single dose of cisplatin produced an increased in chromosomal aberrations and abnormal metaphases noticed at 24th hours.

In the current study, we observed that chromosomal aberrations increased by increasing the dose of cisplatin and the total number of structural and numerical chromosomal aberration in addition aberrant metaphases were observed to be maximum at 24h of treatment. This gradually decreased during the later periods and later phase of cell cycle which support the view as well that antitumor agents cause a high frequency of aberrations in rodents 24h after single exposure which roughly agree with the normal length (22-24h) of the cell cycle [37]. The decrease in the frequency of aberrations in the later hours of treatment could be because of death of damaged cells, clearance of mutagen drug (cisplatin) from the body and post-replication repair process that might be operating for recovery from the cisplatin-caused damage to DNA, in fact [38] have established an involvement of post-replication repair process in cisplatin induced DNA damage.

Cisplatin lesions on O₆ of guanine in normal cells were proposed that to be repaired before replications while in cancer cells the lesions are not removed due to a deficiency in this repair process, so mutation rate increases beyond the limits of survivability [6].

The inhibition of mitotic activity by increasing the dose indicates that, cisplatin binds to DNA, blocks and prolongs the division cells in the G₂ phase of the cell cycle when is injected to the body. The blockage of the cells in

G₂ phase is linked to the inhibition of chromatin condensation [33].

5. Conclusion

According to the previous experiments and results we conclude that cisplatin harmed chromosomes of male mice and caused structural-numerical chromosomal aberrations which increased by increasing the dose of cisplatin after one day and decreased after later period, but mitotic index decreased by increasing the dose of cisplatin.

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