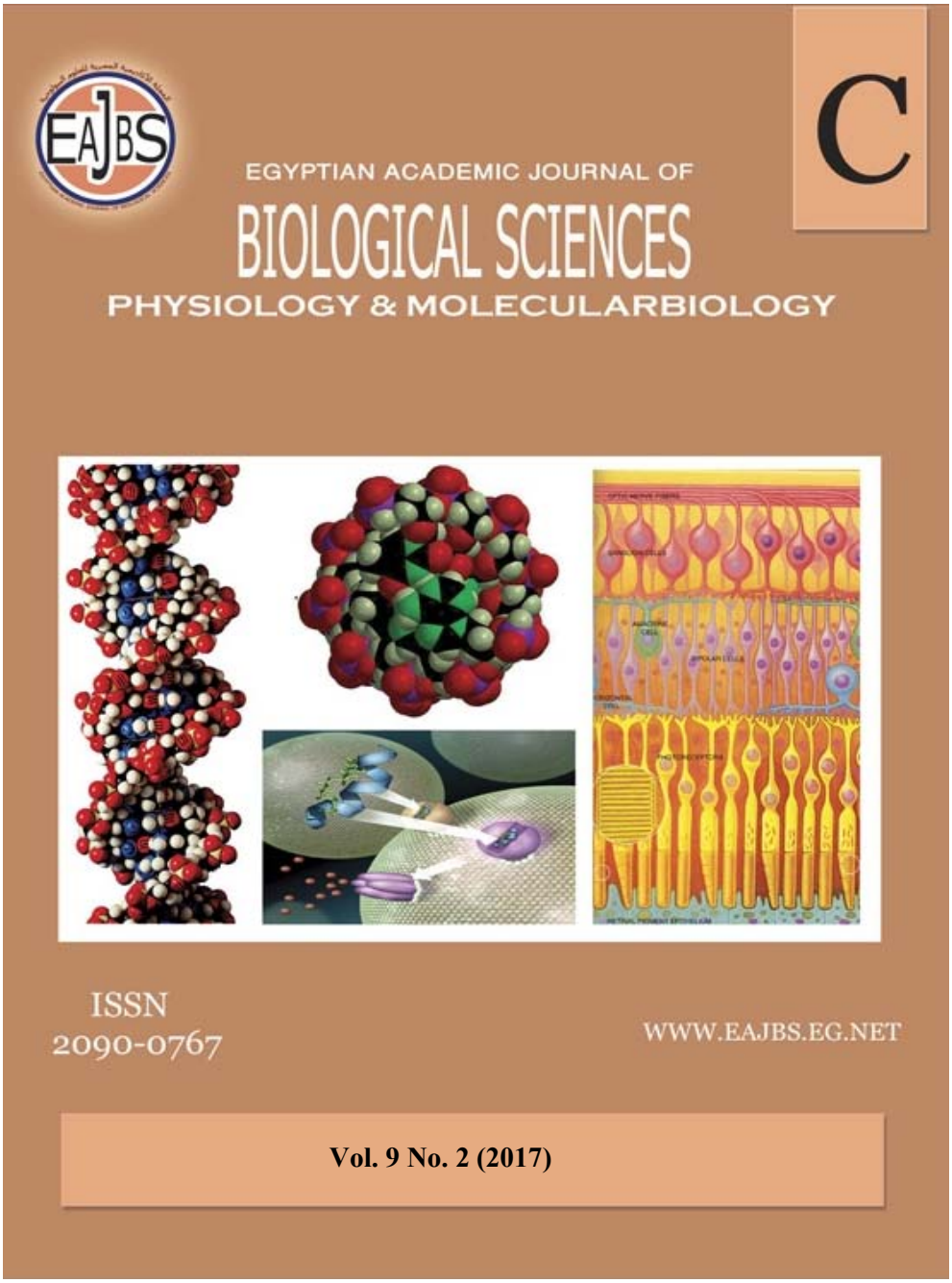

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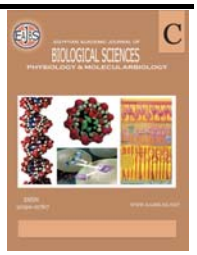

Protective Effects of Zinc Chloride on Cyclophosphamide-Induced Genotoxicity in Male Albino Rat Tissues *in vivo*

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ABSTRACT

The aim of this study was to assess the potential protective effect of zinc chloride ($ZnCl_2$) as an antioxidant against the cytotoxic and mutagenic effects induced by yclophosphamide (chemotherapeutic agent), using mutagenicity tests; molecular assay, chromosomal aberrations (CA), and Mitotic index (MI) *in vivo* as the biomarkers. The experiment was designed as four groups (6 rats per group). Group 1 was injected intraperitoneally (i.p.) with saline solution (1 ml/kg body weight) every other day for 20 days and served as (control group). Group 2 (the treated group) was injected i.p. with a single dose of CP (200 mg/kg b. w.). Group 3 was injected i.p. with a single dose of CP (200 mg/kg b. w.) and treated simultaneous by $ZnCl_2$ (4 mg/kg b. w.) every other day for 20 days, while group 4 (the protective group) was pretreated with $ZnCl_2$ (4 mg/kg b. w.) every other day for 20 days, then treated with a single dose of CP (200 mg/kg b. w.) on the 21st day and was left. The experiment extended for 45 days after the treatment with the cyclophosphamide dose. The results revealed changes in the number, position, and intensity of DNA fragments for liver and kidney tissues in the treated rats with cyclophosphamide, in addition to significant decline in mitotic index and increase in the frequency of chromosomal aberrations compared with the control group. These results may be attributed to the fact that cyclophosphamide can induce genotoxicity through DNA damage in healthy cells. In comparison, rats that were treated simultaneous and pretreated with zinc chloride and then treated with a single dose of cyclophosphamide showed marked improvement in DNA fragments, decrease in the frequency of chromosomal aberration, and an elevation in mitotic index. Furthermore, pretreatment with zinc chloride revealed more protective role in mitotic index and reduce markedly DNA damage and chromosomal aberrations that induced by cyclophosphamide than those showed with the treatment with zinc chloride simultaneity with Cp treatment. Pretreatment of Zinc chloride may open an interesting field concerning its possible use in medicine applications, as a protective treatment to reduce the side effects that can occur by cyclophosphamide treatment and other chemotherapeutic agents.

INTRODUCTION

Cyclophosphamide (CP) is an effective anti-cancer alkylating agent, on the other hand it possesses cytotoxic effects towards normal cells (Kim *et al*., 2013). CP can destroy the normal antioxidant system by active metabolites represented in phosphoramide mustard and acrolein resulting in the accumulation of reactive oxygen species, that can cause DNA damage. Several previous studies reported that cyclophosphamide has carcinogenic effects in healthy human and animal tissues (Rehman *et al*., 2012), it causes gene mutations, chromosomal aberrations in mice tissues *in vivo* and *in vitro* test systems, and an elevated frequency of secondary treatment-related tumors in human cancer survivors (Ember *et al*., 1995;Ridder *et al*., 1998; Sundramoorthy *et al.,* 2004; Kruawanand *et al*., 2006). Numerous studies using the Chromosome aberration (CA) and Mitotic index (MI) *in vivo* and *in vitro* as the biomarkers reported that treatment with cyclophosphamide can induce cytotoxic and mutagenic effects in bon marrow cells of rat and rabbit (Popov *et al.,* 2011; Sharma *et al*., 2012; Sharma V. and Agrawal 2015; Sushma *et al*., 2015; Koura *et al*., 2017). Therefore, it is necessary to develop antioxidant materials to reduce the side effects of CP and the other chemotherapeutics.

Zinc is an essential metal that is integral to many enzymes and transcription factors in the cell. Ii plays an important role in protecting DNA and other cellular components from oxidation and damage through its participating in vital processes such as transcription and replication of DNA through zinc finger proteins (Levine, 1997; Powell, 2000; Prasad, 2003; Ho, 2004; Emily, 2004; Ebisch *et al*., 2007; Leoni *et al*., 2014). DNA transcription is a main part of germ cell development, therefore zinc is affecting important biological processes such as cell division, growth, and differentiation; and it maintains DNA strand from damage (Badkoobeh *et al*., 2013; Grüngreiff 2016).

Other studies (Satoh *et al*., 1993; Sandstrom *et al*., 1994; Lindahl *et al*., 1998; Noh and Koh, 2000; Dhawan and Chadha, 2010; Maremanda, *et al*., 2014) suggest that zinc has a role as an anticancer and its treatment increases resistance against the tumor in mice, thus it has a potential effect on apoptosis. Estı´baliz *et al.,* (2003) reported that pretreatment with zinc showed modulate in DNA damage and gave results near the control levels. For this, in the present study, rats were exposed to $ZnCl_2$ pre and simultaneous treatment via intraperitoneal injection, followed by the injection with cyclophosphamide to evaluate the protective effects of $ZnCl_2$ on DNA fragments in liver and kidney tissues by using inter simple sequence repeat (ISSR) markers. To obtain more effective results, the author assessed the mitotic index and chromosome aberrations using bone marrow cells analysis of the tested rats.

MATERIALS AND METHODS

1- Chemical Materials:

Cyclophosphamide (CP):

Cyclophosphamide was supplied as vials from Baxter Oncology, Germany.

Zinc Chloride ($ZnCl_2$):

$ZnCl_2$ salt from El-Nasr Pharmaceutical Chemical Co.

Primers:

Five ISSR primers were used in the detection of polymorphism among the liver and kidney tissues of tested rat groups. These primers were synthesized by Metabion Corp Germany.

Experimental Animals:

Twenty four of male albino rats of approximate weight (20-25 g) were obtained from the animal house of Veterinary College, south valley

university. The rats were housed in the animal house of the science college, South Valley University, Qena, under normal conditions with a balanced diet and water *ad libitum* until the weight of rats reached of (200-250 g).

Experimental Design:

When the rats reached from 200 to 250 g, 24 rats were divided randomly into 4 groups of 6 rats each.

Group 1: This group is (control group), each rat of this group was injected intraperitoneally with saline solution (0.9 % NaCl, 1 ml/kg b. w.) every other day for 20 days.

Group II: This group is (treated group), each rat of which was injected intraperitoneally by a single dose of CP (200 mg/kg b. w.) and was left for 45 days.

Group III: This group was treated with a single dose of CP simultaneous with the treatment with $ZnCl_2$ (treated group with CP+$ZnCl_2$), this group was injected intraperitoneally with a single dose of CP (200 mg/kg b. w.) followed by $ZnCl_2$ (4 mg/kg b. w., i.p.) every other day for 20 days and was left.

Group IV: This group is (protective group) that received i. p. $ZnCl_2$ (4 mg/kg b. w.) every other day for 20 days before a single dose of cyclophosphamide (200 mg/kg b. w.) on the 21st day and was left for 45 days.

The experiment extended for 45 days after the treatment with the cyclophosphamide dose in the tested rat groups, under normal conditions with a balanced diet and water *ad libitum*.

Molecular Studies:

a- DNA Extraction:

Three rats of each group were dissected to obtain DNA from liver and kidney tissues according to (Zietkiewicz *et al.,* 1994). DNA concentration was determined by diluting the DNA 1:5 in dH2O. 10 µg of a DNA size marker (Lambda DNA digested with HindIII and Phi x 174 DNA digested with HaeIII). This marker can cover from 310 bp to 23130 bp of DNA fragments size, and a range of concentration from 11 ng to 95 ng. Thus, estimation of the DNA concentration in a given sample was achieved by comparing the degree of fluorescence of the unknown DNA band with the different bands in the DNA size marker.

b- ISSR Reaction:

PCR amplification reactions were carried out as described by (Sharaf-Eldeen *et al*., 2006). Reactions in PCR system were performed in 25 µl volume that composed of (1x reaction buffer, 0.2 mM of dNTPs, 1.5 mM $MgCl_2$, 0.2 µM of primer, 0.5 unit of Taq polymerase, and 50 ng of template DNA). The temperature profile in the different cycles was as follows: an initial strand separation cycle at 94°C for 5 min followed by 40 cycles comprised of a denaturation step at 94°C for 1 min, an annealing step at 45°C for 1 min, and an extension step at 72°C for 1.5 min. The final cycle was a polymerization cycle for 7 min at 72°C.

c- Electrophoresis Analysis:

PCR products were mixed with 5 µl gel loading dye and resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 mg/ml) in 1 x TBE buffer at 120 volts. A 100 bp DNA ladder was used as molecular size standard. PCR products were visualized under UV light and documented using a ™XR+ Gel Documentation System (Bio-Rad).

Cytogenetic Studies:

a- Chromosomal Preparations:

After the end of the determined period of the experiment, three rats from each group were injected intraperitoneally with 0.05% colchicine, for 2.5 h. before sacrifice. Bone marrow preparations for the analysis of metaphase cells were obtained as described by Yosida *et al.,* (1977) with

some modifications. 200 metaphase spreads per tested group were screened to determine the number and types of chromosomal aberrations per group.

b- Statistical Analysis:

Number of chromosomal aberrations was expressed as means ± SE. 200 metaphases were counted for three rats in each group. Statistical analysis of cytogenetic was performed on SPSS software (version 24). P values were less than 0.05 levels ($P < 0.05$) were considered to be significant.

c- Calculation of Mitotic Index:

Form mitotic division stages about 5000 cells counted for each animal. Mitotic index was calculated according to the formula that described by Singh and Sankhla (2010).

Mitotic index= A/A+B

Where A= No. of dividing bon marrow cells (metaphase)

B= No. of non dividing bon marrow cells

RESULTS

a-The Molecular Genetic Results:

Inter simple sequence repeats (ISSRs) analysis was performed to evaluate the protective effects of pre and simultaneous treatment with zinc chloride against Cyclophosphamide-induced genotoxicity in rat tissues. Farther, the comparison between the effect of pretreatment and the simultaneity treatment of zinc chloride on cyclophosphamide against its genotoxicity. Two tissues (liver and kidney) of male albino rats were used to obtain DNA. The results of each treated group were examined through inter simple sequence marker in comparison with the control group. Data were illustrated for five primers in (Figures 1 and 2). These primers that used consist of different di- and tri-nucleotide repeats to determine the genetic variation among the tested groups as well as possible (Table 1). The molecular size of amplified bands in liver and kidney were illustrated in (Table 2).

Table (1). The primers code and nucleotide sequences.

Primer	Primer Sequence 5'-3'
ISSR- 1	5'-ACACACACACACACACYA-3'
ISSR- 2	5'-AGAGAGAGAGAGAGAGYT-3'
ISSR- 3	5'-CTCCTCCTCCTCCTCTT-3'
ISSR- 4	5'-CTCTCTCTCTCTCTCTCG-3'
ISSR- 5	5'-TCTCTCTCTCTCTCTCA-3'

nucleotide code: Adenine=A, Cytosine=C, Guanine=G, Thymine=T and Y=Cor T

The results from liver tissue: the five primers (primer ISSR-1, ISSR-2, ISSR-3, ISSR-4, and ISSR-5) were generated 11, 15, 14, 17, and 8 bands, respectively, with an average 13 bands per primer. The five primers developed a total of 209 bands in which 76 of them were polymorphic bands (36.4% polymorphism) as shown in (Figure 1). All the five primers produced polymorphic bands by different percentage, 36.4 %, 62.5 %, 64.3 %, 52.9%, and 25.0% polymorphism (primer ISSR-1, ISSR-2, ISSR-3, ISSR-4, and ISSR-5), respectively (Table 3). The results showed also that all treated groups of rats had disappeared or appeared new bands compared with control group. Control group generated 57 ISSR bands with the five primers opposite to 43, 53, and 57 bands for groups 2, 3, and 4, respectively (Table 2). These results revealed that the number of lost bands in group 2 and 3 was obviously higher than the number of new bands compared with the control group. Animals treated with

cyclophosphamide (group 2) showed the highest lost bands (the total bands was 43 compared to 57 for control group). Thus, group 2 could be an obvious indication for the high genotoxic effect of cyclophosphamide due to losses of alleles compared with the groups that treated by cyclophosphamide plus zinc chloride, groups 3 (52 bands) and 4 (57 bands). Rats in group 3 and 4 with simultaneity and pretreatment with zinc Chloride every other day for 20 days respectively, and followed by treated with CP, showed improvement in DNA strand and increase in bands number (52 and 57 bands, respectively) compared with group 2 (43 bands) that was treated with CP only. These results suggested that the animals treated with cyclophosphamide and received zinc chloride had less genotoxic effect than those treated with cyclophosphamide only, and zinc chloride pretreatment (group 4) exhibited more protective that lead to better result than simultaneity treatment in group 3 compared to the control.

Table 2. Number of obtained bands using five ISSR primers analysis in treated and control groups.

Marker name	Total band Number	Mobility Range (bp)	Number of bands				Mean of band Frequency ±SE
			Group 1 (Control group)	Group 2 (CP treated)	Group 3 (CP+$ZnCl_2$ treated)	Group 4 (CP+ $znCl_2$ Pretreated)	
In liver							
ISSR-1	**11**	**170-670**	**10**	**10**	**9**	**11**	**0.91±0.05**
ISSR-2	**15**	**160-850**	**12**	**7**	**10**	**14**	**0.72±0.08**
ISSR-3	**14**	**190-1000**	**13**	**8**	**10**	**10**	**0.75±0.07**
ISSR-4	**17**	**260-1400**	**14**	**11**	**15**	**14**	**0.79±0.07**
ISSR-5	**8**	**290-1000**	**8**	**7**	**8**	**8**	**0.97±0.03**
Sum			**57**	**43**	**52**	**57**	
In kidney							
ISSR-1	**11**	**170-670**	**11**	**10**	**10**	**11**	**0.95±0.05**
ISSR-2	**12**	**160-850**	**12**	**9**	**11**	**12**	**0.92±0.05**
ISSR-3	**11**	**210-850**	**10**	**7**	**9**	**7**	**0.75±0.11**
ISSR-4	**12**	**260-850**	**12**	**11**	**11**	**12**	**0.96±0.04**
ISSR-5	**7**	**290-770**	**5**	**7**	**5**	**5**	**0.79±0.14**
Sum			**50**	**44**	**46**	**47**	

Table 3. Detected polymorphism for ISSR marker in the tested rats compared to the control group.

Marker Total	Total	Monomorphic Bans	Polymorphic Bands	Polymorphism (%)
In liver				
ISSR-1	**11**	**7**	**4**	**36.4 %**
ISSR-2	**15**	**5**	**10**	**33.3 %**
ISSR-3	**14**	**5**	**9**	**64.3 %**
ISSR-4	**17**	**8**	**9**	**52.9 %**
ISSR-5	**8**	**6**	**2**	**25.0 %**
In kidney				
ISSR-1	**11**	**9**	**2**	**18.2 %**
ISSR-2	**12**	**8**	**4**	**33.3 %**
ISSR-3	**11**	**6**	**5**	**45.5 %**
ISSR-4	**12**	**10**	**2**	**20.0 %**
ISSR-5	**7**	**4**	**3**	**42.9 %**

The results from kidney tissue: these results indicate that kidney tissue showed less genetoxic effect compared to liver tissue with the treatment by cyclophosphamide. With the kidney tissues, the five primers generated 187 bands in which 38 of them were polymorphic bands (20.3% polymorphism) as shown in (Figure 2). All primers produced polymorphic bands with less percentage compared to liver tissue, 18.2 %, 33.3 %, 45.5 %, 20.0%, and 42.9 % (primer ISSR-1, ISSR-2, ISSR-3, ISSR-4, and ISSR-5), respectively (Table 3). Treated groups had disappeared or appeared new bands also compared with control group (Fig. 2). In control group, 50 ISSR bands were obtained opposite to 44, 46, and 47 bands for groups 2, 3, and 4, respectively (Table 2). Results revealed also, the lost bands in treated groups were more than the new generated bands compared with control group. Rats treated with cyclophosphamide (group 2) showed the highest lost bands (the total bands was 44 compared to 50 for control group), which could be an obvious indication for the genotoxic effect of cyclophosphamide in renal tissue due to losses of alleles. Rats in groups 3 and 4 that were treated with doses of zinc chloride every other day for 20 days simultaneitly and pretreatment, respectively, and followed by a single dose of cyclophosphamide, showed increase in the number of bands (46 and 47) bands, respectively, compared with Cp treated group (44 bands). These results suggested that the rats treated with zinc chloride and received cyclophosphamide showed less genotoxic effect in renal tissue than those treated with cyclophosphamide only.

Moreover, the present results showed that liver tissue is more sensitive to toxic actions for CP than kidney tissue. In group 2 (The treated group by cyclophosphamid) liver tissue generated 43 bands, 22 of them were polymorphic bands with percentage 51.2%, while kidney tissue generated 44 bands, 11 of them polymorphic bands with 25% percentage. These results of polymorphism were improved in liver tissue under the effect of zinc chloride to 11.5% and 10.5% in simultaneous and pretreatment treatment with CP (group 3 and 4), respectively. In kidney tissue, the results were improved to 10.9% and 6.4% in group 3 and 4, respectively (Table 4).

Overall, the present study demonstrated that both the pre and the simultaneous treatment with ZnCl2 have a protective role against CP-induced genotoxicity in liver and kidney tissues. This protection was more effective in pretreated rat group with $ZnCl_2$ for 20 days before treated with CP compared to this treated simultaneously with $ZnCl_2$ and CP as shown in Figure 4 that demonstrated the relationships among tested rat groups and control group in liver and kidney tissues. Consequently, pretreatment with $ZnCl_2$ may be applied as a protective agent for people in need of treatment by cyclophosphamide.

Table 4. Detected polymorphism for ISSR primer in the three tested groups compared with control group.

Primer	CP (group 2)			CP+$ZnCl_2$ (group 3)			CP+pre$ZnCl_2$ (Group 4)		
	Total Number	Poly	Polymor %	Total Number	Poly.	Polymor %	Total Number	Poly.	Polymor. %
In liver									
ISSR-1	**10**	**2**	**20**	**9**	**1**	**11.1**	**11**	**1**	**9.1**
ISSR-2	**7**	**5**	**71.4**	**10**	**2**	**20**	**14**	**3**	**21.4**
ISSR-3	**8**	**7**	**87.5**	**10**	**2**	**20**	**10**	**2**	**20**
ISSR-4	**11**	**7**	**63.6**	**15**	**1**	**6.67**	**14**	**0**	**0**
ISSR-5	**7**	**1**	**14.3**	**8**	**0**	**0**	**8**	**0**	**0**
Sum	**43**	**22**	**51.2**	**52**	**6**	**11.5**	**57**	**6**	**10.5**
In kidney									
ISSR-1	**10**	**1**	**10**	**10**	**1**	**10**	**11**	**0**	**0**
ISSR-2	**9**	**3**	**33.3**	**11**	**1**	**9.1**	**12**	**0**	**0**
ISSR-3	**7**	**4**	**57.1**	**9**	**2**	**33.3**	**7**	**3**	**42.9**
ISSR-4	**11**	**1**	**9.1**	**11**	**1**	**9.1**	**12**	**0**	**0**
ISSR-5	**7**	**2**	**28.6**	**5**	**0**	**0**	**5**	**0**	**0**
Sum	**44**	**11**	**25**	**46**	**6**	**10.9**	**47**	**3**	**6.4**

Poly= Polyploidy Polymor= polymorphism

b- Cytogenetic Results:

The antimutagenic potential of zinc chloride against cyclophosphamide genotoxicity was evaluated using *in vivo* assays of rat bone marrow cells. Doses of 4 mg/kg of zinc chloride every other day for 20 days was tested in pre and simultaneously treatment with a single dose of cyclophosphamide compared with the control group and treatment group with CP only. It was observed that in rats administered with cyclophosphamide there was a significant increase in the number of chromosomal aberrations and a decline in mitotic index as compared to the control group. In rats that simultaneous or pretreated with zinc chloride, and followed by administered cyclophosphamide, the number of chromosomal aberrations were less and the mitotic index was nearly equivalent to the control group (Table 5&6 and Figure 3). Table 5 illustrated the results of types and numbers of chromosomal aberrations in bone marrow cells of male albino rats that were injected with CP (200mg/kg b. w.). CP induced several chromosomal aberrations in the tested rats, such as ring chromosomes, chromatide deletion, dicentric chromosomes, chromosomal fragment, end to end, and polyploidy that showed that the mean frequency of total chromosomal aberrations was significantly increased after CP treatment for 45 days (23±3.86) when compared with control (0.67 ±0.33). The treated rats with CP simultaneously plus $ZnCl_2$ treatment or pretreatment with $ZnCl_2$ exhibited significant reduction in chromosomal aberrations induced by CP (10.5 ±2.23 and 5.67±1.41), respectively. Pretreatment of zinc chloride before treatment with cyclophosphamide showed more effective result in chromosomal aberrations than those that concurrently treatments with $ZnCl_2$ and CP (5.67±1.41 opposite 10.5 ±2.23), respectively, and pretreatment of zinc chloride before treatment with cyclophosphamide showed mitotic index nearly equivalent to the control group (0.019 opposite 0.020), respectively, (Table 6).

Table 5. Chromosomal aberrations in rat bone marrow cells after treatment with CP, CP+ $ZnCl_2$ and $ZnCl_2$ pretreatment +CP.

Group	Chromosomal aberrations						Total number of aberrations	Average number of aberrations
	R	D	EE	Dc	F	Poly.		
Control (Group 1)	1	2	0	0	0	1	4	0.67 ±0.33
CP group(Group2	35	30	10	25	24	14	138	23±3.86*
CP+ $ZnCL_2$ sim. Group 3	18	14	4	13	9	5	63	10.5 ±2.23*
$ZnCL_2$ pre.+ CP Group 4	9	8	0	7	7	3	34	5.67 ±1.41*

Sim= simultaneously treatment, pre.= pretreatment
R=chromosomal ring, D= chromatide deletions, EE= end to end, Dc= dicentric chromosome, F= chromosomal fragments and Poly.= polyploidy.
*Significant compared to control at P< 0.05

Table 6: Protective effect of zinc chloride against cyclophosphamide induced decline in mitotic index.

Dose	No. of dividing cells (A)	No. of non dividing cells (B)	Total cells	Mitotic index (MI)
Control (Group 1)	100	4900	5000	0.020
CP group (Group2	59	4940	5000	0.012
CP+ $ZnCL_2$ sim. Group 3	83	4917	5000	0.017
$ZnCL_2$ pre.+ CP Group 4	94	4906	5000	0.019

DISCUSSION

Cyclophosphamide is a chemotherapuetic agent that is widely used as anticancer. Such as most chemotherapeutic agents, it does not distinguish between normal and cancerous cells, so it kills normal cells as well. Acrolein and phosphoramide are active compounds of CP, in spite of these compounds, reduce the growth of cancerous cells by acting at DNA level (Haubitz, 2007). It induce a variety of changes in DNA, that can cause secondary tumors in human and animal healthy tissues (Krishna *et al*., 1987; Ember *et al*., 1995; Ridder *et al*., 1998; Codrington *et al*., 2004). Murata *et al*., (2004) reported that CP and its metabolites induce oxidative stress and react with electron rich areas such as nucleic acids and proteins. Therefore, CP causes disruption of cell growth, mitotic activity, and functions via alkylation of DNA at the N7 position of guanine. Chromosomal aberrations are due to DNA damage caused by phosphoramide mustard which leads to break down of the DNA strand (Sharma *et al*., 2012). The present study demonstrated that the treatment of rats with a single dose of cyclophosphamide caused DNA damage in liver and kidney tissues, as well as decline in mitotic index and increase in the frequency of the chromosomal aberrations in bon marrow cells, these results of the present study are in agreement with all pervious studies.

Numerous studies such as (Murata *et al*., 2004; Abdella, 2008; Sowjanya *et al*., 2009; Popov *et al*., 2011; Sharma *et al*., 2012; Ch and Devi, 2015; Sharma and Agrawa, 2015; Kour *et al*., 2017) found that Bone marrow analysis technique of rat revealed clear signs of

genotoxicity. Several changes in DNA damage, chromosomal aberrations, Sister Chromatide Exchanges, and decrease in mitotic index after intraperitonealy injection of cyclophosphamide. These reports are in agreement with present study that revealed DNA damage and decrease in the DNA bands, decline in the mitotic index, and the occurrence of chromosomal aberrations such as fragmentation, chromosomal ring, chromatid deletions, and dicentric chromosomes in the treated group with CP only. Abdella (2012) reported that despite that most chemotherapy include CP and are necessity for killing cancer cells, they have undesirable toxicity to normal tissues, where CP treatment has the ability to produce chromosome aberrations and decline in mitotic index in animals, current search results may be involved in solving this problem.

Over all, Cyclophosphamide belong to a class of drugs known as alkylating agent, which have been used to treat some kind of cancer, several studies confirmed that it is a potent anticancer drug but it also affects normal cell division by decreasing the immune responses. The present results indicated that albino rats treated with single dose of CP and left for 45 days showed several times increase in frequency of chromosome aberrations and decrease in the mitotic index. This results are in agreement with the previous investigations which confirmed that CP had the side effects as oxidation and genotoxic effects in normal tissues of treated animals. Therefore, the developing of anti-cancer drug or manufacturing new drugs with low or no side effects has become an important aim in modern studies to overcome chemotherapeutic side effects.

Zinc is a component of chromatin structure, integral in DNA replication and transcription. Thus, zinc is involved in protecting cell and its components against oxidative material and preserving DNA, regulation of transcription, and replication of DNA through zinc finger proteins (Pavletich *et al*., 1993; Falchuk, 1998; Dong *et al*., 1999; Ho, 2003; Emily, *et al*., 2004; Witkiewicz-Kucharczyk and Bal, 2006; Song *et al.,* 2009; Dhawan and Chadha, 2010). Many studies reported that zinc salts have the ability defend against oxidative harm to the different tissues (Sunderman, 1995; Bagchi *et al*., 1998; Zago and Oteiza, 2001; Badkoobeh *et al*., 2013).

Other studies reported that Zn supplementation in rats reduced and ameliorated various types of CP-induced DNA damage (Ishida, 2017; Torabi *et al.,* 2017), and inhibit apoptosis induced by chemotherapeutic agents (Maremanda *et al*., 2014; Ghobadi *et al*., 2017). In the present study, doses of (4 mg /kg b. w.) of zinc chloride simultaneous and pretreatment associated with cyclophosphamide exposure, clearly reduced DNA damage, chromosomal aberrations, and elevated of mitotic index. These results indicate zinc chloride has provided protection against cyclophosphamide induced cytotoxicity using various cell lines *in vivo* assay that are in agreement with the previous findings. (Garufi *et al.,* 2015) reported $ZnCl_2$ could be a valuable element in chemotherapeutic against cancer and reduce the side effects of these drugs. This reports also are agree with the obtained results of the present study that showed DNA fragments, mitotic index, and chromosomal aberrations together indicate the best protective effect of zinc chloride especially in pretreatment against CP induced genotoxicity in rat tissues.

CONCLUSION:

The present data indicated that the pretreatment by zinc chloride or simultaneous treatment have good antimutangenic activity against cyclophosphamide induced genotoxicity, zinc being antioxidant or acting on DNA

replication, resulting in increase in DNA fragments, significant inhibition in the formation of chromosomal aberrations, and elevation in mitotic index that induced by the treatment with cyclophosphamide. Furthermore, pre-treatment with zinc chloride showed more protective effects in all tested rat tissues (liver, kidney, and bon marrow) as well as elevation in mitotic index than simultaneous treatment compared to the treatment with cyclophosphamide alone. The present results have demonstrated the capability of zinc chloride, as an essential element in the healthy body, and its ability to maintain the normality of DNA fragment and the bone marrow cells in cyclophosphamide-treated rat tissues. Over all, it appears that $ZnCl_2$ is a potential antioxidant as an additive to chemotherapeutic drugs that are toxic to human organs. Present findings indicate favorable prospects for clinical applications for zinc chloride in combination with chemotherapeutic agents like CP.

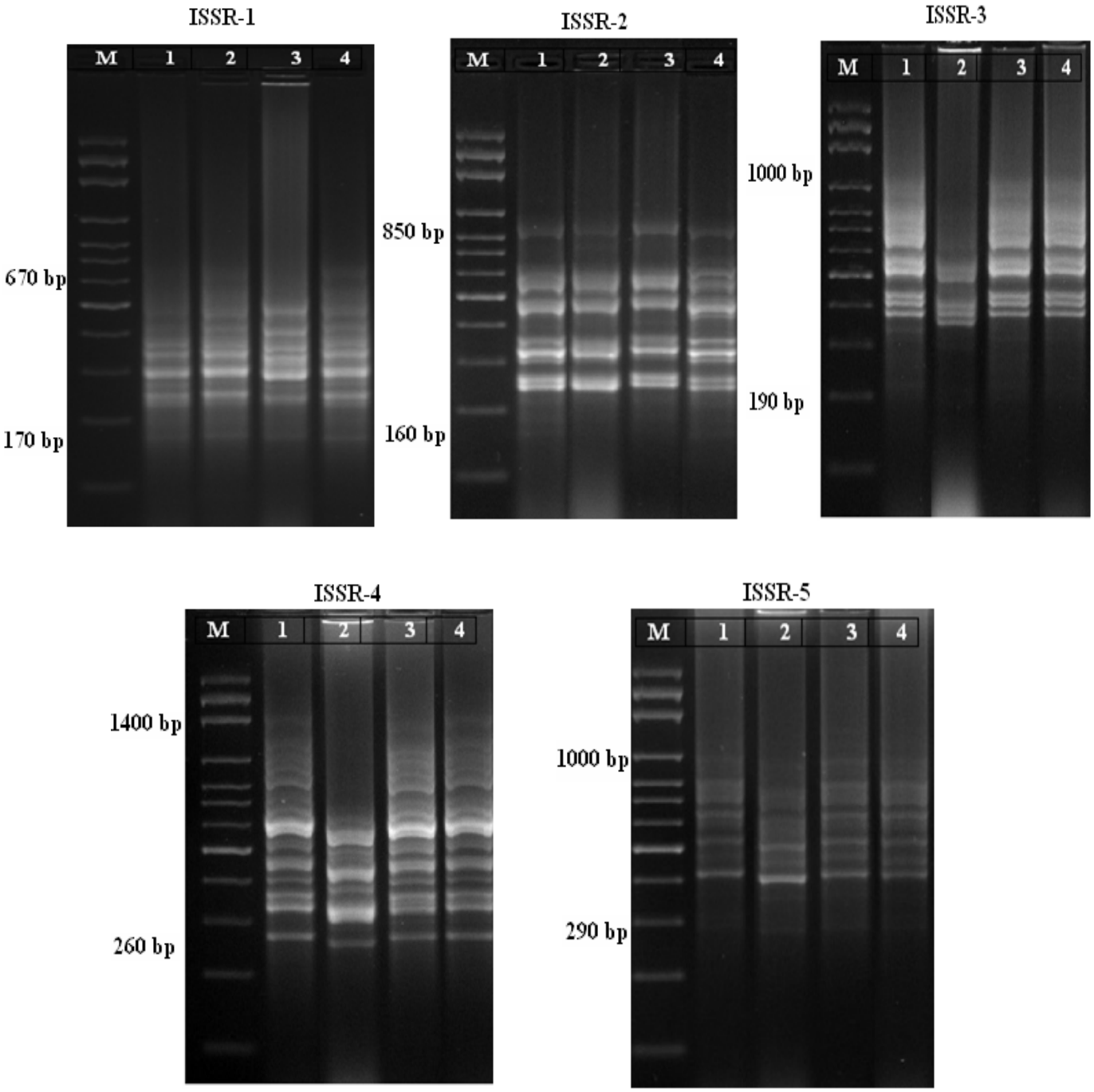

Fig. 1. PCR products of genomic DNA from liver of tested rat with five ISSR primers. Lane (1) control liver, lane (2) rat liver injected intraperitoneally with a single dose of CP (200 mg/kg b. w.), lane (3) injected intraperitoneally with a single dose of CP (200 mg/kg b. w.) and simutlaneosly injected intraperitoneally with $ZnCl_2$ (4 mg/kg b.w.), lane (4) rat liver pretreated with $ZnCl_2$ (4 mg/kg b.w.) for 20 days, then injected intraperitoneally with a single dose of CP (200 mg/kg b. w.) and M is DNA Marker.

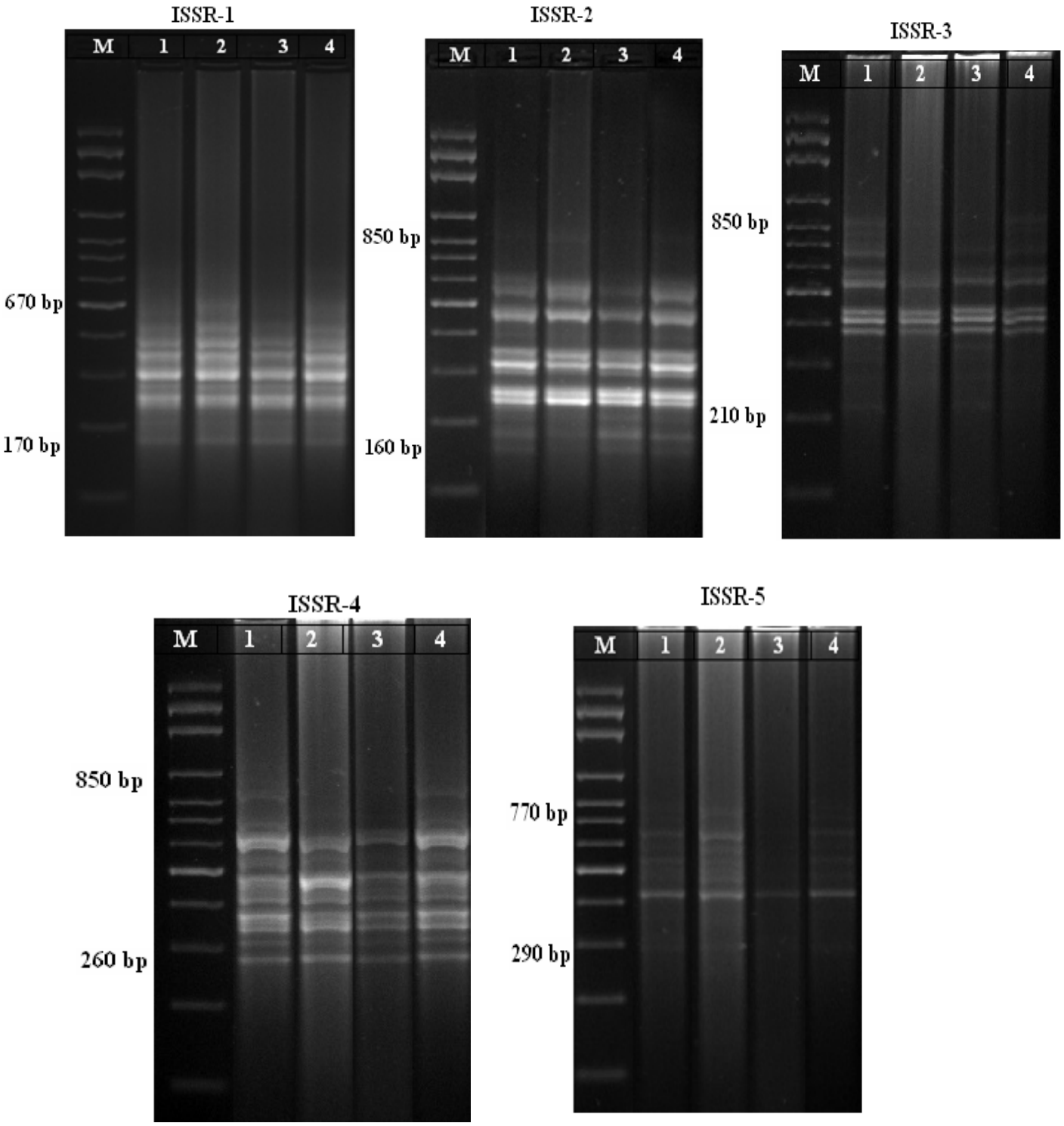

Fig. 2. PCR products of genomic DNA from kidney of tested rat with five ISSR primers. Lane (1) control kidney, lane (2) rat kidney injected intraperitoneally with a single dose of CP (200 mg/kg b. w.), lane (3) injected intraperitoneally with a single dose of CP (200 mg/kg b. w.) and simutlaneosly injected intraperitoneally with $ZnCl_2$ (4 mg／kg b.w.), lane (4) rat kidney pretreated with $ZnCl_2$ (4 mg／kg b.w.) for 20 days, then injected intraperitoneally with a single dose of CP (200 mg/kg b. w.) and M is DNA Marker.

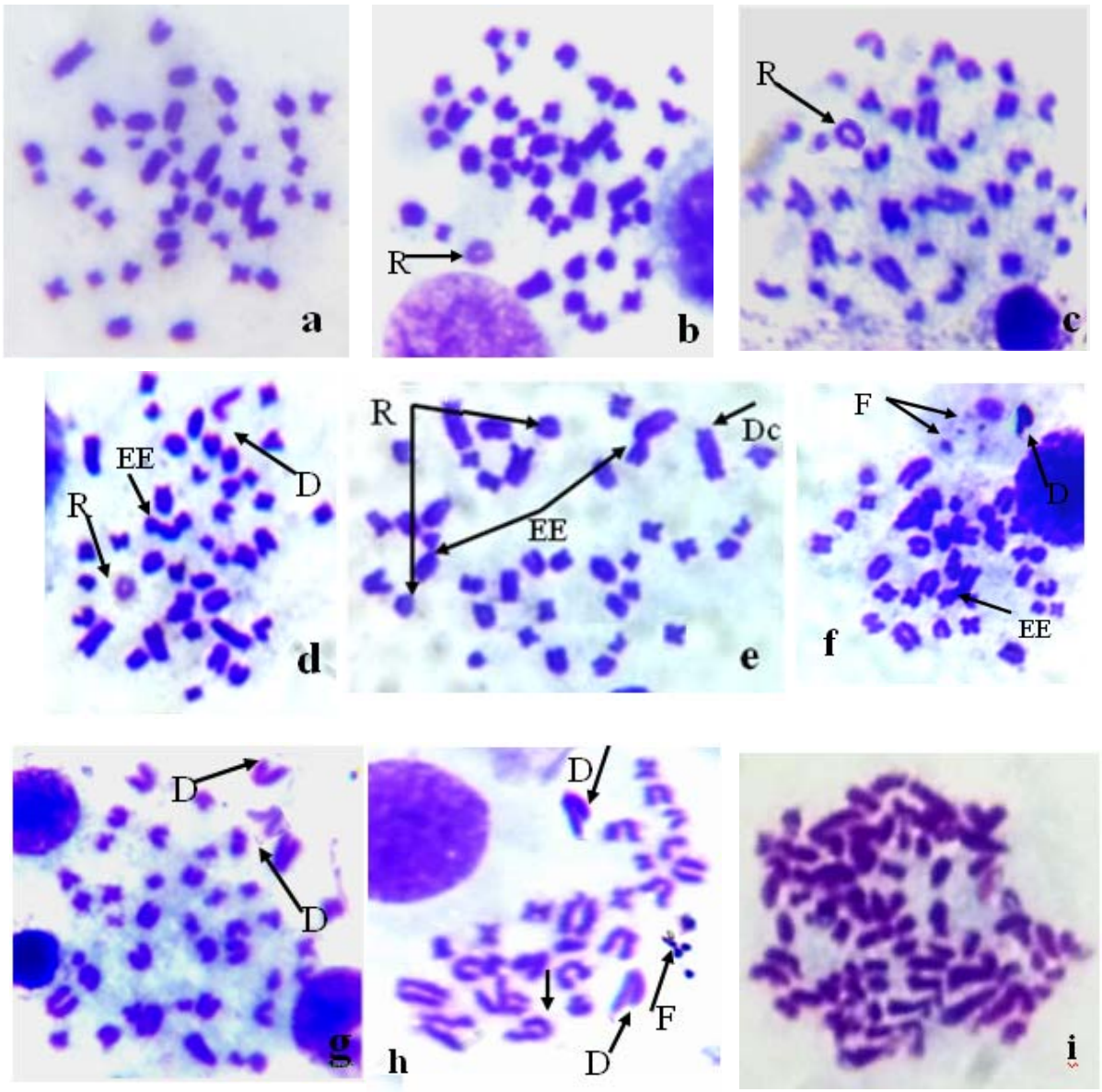

Fig. 3. Metaphase figures of chromosomal aberration of bone marrow cells induced by cyclophosphamid treatment showing: (a) normal metaphase and (i) polyploidy chromosomes; the letters (R), (D), (EE), (Dc) and (F) refer to chromosomal ring, chromatide deletion, end to end, dicentric chromosome and chromosomes fragment respectively.

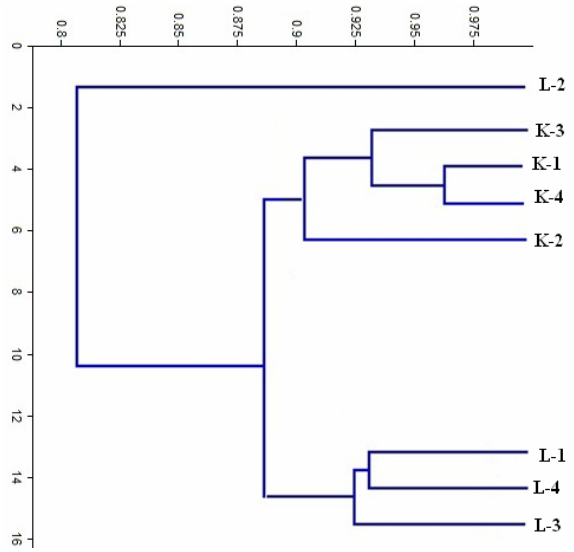

Fig. 4. Dendogram demonstrating the relationships among tested rat groups and control group in liver and kidney tissues. (L and K) refer to liver and kidney and 1, 2, 3 and 4 refer to group 1, group 2, group 3 and group 4 respectively.

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ARABIC SUMMARY

التأثيرات الوقائية لكلوريد الزنك على السمية الجينية الناتجة من السيكلوفوسفاميد فى انسجة الجرذان البيضاء فى الجسم الحى

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السيكلوفوسفاميد (Cyclophosphamide) هو أحد العقاقير الشائعة الاستعمال فى علاج العديد من أنواع الأورام السرطانية واختلالات المناعة الذاتية. وهو يمثل جزء من أدوية العلاج الكيميائي للسرطان، ويصنف ضمن الأدوية ذات التأثير المباشر على البنية الجزيئية للحمض النووي (DNA) للخلايا السرطانية. فهو ينتمى لمستحضرات فئة عوامل الآلكلة (Alkylation agents) التى تضيف مجموعة آلكيل (Alkyl) إلى الدنا مما يمنع عملية مضاعفة الحمض النووي و تكاثر الخلايا السرطانية، حيث يقوم بتكسير هيكلة الحمض النووي في كل مراحل دورة حياة الخلية السرطانية، بما يوقف نموها و يتسبب فى موتها.

ومن المعروف أن الزنك هو احد المعادن الأساسية التى تساهم فى تكوين عدد كبير من الإنزيمات وعوامل النسخ ويدخل فى تركيب جميع انسجة الجسم. وقد اثبتت الأبحاث المعملية أن الزنك له تأثير مضاد للإجهاد التأكسدى ويلعب دور هام فى نسخ وتكرار وإصلاح الدنا (DNA) حيث أن بروتينات أصابع الزنك (zinc finger proteins) تنظم عمليات النسخ والتكرار وإصلاح الضرر فى الدنا (DNA). لذا فهو له دور هام فى حماية الخلايا ومحتوياتها.

وقد كان الهدف من هذه الدراسة. إلقاء الضوء على التأثير العلاجي والوقائى لكلوريد الزنك ضد الآثار الجانبية المترتبة على استعمال عقار السيكلوفوسفاميد فى بعض العلاجات الدوائية لبعض الأمراض.
وتناولت الدراسة التقنيات التالية:
1- تقنية (Inter Simple Sequence Repeat ISSR) باستخلاص الدنا من نسيج الكبد والكلى لذكور الجرذان البيضاء باعتبارها من الاعضاء التى تتأثر من جراء المعالجة بعقار السيكلوفوسفاميد
2- اعداد التحضيرات الكروموسومية للانقسام الميتوزى لخلايا نخاع العظام لتحديد انحرافات الكروموسوومات فى الاتجاهين التركيبى والعددى (Chromosomal aberrations) وتقييم مؤشر الانقسام للخلية (mitotic index).

ولقد تم تصميم تلك الدراسة باستخدام عدد 24 من ذكور الجرذان البيضاء، تم تقسيمهم إلى أربعة مجموعات بكل مجموعة 6 جرذان.
المجموعة الأولى :تعتبر المجموعة الضابطة وقد تم حقنها فى الغشاء البريتونى بمحلول ملحى فسيولوجى بجرعة (١ مللتر/كجم) من وزن الجسم كل يوم بعد يوم لمدة عشرون يوما.
المجموعة الثانية : تعتبر المجموعة المعالجة بعقار السيكلوفوسفاميد وقد تم حقنها خلال الغشاءالبريتونى بجرعة واحدة من السيكلوفوسفاميد (٢٠٠ مجم/كجم من وزن الجسم) ثم تركت لمدة خمسة واربعون يوما.
المجموعة الثالثة :تعتبر المجموعة المعالجة بالسيكلوفوسفاميد وكلوريد الزنك، وقد تم حقنها بجرعة واحدة من السيكلوفوسفاميد (٢٠٠ مجم/كجم من وزن الجسم) وفى نفس الوقت بدأ حقنها بمادة كلوريد الزنك خلال الغشاءالبريتونى بجرعة (٤مجم/كجم من وزن الجسم) كل يوم بعد يوم لمدة عشرون يوما.
المجموعة الرابعة :وتعتبر المجموعة الوقائية حيث تم حقنها بمادة كلوريد الزنك (٤مجم/كجم من وزن الجسم) كل يوم بعد يوم لمدة عشرون يوما قبل حقنها بعقار السيكلوفوسفاميد. وقد تم حقنها بالسيكلوفوسفاميد فى اليوم الحادى والعشرون بجرعة واحدة (٢٠٠ مجم/كجم من وزن الجسم) ثم تركت لمدة 45 يوما.

بعد 45 يوما من الحقن بالسيكلوفوسفاميد للمجموعات الثلاث، تم اجراء الفحوصات والتحاليل
وقد اظهرت الدراسة بتقنية (ISSR) ان المجموعة المعالجة بعقار السسيكلوفوسفاميد (مجموعة 2) حدث بها تحلل للدنا (DNA) فى خلايا الكبد والكلى وكذلك وجدت اختلافات معنوية فى عدد الكروموسووومات الشاذة ونوعيتها فى التحضيرات الكروموسومية لنخاع العظام وانخفاض واضح فى مؤشر الانقسام مقارنة بالمجموعة الضابطة. وبمقارنة نتائج المجموعة المعالجة بعقار السيكلوفوسفاميد بالمجموعتين المعالجتين بكلوريد الزنك المتزامن مع العلاج بعقار السيكلوفوسفاميد (مجموعة 3) أو المعطى قبل العلاج بالسيكلوفوسفاميد (مجموعة 4) وجد تحسنا واضحا فى الدنا ظهر فى زيادة عدد جزم الدنا الناتجة وكذلك انخفاض فى انحرافات الكروموسومات الشاذة وارتفاع فى مؤشر الانقسام.

وقد خلصت الباحثة إلى أن كلوريد الزنك المتزامن فى العلاج مع السيكلوفوسفاميد والمعطى مسبقا قبل العلاج بالسيكلوفوسفاميد له نشاط مضاد للطفرات الجينية فى انسجة الجرذان.
وأن العلاج بكلوريد الزنك قبل البدء فى العلاج بالسيكلوفوسفاميد له تأثير أكثر فاعلية على السمية الجينية للسيكلوفوسفاميد مقارنة بالعلاج بكلوريد الزنك المتزامن مع العلاج بالسيكلوفوسفاميد.

ونستخلص من هذه الدراسة أن كلوريد الزنك ينبغى أن يستخدم فى توليفة علاجية مع العلاج بالسيكلوفوسفاميد كعلاج وقائى قبل العلاج بالسيكلوفوسفاميد للمرضى الذين تتطلب حالاتهم ضرورة العلاج بالسيكلوفوسفاميد.