Genetical and biochemical changes of the anticancer drugs and the protective effect of curcumin in tumor bearing mice

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ABSTRACT

This study was aimed to investigate the genotoxicity and hepatotoxicity of the anticancer drugs "cis-diamminedichloro platinum (CDDP) (8 mg/kg b.w.) and Doxorubicin (DOX) (10 mg/kg b.w)" alone and the protective action of Curcuma longa (Turmeric) (250 mg/kg b.w.) in combination with CDDP and DOX in Ehrlich ascites tumor bearing male mice. The results illustrated that the effect of CDDP and DOX alone decreased total protein, albumin and globulin concentrations in serum. Also, there are no significant changes in serum protein banding patterns between treatments on SDS-PAGE. On the other hand, the lysosomal enzymaic activities of β- N-acetyl glucosaminidase (β-NAG) and acid phosphatase (ACP) as well as glutathione-S-transferase (GST) in liver homogentae were increased. Total protein content in liver homogenate was reduced as compared to the control. The percentage of structural aberrations of chromosomes of bone marrow cells after 24 hours of drugs administration were increased by 261.0% and 149.1% for CDDP and DOX, respectively . On the other hand, the turmeric extract were increased in mixture with each drug resulted in increasing the total protein, albumin and globulin concentrations in serum. Also, the enzymes activity of ACP, β-NAG and GST were decreased. Total protein contents was increased in liver homogenate as compared to the control. In addition to, the percentage of structural and numerical aberrations of chromosomes of bone marrow cells after 24 h of curcumin administration were decreased, CDDP and DOX showed damage and smear bands of genomic DNA as compared with negative control group, while the mixture of turmeric showed less damage of genomic DNA bands.

Key words: *Cisplatin, Doxorubicin, Curcumin, Ehrlich ascites tumor, Chromosomal aberrations.*

INTRODUCTION

Carcinoma is a type of cancer that represents 80-90% of cases and originates in epithelial tissue, which includes the skin, the covering, lining of the organs and internal passageways (Murphy *et al.*, 1997). Ehrlich Ascites Tumor (EAT) is a type of tumors originates from the carcinomas tumors. Cis-diaminedichloroplatinum (II) (CDDP) is chemotherapeutic agent. It is widely used in the treatment of different types of malignant tumors, such as testicular cancer, lung cancer and breast carcinoma (De Pas *et al.*, 2001). Cisplatin has a genotoxic effect in germ cells, causing the development of various

forms of abnormalities in the sperm heads in mice (Khynriam and Prasad, 2003). Edelweiss *et al.* (1995) investigated the clastogenicity of CDDP on Wistar rat bone marrow cells, the most impressive effect of a single dose of CDDP was an increase in the frequency of chromosomal aberrations and in the number of abnormal metaphases after 24 hr of interapertoneal administration (Choudhury *et al.*, 2000). Cisplatin causes oxidative stress mainly by inceasing lipid peroxidation and depletion of glutathione, which induces apoptosis of renal proximal tubule cells and consequent kidney dysfunction (Zhang and Lindup, 1993 and Chang *et al.*, 2002).

Doxorubcin is anti-tumor agent, it is cytotoxic anthracycline antibiotic isolated from cultures of *Streptomyces peucetius* var. caesius. It is supplied in the hydrochloride form as a ready to use solution (Kapusta et al., 2000). Structurally, Doxorubicin is related to Daunomycin (daunorubicin) and differs only in hydroxyl group substitution (instead of hydrogen) at the alkyl side chain at position nine of A ring (Kapusta *et al.*, 2000). Dox-induced changes in the expression of apoptosis-regulating gene products Bcl-xL and p53, reflect considerable perturbation in genome integrity. The anti-apoptotic role of Bcl-xL relates to its sequestration of the pro-apoptotic family members and prevention of the oligomerization required for the initiation of apoptosis (Ray *et al.*, 2006).

Curcumin induces suppression of cell proliferation by G1/S arrest, which is based on downregulation of the expression of cyclin D1 and phosphorylation on CDK4-mediated retinoblastoma protein (Mukhopadhyay *et al.*, 2002). Gupta *et al.* (2004) studied the effect of antioxidants in preventing as well as providing protection against infection and degenerative diseases. Curcumin is able to prevent the toxic effect of free radicals which interact with a number of cellular molecules. Also, it has a biological function prevention through lipid peroxidation, DNA damage, and enzymes failure (Prasad, 1995).

This study aims to investigate the effect of CDDP and DOX alone and in combination with *Curcuma longa* extract on the total protein, enzyme activities, DNA and chromosomal changes of bone marrow cells in Ehrlich ascites tumor bearing female mice.

MATERIALS AND METHODS

Chemicals

Cis-diaminedichloro platinum (II) (CDDP) was imported by Star International Company, manufactured by CIPLALTD Verna Industrial Estate Goa 403722, India. Doxorubicin (DOX) manufactured by Pfizer Australia Ptyltd, 1-chloro 2,4 dinitrobenzene (CDNB) (Fluka, India), ρ-nitrophenyl-phosphate-sodium salt (Sigma, USA) and bovine serum albumin (Sigma, USA), 4-nitrophenol-N-acetyl-β-D- glucosaminidase (Sigma), were used.

Mice and tumor maintenance

Inbred male albino mice (*Mus musculus*), weighting 25±2g, 10-12 weeks old were used. Animals were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR), Giza, Egypt; they were kept under environmental and nutritional conditions for 2 weeks before used.

The tumor was maintained in female Swiss albino mice by weekly interapertoneal (i.p) transplantation of 2.5 X 10^6 cells in the National Cancer Institute (NCI), Cairo, Egypt. Tumor cells were taken from transplanted animals after 4 days of transplantation and resuspended by appropriate volume of saline, then 2 X10^6 cells (approximately 0.2 ml) of this suspension were injected (i.p) in each male mouse.

Curcumin

Curcumin *(Curcuma longa)* was extracted with 70% of ethanol and lyophilized by Freeze dryer (Snijders).

Experimental design

This study was carried out on 60 male albino mice, each one was injected (i.p) with 2.5 X 10^6 (0.2 ml) of EACCs, after 4 days of tumor transplantation; animals were divided into 6 groups, each group contained 10 animals:

Group (1): This group received NaCl (0.9% i.p) (Negative control).

Group (2): This group received Curcumin (250 mg/kg orally for 9 days consequently) .

Group (3): This group received Cisplatin (8mg/kg (i.p) on the ninth day of curcumin administration).

Group (4): This group received a mixture of Curcumin+ CDDP of the same concentrations.

Group (5): This group received Doxorubicin (10mg/kg on the ninth day of curcumin administration).

Group (6): This group received Turmeric + Doxorubicin

The mice were scarified after 24 hours of CDDP and Doxorubcin administrations. Blood was collected after 24 hrs of anticancer drugs administrations. Also, cervical dislocation was carried out after 24 hrs of CDDP and DOX administration.

Preparation of tissue liver homogenate

Animals were killed by decapitation and bleeding. Liver was rapidly removed and weighed and homogenized in saline (0.9%) using an electric homogenizer to prepare 10% w/v (0.5 g/5 ml) homogenate. The homogenate was centrifuged at 5000 rpm for 15 min at 4°C and the supernatant was used for determining the different parameters.

Determination of Albumin

Albumin was determined in serum according to Thomas (1998). In the presence of bromocresol green at a slightly acid pH, serum albumin produces a color hange of the indicator from yellow-green to green-blue.

Determination of Glutathione S-Transferase (GST) activity

The enzyme activity of GST was determined according to Habig *et al.* (1974). It is based on the fact that GST enzyme catalyzes the conjugation of glutathione with 1-chloro 2, 4 dinitrobenzene (CDNB) and forms a complex which has an absorbance at 340 nm. The mean decrease of absorbance per min was calculated.

Determination of total protein

Total protein in liver homogenate was measured according to Lowry *et al.* (1951).

Protein separation by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method

It was used for determining the protein fractionation in liver homogenate and serum Sodium dodecyl sulphate polyacrylamide gel electrophoresis was done according to Laemmli (1970) on 12.5% concentration of gel.

Extraction and purification of genomic DNA

Genomic DNA was isolated from the mouse liver and kidney tissue according to Surzycki (2000). Genomic DNA was fractioned on agarose gel electrophoresis (1.5%) and ethidium bromide staining.

Cytogenetic analysis

The cytogenetic analysis of chromosomes of bone marrow cells was carried out according to Nichols *et al.* (1972). Mice were injected 0.1ml of colchicine (0.5%) after 1-1.5 hr; they were killed. The chromosomes were

prepared and stained by Giemsa stain using phosphate buffer (pH 6.8).

Statistical analysis

Data were subjected to statistical analysis as mean±S.E. according to Fisher and Yates (1957) and Snedecor and Cochran (1967) and practicing statistical analysis of Student's *t*-test. Differences were regarded as insignificant at $P>0.05$, significant at $P>0.025$, highly significant at $P>0.0025$ and very highly significant at $P<0.0005$.

RESULTS

The following results illustrated the genotoxicity and hepatotoxicity induced by of cisplatin (8 mg/kg), doxorubicin (10 mg/kg) alone and in combination with *Curcuma longa* (Turmeric) croud extract (250 mg/kg) in Ehrlich ascites tumor (EAT) bearing male mice.

Effect of CDDP, DOX alone and in combination with turmeric on total protein, albumin and glubulin in serum.

Table (1) revealed that the total protein of serum in mice treated with Turmeric alone was increased by 15.86%. CDDP and DOX had reduced total protein concentration by 14.89% and12.62%, respectively after 24 hrs of administration, but total protein increased by 6.47% and 12.78 %, respectively in combination with Turmeric.

On the other hand, albumin and glubulin were increased by Turmeric alone with a percentage of 23.51% and 10.00%, respectively, but the treatment of the two anticancer drugs alone decreased them by 9.33 % and 19.14% for CDDP, 6.72% and 17.14% for DOX, respectively. The combination with Turmeric, albumin and globulin were increased by 24.63% and 7.43% with CDDP and 44.03% and 11.14% with DOX, respectively.

Effect of CDDP and DOX alone and in combination with Turmeric on serum protein banding patterns

Fig. (1) showed that protein profiles have changed drastically, the serum protein patterns exerted variability in molecular size between 6.5 to 200 KDa, the most intensive bands observed were between 116-200 KDa, while the protein bands were decreased ranging from 21 to 45 KDa. Significantly, there are several changes in serum protein banding patterns in the low molecular weight proteins ranged from 29 to 6.5 KDa 24 hrs of administration, where the CDDP and DOX alone cause significant changes in protein content of these organs. Animals of the combination group, however, experienced significantly alleviated decrease in protein values compared to cisplatin group.

Effect of CDDP and DOX alone and in combination with Turmeric on the total protein content and GST activity in liver homogenate

Table (2) revealed that the total proteins extracted from liver of mice treated with CDDP and DOX was reduced with a percentage of 10.21 % and 0.9 %, respectively after 24 hrs of administration. The mixtures of Turmeric with CDDP and DOX appeared to be of less enhancements by 5% and 3.43%, respectively. The protein banding pattern on 12.5 % gel of SDS-PAGE, after 6 days of administration was shown in (Fig. 2).

The Turmeric extract decreases the GST activity by 4.71%, while under the effect of drugs "CDDP, DOX" the GST activity was increased by 8.84 and 16.72%, respectively after 24 hrs of administration. The mixture of Turmeric with CDDP and DOX decreased GST activity by 2.10% and 5.24%, respectively.

Table (1): Effect of CDDP or DOX alone and in combination with Turmeric on total protein, albumin and glubulin concentrations in serum and their percentage (n=9) .

Treatment	Total protein (g/dl)	Albumin (g/dl)	Glubulin (g/dl)
Negative control (-V)	6.18±0.04	2.68±0.09	3.50±0.08
Turmeric (250mg/kg)	7.16±0.06***	3.31±0.10***	3.85±0.13*
% change [a]	15.86%	23.51%	10.00%
CDDP (8 mg/kg)	5.26±0.02***	2.43±0.20†	2.83±0.22***
% change [b]	14.89%	9.33%	19.14%
CDDP+ Turmeric	6.58±0.02***	3.34±0.08***	3.24±0.15*
% change [c]	6.47%	24.6%	7.43%
b & c % change	20.10%	27.25%	12.65%
DOX (10 mg/kg)	5.40±0.12***	2.50±0.19†	2.90±0.22*
% change [d]	12.62%	6.7%	17.14%
DOX+ Turmeric	6.97±0.03***	3.86±0.11***	3.11±0.09**
% change [e]	12.78%	44.03%	11.14%
d & e % change	22.53%	35.23%	6.75%

The % change of a, b, c, d and e % was calculated from negative control
† Insignificant at P>0.05 , * significant at P>0.025
** highly significant at P>0.002 , *** very highly significant at P<0.0005.

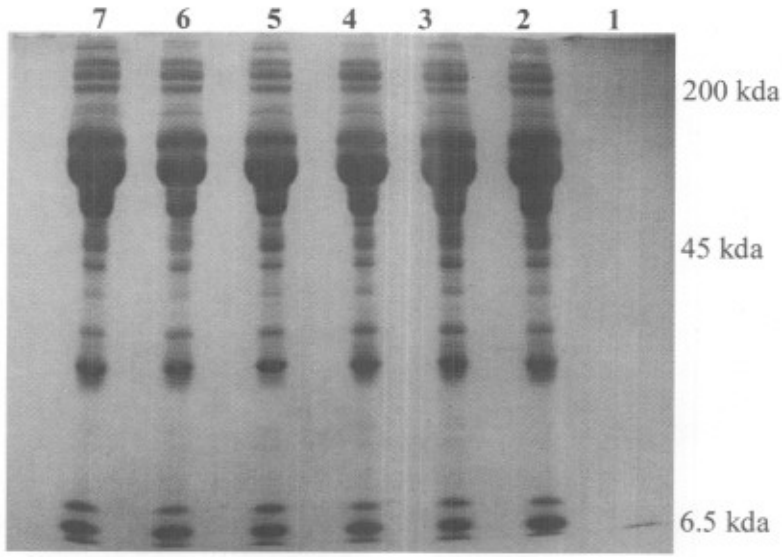

Fig. (1):Serum protein profile by SDS-PAGE (12.5%). (1) Marker (6.5-200KDa), (2) Negative control, (3) Turmeric (250mg/kg), (4) CDDP (8mg/kg), (5) CDDP+Turmeric (6) DOX (10 mg/kg) and (7) DOX+Turmeric in Ehrlich ascites tumor bearing male mice.

Table (2): Effect of CDDP or DOX alone and in combination withTurmeric on the total protein content and GST activity in liver homogenate (n=9).

Treatment	Total protein (mg/ml)	GST activity (M/min)
Negative control (-V)	109.37±1.21 -	103.49±0.66 -
Turmeric (250mg/kg) % change [a]	119.90±2.36** 9.63%	98.62±9.62† 4.71%
CDDP (8 mg/kg) % change [b]	98.20±0.21*** 10.21%	112.64±0.14** 8.84%
CDDP+ Turmeric % change [c]	114.83±0.39*** 5.00%	101.32±2.51† 2.10%
b & c % change	14.48%	11.17%
DOX (10 mg/kg) % change [d]	108.44±0.48† 0.90%	120.79±2.99*** 16.72%
DOX+ Turmeric % change [e]	113.12±0.76** 3.43%	108.91±8.37† 5.24%

The % change of a, b, c, d and e % were calculated from negative control † Insignificant at P>0.05 , * significant at P>0.025 ** highly significant at P>0.002 , *** very highly significant at P<0.0005.

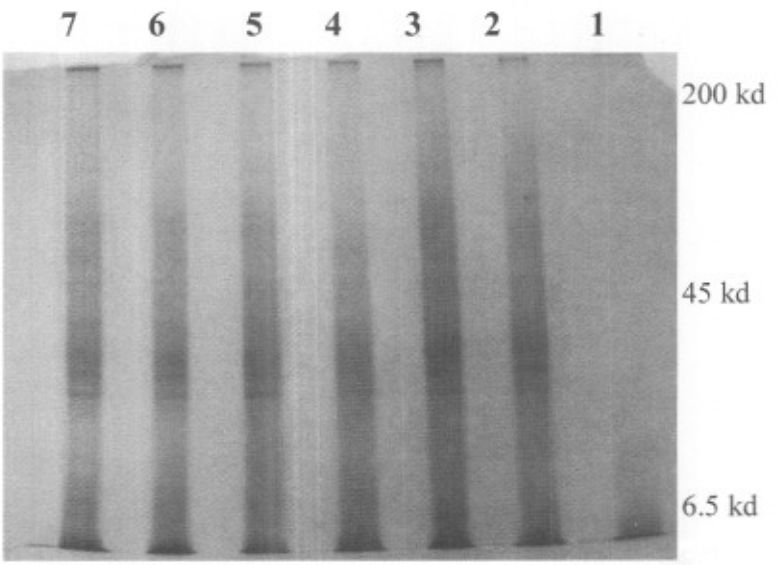

Fig. (2): Liver protein profiles by SDS-PAGE (12.5%). (1) Marker (6.5-200KDa), (2) Negative control, (3) Turmeric (250mg/kg), (4) CDDP (8 mg/kg), (5) CDDP+Turmeric (6) DOX (10 mg/kg) and (7) DOX+Turmeric in Ehrlich ascites tumor bearing male mice.

Effect of CDDP and DOX alone and in combination with Turmeric on protein banding patterns isolated from liver homogenate

Figure (2) showed the same protein bands, ranging from 6.5-200 KDa, further very few higher molecular weight protein bands than 200 KDa. Therefore, there is no significant difference between treatments in protein banding pattern in SDS-PAGE.

Effect of CDDP and DOX alone and in combination with Turmeric on genomic DNA isolated from liver tissue

The isolation and purification of genomic DNA in Figure (3) illustrated the different lane profiles on 1.5 % Agarose gel. The genomic DNA was isolated from liver tissue after 6 days of administration. However, mice treated with CDDP and DOX and showed clearly damage and smear bands as compared with the genomic DNA of negative control group. This may be due to the cytotoxicity of these drugs on genomic DNA. While the mixture of CDDP + Turmeric as well as DOX and turmeric showed less smear bands of genomic DNA.

Effect of CDDP or DOX alone and in combination with Turmeric on the chromosomes of bone marrow cells

Cancer occurs after normal cells have been transformed into neoplastic cells through alteration of their genetic material and the abnormal expression of certain genes. Neoplastic cells usually exhibit chromosomal abnormalities and the loss of their differentiated properties. These changes lead to uncontrolled cell division and may result in the invasion of previously unaffected organs, a process called metastasis.

As indicated from data in Table (3), cell metaphase in turmeric (Fig. 4-b) as compared to negative control (Fig. 4- a). CDDP exerted a significant increase for all the structural forms of the chromosomes by 341% (deletion), 150.0 % centromeric attenuation, 162.5 % centromeric fusion, 250.0 % ring chromosome, 230.0 % end to end association, 425.0 % fragments and 191.7 % gap. The total chromosomal structure aberrations were 261.0 % as shown in Fig. (5-a).

The mixture of CDDP with turmeric exerted a less inhibitory effect on these structural forms of chromosomes by 150.0 % deletion, 80.0% centromeric attenuation, 37.5 % centromeric fusion, 200.0% ring chromosome, 170.0% end to end association, 255.0 % fragment and 91.7 % Gap. The total chromosomal structure aberrations observed were 131.0 % as shown in Fig. (5-b).

On the other hand, DOX increased all forms of chromosomal structure aberration by the percentage of 104.55 % deletion, 215.0 % centromeric attenuation, 112.5 % centromeric fusion, 300.0 % ring chromosome, 210.0 % end to end association, 120.0 % fragment and 208.33 % gap. The total chromosomal structure aberrations observed were 149.1 % as shown in Fig. (6-a).

The mixture of DOX with turmeric revealed a significant reduction in all of these items of chromosomal aberrations by 72.73 % deletion, 105 % centromeric attenuation, 75.0 % centromeric fusion, 140.0 % ring chromosome, 160.0 % end to end association, 180.0 % fragment and 150.0 % gap. The total chromosomal structure aberrations observed were 92.73% as shown in Fig.(6-b).

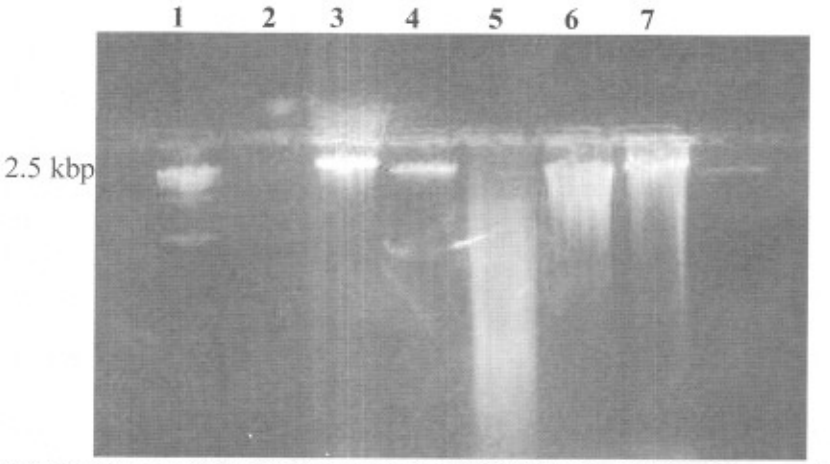

Fig.(3): DNA fragmentation from liver tissue. (1) Marker(2.5Kbp), (2) Negative control, (3) Turmeric (250mg/kg), (4) CDDP (8mg/kg), (5) CDDP+Turmeric, (6) DOX (10 mg/kg) and (7) DOX+Turmeric in Ehrlich ascites tumor bearing male mice.

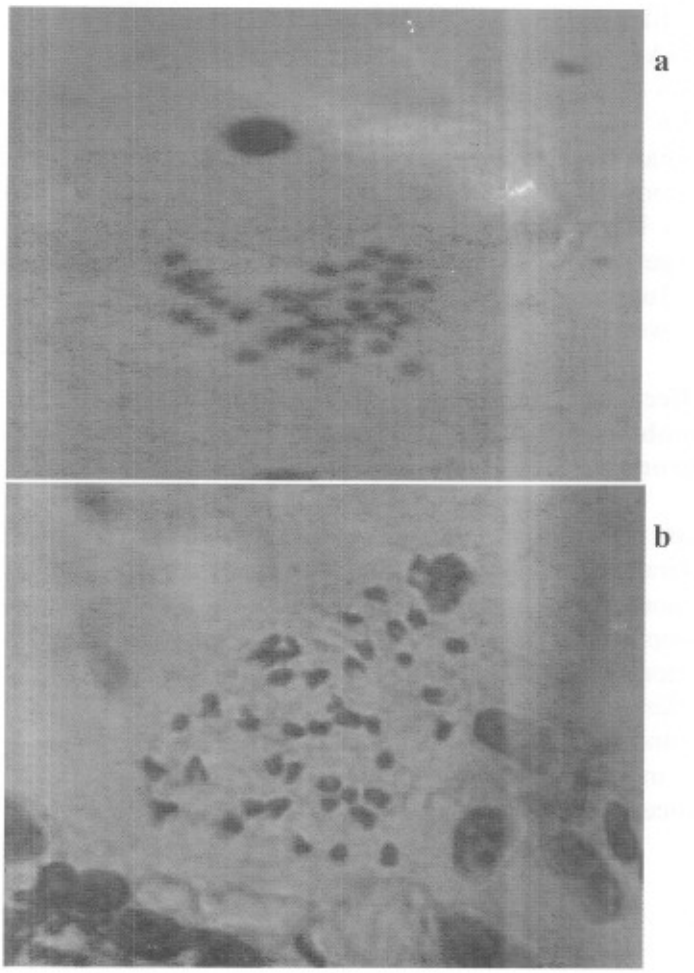

Fig.(4):Cell metaphase of a) Neg. control, b) Turmeric (250 mg) in mouse bone marrow cells.

Table (3) : Effect of CDDP or DOX alone and in combination with turmeric on the chromosomal structures of bone marrow cells (n=9).

Treatment	Structural chromosome aberrations							Total aberrations
	Deletion	Centromeric attenuation	Centromeric fusion	Ring chrom.	End to end association	Fragment	Gap	
Negative control (-V)	2.2±0.21 -	2.0±0.20 -	1.6±0.13 -	1.0±0.15 -	1.0±0.14 -	2.0±0.22 -	1.2±0.22 -	11.0±0.95 -
Turmeric (250mg/kg) % change [a]	2.4±0.22† 9.1%	1.6±0.16† 20.0%	1.4±0.20† 12.5%	1.0±0.20† 0.0%	0.9±0.21† 10.0%	1.3±0.13† 35.0%	0.8±0.01† 33.3%	9.4±0.8† 14.6%
CDDP (8 mg/kg) % change [b]	9.7±0.64*** 341.0%	5.0±0.44*** 150.0%	4.2±0.30*** 162.5%	3.5±0.13*** 250.0%	3.3±0.21*** 230.0%	10.5±0.41*** 425.0%	3.5±0.31*** 191.7%	39.7±0.35*** 261.0%
CDDP+ Turmeric % change [c]	5.5±0.40*** 150.0%	3.6±0.31*** 80.0%	2.2 ±0.12** 37.5%	2.0±0.13*** 200.0%	2.7±0.21*** 170.0%	7.1±0.30*** 255.0%	2.3±0.17*** 91.7%	25.4±2.30*** 131.0%
b & c % change	150.0%	38.9%	91.0%	75.0%	18.18%	47.9%	52.17%	56.3%
DOX (10 mg/kg) % change [d]	4.5±0.36*** 104.55%	6.3±0.61*** 215.0%	3.4±0.37*** 112.5%	3.0±0.23*** 300.0%	3.1±0.26*** 210.0%	3.4±0.37*** 120.0%	3.7±0.45*** 208.33%	27.4±3.70*** 149.1%
DOX+ Turmeric % change [e]	3.8±0.30*** 72.73%	4.1±0.42*** 195.0%	2.8±0.21** 75.0%	2.4±0.21*** 140.0%	2.6±0.21*** 160.0%	2.8±0.40*** 180.0%	3.0±0.21*** 150.o%	21.2±2.10*** 92.73%
d & e % change	18.42%	51.23%	21.43%	25.0%	19.23%	21.43%	23.33%	29.24%

The % change of a, b, c, d and e % were calculated from negative control

Insignificant at P>0.05, significant at P>0.025, ** highly significant at P>0.0025, and *** very highly significant at P<0.0005.

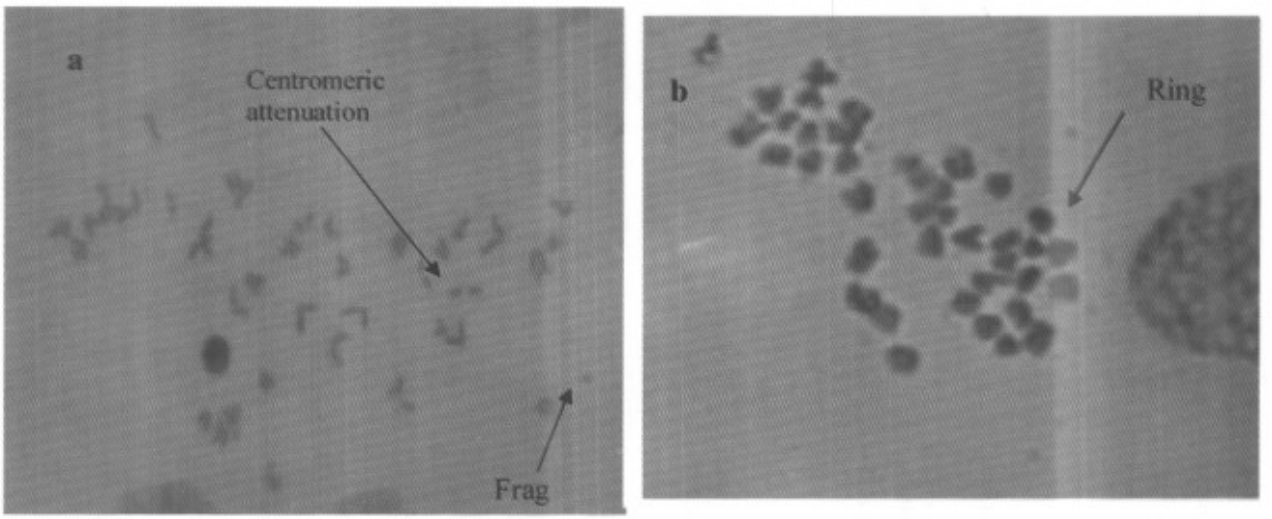

Fig.(5): Cell metaphase of a) CDDP (8mg), b) CDDP+ turmeric in mouse bone marrow cells.

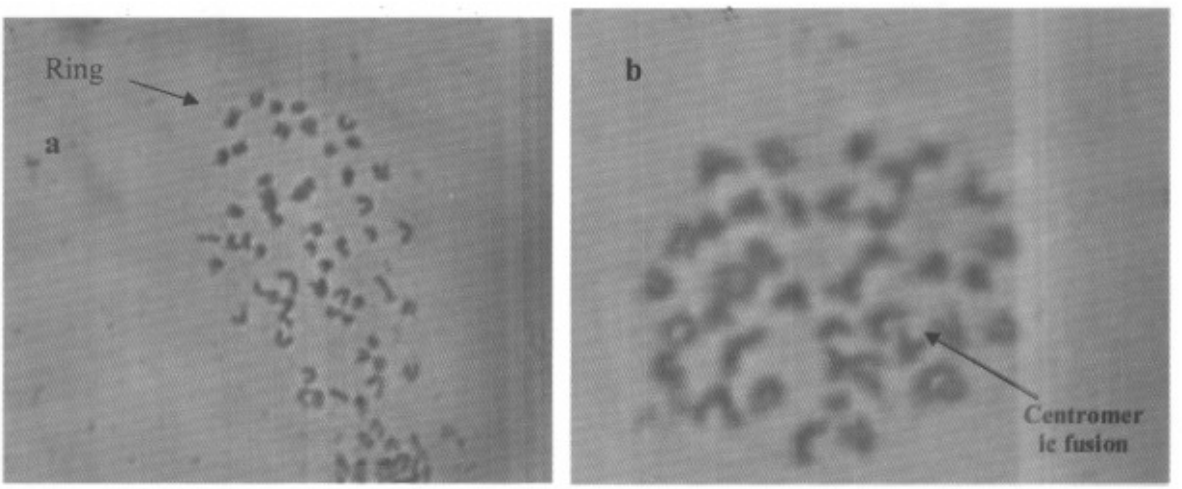

Fig.(6): Cell metaphase of a) DOX (10 mg) and b) DOX+turmeric in mouse bone marrow cells.

DISCUSSION

Shabon (2008) investigated the oral curcuma administration (100 mg/Kg) for 14 successive days pre-irradiation (with doses 0.1, 0.75 and 2 Gy) for each group and showed a decrease in serum total proteins in irradiated groups with 2 Gy which sacrificed after 1 and 48 hours in comparison with control group. There was also a significant increase in serum total proteins in groups injected with curcuma pre-irradiation in comparison with its identical irradiated group. There was a significant decrease in serum albumin of irradiated groups with 0.75 and 2 Gy as compared to control group. There was also a significant increase in serum albumin in all groups that injected with curcuma pre-irradiation in comparison with its identical irradiated group. The mechanism of cisplatin nephro-toxicity remains uncertain.

Cisplatin could cause decreased protein synthesis, membrane peroxidation, mitochondrial dysfunction, and/or DNA injury and thereby cause tubular injury. Cytochrome P450, a group of heme proteins, may serve as a significant source of catalytic iron in cisplatin-induced nephroto-xicity (Hanigen and Devarajan, 2003). Cisp-latin nephrotoxicity has also been demo-nstrated to be mediated by DNase I a highly reactive renal endonuclease (Basnakian *et al*., 2005 and Ali and Al-Moundhri, 2006).

Cisplatin causes the generation of reactive oxygen species (ROS), such as super oxide anion and hydroxyl radical and conesquently, depletes glutathione (GSH) and inhibits the activity of antioxidant enzymes in renal tissues (Badary *et al*., 2005 and Ajith *et al*., 2007). Glutathione is one of the essential compounds for regulation of a variety of cell functions. It has a direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized glutathione (GS-SG) and other disulfides. Glutathione S-transferase (GST) and glutathione peroxidase (GSH-Px) are GSH-dependent antioxidant enzymes (Karthikeyan *et al*., 2007).

The combination of flavonoids with drugs has clearly reduced the effect of drug toxicity. Flavonoids seem to act by activation of the turnover of the glutathione and enzymes stimulating particularly GST permitting the capitation of the reactive metabolites of the studied drugs. In agreement with Khynriam and Prasad (2003), the decreased cellular GSH concentration after the treatment with cisplatin alone, may hint the possibility of a less conjugation of cisplatin with GSH and availability of more drug to bind with DNA and causing genotoxic effects.

Ippoushi *et al*. (2003) have investigated the antioxidant compounds and found inhibitory activities against DNA damage induced by reactive oxygen species (ROS); this is due to the phenolic compounds. Oxygen free radicals are known to cause peroxidation of membrane polyunsaturated fatty acid chains, modification of DNA including base alteration, single strand breaks, sister chrom-atid exchange and DNA protein cross linked and loss of sulphhydryl's in proteins.

Moreover, cisplatin-induced transcription high jacking is another reason for the inhibition of protein synthesis associated with cisplatin. Transcription high jacking refers to the consequences of the ability of certain transcription factors to bind to DNA adducts caused by organoplatinum compounds. This leads to the sequestration of these transcription factors from their usual promoter binding sites (Tarloff and Lash, 2004).

Curcumin has profound effects on modulation of TNF-induced signaling, as well as inhibition of expression of TNF. Curcumin treatment inhibited lipopolysaccharide (LPS) or phorbol methyl acetate (PMA)-induced TNF-a levels in dendritic cells, macrophages, monocytes alveolar macrophages, and endothelial and bone marrow cells. Recent reports suggest that curcumin inhibited the expression of TNF mRNA in the livers of copper uploaded rats and that CCl4-induced hepatic fibrosis (Fu *et al*., 2008).

The mechanisms of anthracycline cytotoxicity, particularly of Dox, in cancer cells include: (i) intercalation into DNA with inhibition of DNA replication and RNA transcription, (ii) generation of free radicals with DNA damage and lipid peroxidation, (iii) DNA binding and arylation, (iv) DNA crosslinking, (v) interference with DNA unwinding, DNA strand separation, and helicase activity, (vi) direct membrane damage due to oxidation of lipids, and (vii) inhibition of topoisomerase II (Patel *et al*., 2010).

The chromosomal aberration pattern revealed that chromatid breaks and gaps

occurred more frequently. The total number of aberrant metaphases as well as chromosomal aberration was noticed to be highest at 24 hour of treatment which decreased appreciably during later periods (Khynriam and Prasad, 2003). It was investigated that the total of chromosomal aberrations and the number of abnormal metaphases decreased significantly in the animals that received pretreatment with olive, extra virgin olive and canola oils plus cisplatin, when compared to cisplatin alone. Olive and extra virgin olive oils were the most effective in the inhibition of chromosomal aberrations induced by cisplatin. These vegetable oils have many antioxidant compounds that may exert a protective effect by acting as a direct antimutagen inhibiting oxidative damage induced by reactive oxygen species generated by cisplatin (Owen *et al.*, 2003).

The present study recommends that, not only anticancer drugs, but also all drugs have dangerous side effects in general if are taken by high doses and for long period, for reducing these effects, antioxidants should be taken. Antioxidants agents and medicinal plants have protective action against free radicals and toxicity induced by different drugs. On the other hand, antioxidants and medicinal plants should not be taken by high doses for long periods.

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الملخص العربي

التغيرات الوراثية والكيميائية للأدوية المضادة للسرطان والفعل الوقائي للكركم في الفئران الحاملة للورم

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تهدف هذه الدراسة إلى معرفة التغيرات الكيميائية والوراثية للأدوية المضادة للسرطان كالسسبلاتين(8 مجم/كجم وزن الجسم) والدوكسروبسن (10 مجم/كجم وزن الجسم) والفعل الوقائى لمستخلص فلص الكركم الإيثانولى (250 مجم/كجم وزن الجسم) على الأنشطة المضادة للأكسدة، DNA وكروموسومات خلايا نخاع العظام في الفئران الإرليش الصغيرة البيضاء الحاملة للورم. حيث تم استخدام مستخلص الكركم لمدة 9 أيام متتالية بعد 4 أيام من إستحداث الورم بالفئران، وتم إعطاء السسبلاتين والدوكسروبيسن كجرعة واحدة فى اليوم التاسع من المعاملة بالكركم. وبعد 24 ساعة من المعاملة بهذه العقاقير وجد أن كلا منهما قد ادى إلى إنخفاض تركيز كل من البروتين، الألبيومين و الجلوبيولين فى السيرم. كذلك أوضح الفصل الكهربي لبروتينات السيرم وجود بعض الإختلافات في بعض حزم البروتينات لهذه المعاملات مقارنة بالمجموعة الضابطة بالإضافة إلى زيادة نشاط بعض إنزيمات الليسوسوم مثل الأسيد فوسفاتيز والبيتاناج كذلك زيادة نشاط إنزيم جلوتاثيون ترانسفيرازوكذلك إنخفاض تركيز البروتين في مستخلص الكبد المتجانس. كذلك أوضح الفصل الكهربي لبروتينات الكبد وجود بعض الإختلافات في بعض حزم البروتينات لهذه المعاملات مقارنة بالمجموعة الضابطة. كما أدت هذه العقاقير إلى زيادة الانحرافات الكروموسومية لخلايا نخاع العظام مقارنة بالمجموعة الضابطة. كما وجد أن تأثير هذه العقاقير يعتمد على الجرعة المعطاة للحيوان. أما عند المعاملة بمستخلص الكركم مع هذه العقاقير فقد ادى إلى زيادة تركيز كل من البروتين، الألبيومين و الجلوبيولين فى السيرم. كذلك أوضح الفصل الكهربي لبروتينات السيرم انه ما زال لا يوجد تغييرفي حزم البروتينات لهذه المعاملات مقارنة بالمجموعة الضابطة بالإضافة إلى إنخفاض نشاط بعض إنزيمات الليسوسوم مثل الأسيد فوسفاتيز والبيتاناج كذلك إنخفاض نشاط إنزيم جلوتاثيون ترانسفيرازو إرتفاع تركيز البروتين في مستخلص الكبد المتجانس. كذلك أوضح الفصل الكهربي لبروتينات الكبد وجود بعض الإختلافات في بعض حزم البروتينات لهذه المعاملات مقارنة بالمجموعة الضابطة.ايضا حدوث تكسير لحزم DNA عن طريق الادوية لكن مع استخدام مستخلص الكركم ادى الى انخفاض هذا التكسير، كما أدت هذه العقاقير إلى إنخفاض الانحرافات الكروموسومية لخلايا نخاع العظام مقارنة بالمجموعة الضابطة. كما وجد أن تأثير هذه العقاقير يعتمد على الجرعة المعطاة للحيوان.