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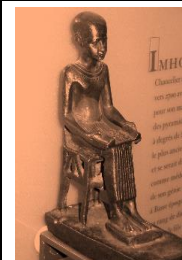


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Original Article

Induced Chromosomal Aberrations of Cyclophosphamide in Bone Marrow Cells of Albino Rats and Possible Protective Role of Zinc Chloride

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ABSTRACT

Article information

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Background: Chemotherapeutic agents are associated with different unwanted effects; the dangerous are mutagenic actions. Researchers do their best to ameliorate such effects by different supportive agents.

Objective: The study designed to demonstrate the mutagenicity of the chemotherapeutic drugs especially cyclophosphamide [CP] and possible protective effects of Zinc Chloride [Zn Cl]

Materials and Methods: The experiment was designed as 4 groups each group contain 12 animals, the first group [control group] was injected intraperitoneal normal saline [1 ml/kg] every other day for 20 days, the second group was given intraperitoneal Zn Cl [4 mg/kg] every other day for 20 days. The third group [Test group] injected by intraperitoneal cyclophosphamide [200 mg/kg] as a single dose on 15th day of experiment, Fourth group [protective group] was given intraperitoneal ZnCl [4 mg/kg] every other day for 20 days and injected intraperitoneal single dose by CP [200 mg/kg] on 15th day.

Results: There were significant changes in blood cells, mitotic index and increased frequency of structural and numerical changes in chromosomes compared with the control group and these outcomes attributed to effects of free radicals of CP on health cells. The protective effect of Zn Cl which decrease blood cell changes, the structural and numerical aberrations in relation to the test group.

Conclusion: CP has harmful effects on the blood cells and chromosomes. Zinc Chloride has a protective effect against the harmful effects of CP.

Keywords: Cyclophosphamide; Zinc Chloride; Bone Marrow Cells; Mutagenic; Chromosomes.



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INTRODUCTION

Cyclophosphamide [CP] has anticancer effects and harmful to normal cells, this bad effects may due to the imbalance of antioxidant system resulting in the accumulation of free radicals, Free radicals can cause DNA fragments, chromosomal aberrations [1]. Several studies demonstrated chromosomal aberrations caused by anticancer drugs in bone marrow cells [2,3]. So, it was necessary to use antioxidant materials to decrease the harmful impacts of CP and other anticancer drugs on normal tissues. CP Also destroy the antioxidant system in normal cells and has a carcinogenic effect on normal tissues in animals, it also causes gene mutation and abnormal gene expression and chromosomal aberrations in normal tissues of the mice and secondary tumors in animals treated by anticancer drugs [4].

DNA replication and transcription play an important role in cell division in bone marrow cells development and cell growth. The antioxidant system play an important role in these biological processes to protect DNA from harmful effect of anticancer drug [5,6]. There are other investigations suggest that Zn Cl has anticancer effects and increase the resistance to tumor formation in mice, so it has potential effect in the apoptosis process [7,8].

Pretreatment with Zn Cl has protective effects in chromosomal aberrations and give similar results as the control animals and so in this study animals treated by Zn Cl before injection I.P. by CP to evaluate the protective effect of Zn Cl against the harmful effect of CP on normal tissue of albino rat [9].

THE AIM OF THE STUDY

The current work designed to investigate the protective effect of ZnCl against the harmful effect of CP on bone marrow cell.

MATERIALS AND METHODS

Forty-eight male albino rats [120±10 g] from pharmacology department of the faculty of medicine, Al-Azhar University [Egypt], were used in this study. Animals were housed under the same environmental conditions and were fed on standard materials and tap water. Animals were divided into 4 groups [12 in each groups].

Group I [control group]: animals received an intraperitoneal injection of normal saline [1ml/ kg/BW] every other day for 20 days.

Group II: [Zinc chloride group]: animals received intraperitoneal ZnCl [4gm/kg/BW] every other day for 20 days. Zn Cl was purchased from El- Gomhoria co.

Group III [CP group]: Animals received single dose of intraperitoneal injection of CP 200mg/kg/BW at the beginning of the third week of the study

Group IV [ZnCl plus CP]: Animals received intraperitoneal ZnCl [4gm/kg/BW] every other day for two weeks before CP injection which had been administrated one week before the end of the study

All animals received intramuscular injection of colchicine [0.25 ml of 0.5% solution/kg BW] 2 hours before scarifying to stop cell division at the metaphase stage. Retro-orbital blood sample was obtained for the analysis of blood cells and bone marrow samples were processed using methods described by Rabello and Ahmed [10] to determine chromosomal abnormalities. A sample of bone marrow cells was collected in a test tube containing normal saline to analyze the effect of CP on the cells. Numbers of chromosomal abnormalities were expressed as means ± SE, and 300 metaphases were counted for each rat.

The statistical comparison between groups had been performed by one way analysis of variance [ANOVA test] and for comparison between two groups, the independent samples student *t*-test was used. P value < 0.01 was considered significant.

RESULTS

Data in table [1] showed that, CP lead to a significant reduction in the RBCs count after 3 weeks. This reduction attributed to CP toxicity and antimitotic effects on bone marrow cells. The use of zinc was associated with an increase in RBCs and white blood cells than the control group. However, the difference was statistically non-significant. The use of zinc supplementation with cyclophosphamide was associated with increased cells towards values registered by the control group.

The protective effect of ZnCl against the CP chromosomal abnormalities was evaluated by using assays of rat bone marrow cells. ZnCl group, CP group and ZnCl + CP were tested and compared with the control group. It was observed that group ZnCl is comparable or even similar to the control group. However, the comparison between Group III and the control group showed that, the number of chromosomal abnormalities were increased substantially in the cyclophosphamide treated group. The number of chromosomal abnormalities in the zinc chloride-supported group were less than CP and comparable to the control group [figures 1 to 7].

Data in table [2] demonstrated that, the numbers and types of chromosomal abnormalities in bone marrow cells of rats of the CP-treated group had several chromosomal abnormalities such as ring chromosomes, deletion, fragmentation and polyploidy. When compared to the control group, the mean frequency of overall chromosomal abnormalities was considerably higher following CP therapy [0.34±0.40 versus 9.67±1.85]. The pretreatment with ZnCl with CP showed significant reduction of chromosomal abnormalities that induced by CP [1.97 ±0.75]. However, it still higher than the control group.

Data in table [3] showed that, pretreatment by ZnCl before CP showed significant reduction of the mitotic index in CP-treated group than the control group [13.00 ± 0.57 vs 17.50 ± 0.29]. The supplementation of ZnCl was associated with an increase in mitotic index, which was close to the normal group.

Cellular changes observed by cyclophosphamide may be due to drug induced decrease in stem cells of RBCs. Also, these changes could be attributed to hemolysis and decreased RBCs production with subsequent anemia. Blood cell reduction also might cause by free radicals induced peroxidation of phospholipid of cell membrane which reduced by Zn Cl.

Table [1]: The protective effect of Zn Cl against Cyclophosphamide on peripheral blood cells at the end of study.

| Blood cells | Group I | Group II | Group III | Group IV |
|--------------------------------------|-----------------|------------------|---------------------|-----------------|
| Red blood corpuscle $\times 10^6/CC$ | 9.22 ± 0.94 | 10.82 ± 0.43 | $6.8 \pm 1.2^*$ | 8.06 ± 0.71 |
| White blood cells $\times 10^3/CC$ | 6.74 ± 0.30 | 8.21 ± 1.17 | $4.6 \pm 0.17^{**}$ | 6.27 ± 0.56 |

$P < 0.05 = *$, $P < 0.01 = **$



Figure [1]: Metaphase spread from albino rat bone marrow cells of the control group [42 XY] [Giemsa stain X 1000].

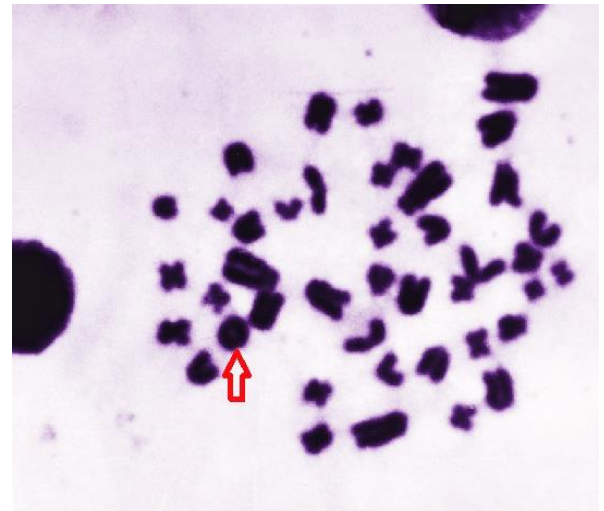


Figure [2]: Metaphase spread from albino rat bone marrow cells [CP-treated group]. The red arrow refers to ring chromosome [Giemsa stain X 1000].



Figure [3]: Metaphase spread from albino rat bone marrow cells [CP-treated group]. The red arrow refers to chromatid break [Giemsa stain X 1000].



Figure [4]: Metaphase spread from albino rat bone marrow cells [CP-treated group]. The red arrow refers to chromatid fragment [Giemsa stain X 1000].

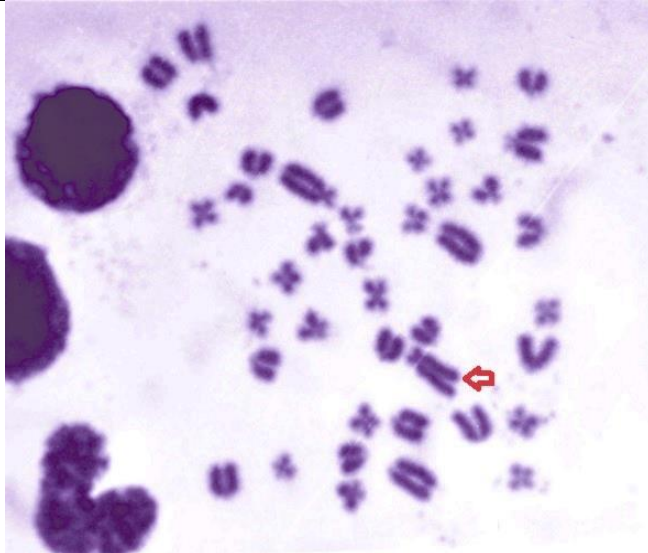


Figure [5]: Metaphase spread from albino rat bone marrow cells [CP-treated group]. Red arrow refers to chromatid deletion [Giemsa stain X 1000].

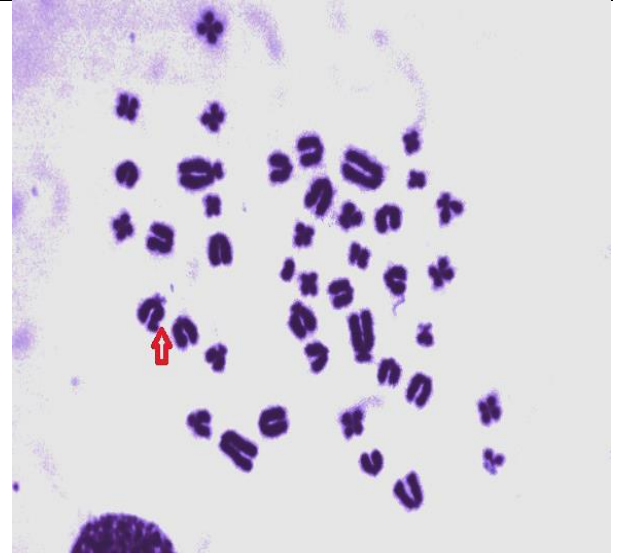


Figure [6]: Metaphase spread from albino rat bone marrow cells [CP-treated group]. The red arrow refers to chromatid gap [Giemsa stain X 1000].

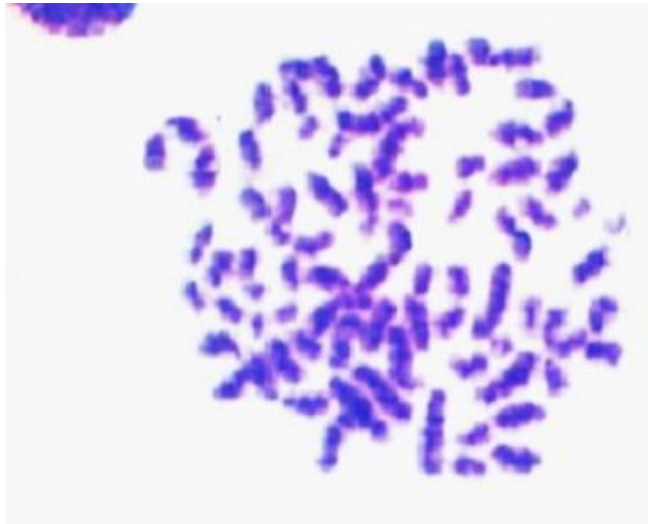


Figure [7]: Metaphase spread from albino rat bone marrow cells [CP-treated group] showed polyploidy [Giemsa stain X 1000].

Table [2]: Structural and numerical chromosomal abnormalities of bone marrow cells of albino rats

| | Structural abnormalities of chromosomes | | | | | Total Abnormalities | Total damage Mean±SE |
|------------------|---|-----------------|--------------------|--------------------|---------------|---------------------|----------------------|
| | Ring chromatid | Chromatid Break | Chromatid fragment | Chromatid deletion | Chromatid Gap | | |
| Group I | - | 1 | - | - | 1 | - | 0.34±0.40 |
| Group II | - | 1 | - | - | - | - | 0.17±0.20 |
| Group III | 4 | 6 | 6* | 21* | 11* | 10* | 9.67±1.85** |
| Group IV | 2 | 3 | 5 | 4 | 1 | - | 1.97±0.75** |

P < 0.05 = *, P < 0.01 = **

Table [3]: Mitotic index efficacy by cyclophosphamide and/or ZnCl in bone marrow cells of albino rats [Mean±SE].

| Groups | Mitotic index [mean±SE] |
|------------------|-------------------------|
| Group I | 17.50± 0.29 |
| Group II | 16.59±0.29* |
| Group III | 13.00± 0.57** |
| Group IV | 15.50±0.29 |

P < 0.05 = *, P < 0.01 = **

DISCUSSION

Anticancer chemotherapeutic drugs such as cyclophosphamide are commonly utilized. Because it can't tell the difference between normal and malignant cells, it's also detrimental to healthy cells. CP's active components are phosphate and acrolein. Although these substances work at the DNA level to slow the formation of malignant cells, they also generate mutations in DNA that may lead to secondary cancers in human and animal cells [11,12].

CP and its metabolites cause oxidative stress by reacting with electron-rich regions of the body, such as proteins and nucleic acids. As a result of hydroxylating DNA at the N7 position of the guanine base, CP impairs cell development, mitotic activity, and functionality. CP causes chromosomal abnormalities owing to DNA strand damage and disintegration [13].

This research found that injecting a single dosage of CP into mice resulted in a reduction in blood cells, increased mitotic index, increased DNA damage, and chromosomal abnormalities in bone marrow cells. These findings are in line with those of a number of other researches, like Popov *et al.* [14], Sushma and Devi [15] and Kour *et al.* [3].

Other research discovered that the rat's bone marrow tissue analysis approach indicated significant indicators of genotoxicity. After CP therapy, there were many changes in DNA damage, chromosomal aberrations, and a drop in mitotic index. These findings are consistent with the current research, which found DNA strand damage and a drop in the mitotic index, as well as chromosomal abnormalities such as ring chromatid fragmentation, chromatid deletions, and polyploidy in the C.P.-only group [16].

Despite the fact that most chemotherapy, particularly CP, is important for destroying tumor cells, it is hazardous to normal cells, with C.P. treatment causing chromosomal aberrations and a drop in mitotic index in rats, current search results may be useful in resolving this issue [17].

In general, CP is an alkylating medication that is utilized to treat certain types of malignancies such as carcinoma, according to several studies.

Although CP is an effective cancer therapy, it also has side effects on normal tissues, cell division, and mitotic index. The current findings revealed that albino rats given a single dosage of CP and left for seven days displayed changes in blood cells, mitotic index, and chromosomal abnormalities.

These findings are consistent with prior research, which found that CP has a negative impact on normal healthy tissues called genotoxicity. As a result, developing tumor treatments or improving existing therapies that are free of or have few side effects has been

a major goal in recent research to reduce the negative effects of chemotherapy [18,19].

Zinc chloride has a role in the integrity of DNA replication and transcription. As a result, Zn Cl is employed to protect cells against oxidative material while also conserving DNA, regulating transcription, and replicating DNA [20-23].

Many researchers have shown that zinc chloride [ZnCl] has a protective effect against oxidative stress in many organs [5,24].

Other findings show that ZnCl supplementation in rats decreased several forms of CP-induced DNA damage and inhibited chemotherapeutic-induced apoptosis [8, 18,19, 25].

In this work, providing Zn Cl concurrently with cyclophosphamide exposure reduced DNA damage, chromosomal abnormalities, and increased mitotic index, resulting in protection against cyclophosphamide-induced cytotoxicity in multiple cell lines tests, which is consistent with earlier results [26].

Conclusion:

The current findings show that Zn Cl pretreatment or simultaneous treatment has good anti-mutagenic activity against cyclophosphamide-induced genotoxicity. It exerts its action by its anti-oxidant properties and its work on DNA replication, resulting in an increase in DNA fragments, significant inhibition of chromosomal aberration formation, and an increase in mitotic index. The current results point to promising clinical uses for Zn Cl in conjunction with chemotherapeutic drugs such as CP.

Financial and non-financial activities and relations of interest

None

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