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Chlorophyllin Protects Against Cisplatin and 5-Fluorouracil Regimen Induced Genotoxicity and Cytotoxicity Without Affecting Their Antitumor Activity

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Abstract: Two combination therapies based on cisplatin and 5-fluorouracil might improve treatment efficacy for several types of malignancies, but at risk of increasing toxicity. The possible protective effects of chlorophyllin (CHL) against the genotoxicity and cytotoxicity of the widely used cisplatin (CIS) and 5-fluorouracil (5-FU) regimen were evaluated. Mice were injected i.p. with CIS (6 mg/kg b.w.) and 5-FU (10 mg/kg b.w.) while CHL (2, 4 and 6 mg/kg b.w.) was administered as single i.p. injection 1 hour before the treatment with the two anticancer drugs. The results showed that CHL administered at dose levels of 2, 4 and 6 mg/kg b.w. 1h before the treatment with CIS and 5-FU did not induce any significant change in the antitumor activity of CIS and 5-FU combination. Moreover, CHL pretreatment significantly decreased the levels of chromosomal aberrations and micronuclei induced by CIS and 5-FU treatment in mice bone marrow cells. In addition, CHL pretreatment markedly restored the mitotic activity of bone marrow cells that had been suppressed by the two anticancer drugs in a dose-dependent manner. It could be concluded that CHL acts as a potent protective agent against the genotoxicity and cytotoxicity of CIS and 5-FU combination without affecting their antitumor activity. This protective effect of CHL may be attributed to free radicals scavenging ability of CHL. Therefore, more and extensive studies on the possibilities of using CHL supplement in cancer patients treated with cisplatin + 5-FU regimen are recommended.

Key words: Anticancer drugs · Antitumor activity · Chlorophyllin · Cisplatin and 5-Fluorouracil regimen · Genotoxicity

INTRODUCTION

Combination chemotherapy involves treating a patient with a number of different drugs simultaneously, differ in their mechanism and side effects to minimize the chances of resistance developing to any one agent, also to achieve greater dose intensity, greater cell kill and improve response rates [1, 2]. The combination of cisplatin (CIS) and 5-fluorouracil (5-FU) has been shown to elicit synergistic antitumor activity against several experimental tumors *in vivo*. Furthermore, this combination showed marked therapeutic effects on human malignancies that are relatively refractory to chemotherapy, such as head and neck cancer, colorectal cancer and esophageal carcinoma [3, 4]. Also, excellent results have been reported after treatment with CIS +5-FU chemotherapy in hepatocellular carcinoma patients [5,6]. Recently, Jung *et al*. [7] reported cases of recurrent metastatic sebaceous carcinoma patients who respond to 5-FU and CIS combination chemotherapy. Thus, the two –drug combination therapy based on CIS + FU might improve treatment efficacy for many types of cancers, but at risk of increasing toxicity. Moreover, their *in vivo* genotoxicity and cytotoxicity have been reported in several studies using chromosomal aberrations, sister chromatid exchanges and micronucleus assays in both bone marrow cells and germ cells [8-13].

It is common to find patients taking food supplements to diminish the intensity of the side effects of chemotherapy. One class of food products that is commonly used by patients contains high level of chlorophyll. Additional concentrated chlorophyll may also be used in conjunction with a regular diet as a "health food" supplement [14]. Chlorophyllin (CHL) is a synthetic water soluble sodium-copper salt of chlorophyll. It is widely used as food coloring agent and, health food

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additive. Moreover, CHL has been successfully used as a cancer chemo-preventive agent in human population residing in certain parts of China who are at high risk of eating aflatoxin-contaminated food [15]. CHL has been demonstrated to be highly efficient antimutagenic and anticarcinogenic agent [16-18]. Both *in vitro* and *in vivo* short-term genotoxicity assays have demonstrated the potent antigenotoxic activity of CHL against a variety of mutagens. Under *in vitro* conditions, CHL has been reported to eliminate the mutagenic activity of a range of chemical mutagens/carcinogens, e.g. benzo {a] pyrene [19] and tobacco specific nitrosamines [20] in Chinese hamster V79 cells. In *in vivo,* CHL has been reported to reduce the chromosomal aberrations induced by the powerful mutagen thiotepa in Chinese hamster [21], the micronuclei induced by chromium trioxide [22]. Moreover CHL has been reported to inhibit chromosomal aberrations and micronuclei induced in mice bone marrow cells after cisplatin [12] and 5-fluorouracil treatment [13].

This study was designed to investigate the possible protective role of CHL against the genotoxic and cytotoxic effects of the widely used CIS and 5-FU regimen in mice. Chromosomal aberrations and micronucleus assays were used as indicators for genotoxicity. Also, the effect of CHL on the antitumor activity of the used anticancer drugs was evaluated in mice bearing Ehrlich Ascites Carcinoma (EAC) cells *in vivo.*

MATERIALS AND METHODS

Animals: Male and female Swiss Webster mice aged 10-12 weeks (25-30g) were obtained from the animal house of National Organization for Drug Control and Research (NODCAR). Animals were supplied with standard diet pellets and water that were given *ad libitum.* Animals were kept in plastic cages for 7 days to be accommodated with our laboratory conditions before being treated.

Chemicals and Doses: CIS (*Cis*-diamminedichloroplatinum (II)) was purchased in the form of colorless, clear solution in amber vials under the trade name of Unistin (KUP-United Douglas Pharm., USA, Pharmaceutical CO.) and administered i.p. at a dose level of 6 mg/kg b.w. [23]. 5-FU was purchased in the form of yellow clear solution in ampoules under the trade name of 5-Fluorouracil Biosyn (Pharma Hameln Gmbh, Germany) and administered i.p. at a dose level of 10 mg/kg b.w [8]. CHL was purchased in the form of dark green powder (Sigma chemical Co., St. Louis, MO, USA) and administered i.p. at dose levels of 2, 4, or 6 mg/kg b.w. [17].

Ehrlich Ascites Carcinoma (EAC) Cell Line: Ehrlich Ascites Carcinoma (EAC) cell line was obtained from Cancer Biology Dept., National Cancer Institute, Cairo University. It was kindly supplied from Netherlands Cancer Institute (Holland). It is a mouse tumor cell line of a mammary origin. A spontaneous breast cancer was served as the original tumor from which an Ascites variant was withdrawn. EAC cell line was maintained by serial intraperitoneal injections (inoculations) in female Swiss Webster mice at 7-10 days intervals. After the tumor inoculation, an Ascites rich with tumor cells were produced. The inoculated mice lived for about 10-20 days.

Antitumor Activity Test: To assess the effect of CHL pretreatment on the antitumor activity of CIS + 5-FU regimen, 160 female Swiss Webster mice weighting 28-34 gm were i.p. inoculated on day zero with 2.5 x 10^6 viable EAC cells/mouse. After 24 hours of EAC cells injection (on day one); animals were randomly divided into eight groups (twenty mice per each): negative control (group 1) injected i.p with distilled water and CHL control groups (groups 2,3 and 4) were injected with CHL (2, 4 or 6 mg/kg b.w), respectively. One group was injected with CIS and 5-FU simultaneously (group 5). The remaining three groups were treated with CHL 1 h before CIS and 5-FU injections (groups 6, 7 and 8). Then the number of died and alive animal (survival) was recorded every day up to 60 days. The mean survival time (MST) of each group was calculated using the survival analysis test [24].

Genotoxicity Tests

Treatment Schedule: Forty mice were randomly divided into 8 groups of five animals each. The same treatment schedule used for the eight groups of the antitumor activity test was followed.

Chromosomal Aberrations Analysis: Chromosomes were prepared according to the method described by Adler [25] with some modifications. Animals were injected with colchicine (2mg/kg b.w.) 2h before sacrifice. The harvested bone marrow cells were incubated in 5ml of 0.075 M KCl for 20 min at 37°C. At the end of incubation period, 3ml of fixative (glacial acetic acid – methanol 1:3 v/v) was added to each tube and then centrifuged for 5 min at 1000 x g. The supernatant was discarded and 3 ml of fresh fixative was gently pipetted onto the cells. Two to three changes in fixative were required before preparing the slides. Finally the cells were suspended in a small volume of fixative and burst opened on a clean slide to release chromosomes. The slides were air dried and stained in 10% buffered Giemsa and coded before

observations. One hundred metaphase spreads were scored per animal for chromosomal aberrations. Only cells with well spread chromosomes were selected for scoring. All metaphase spreads were examined for both structural and numerical aberrations. Both number of chromosomal aberrations and number of cells with chromosomal aberrations were recorded.

Mitotic Activity: The mitotic activity of bone marrow cells was obtained by counting 3000 cells per animal (of total 15000 per group) and the number of dividing cells including late prophase and metaphase was determined. The mitotic activity is expressed as mitotic index (MI), which is the number of dividing cells in 1000 cells.

Micronucleus Assay: Bone marrow slides for micronucleus assay were prepared and stained according to the method described by Schmid [26]. The bone marrow cells were flushed out with fetal calf serum, centrifuged, smeared and stained for 5 minutes in May-Grunwald–Giemsa stain mixture. Finally wash in distilled water and mounted with DPX. For each animal, 2000 polychromatic erythrocytes (PCE_S) were scored and the number of micronucleated polychromatic erythrocytes (MNPCEs) was determined. The results are expressed as mean percentage of MNPCEs. In addition, the rate of PCES with respect to normochromatic erythrocytes (NCE_S) was scored in 1000 erythrocytes. The values were expressed as mean percentage of PCEs to the total erythrocytes counts.

Statistical Analysis: Statistical analysis was performed using the Analysis of Variance (ANOVA) and Duncan's Multiple Range Test to determine differences between group means. T-test was also computed to test the significance level between groups. Survival analysis was carried out to compute MST of control and treated groups. All statistics were carried out using Statistical Analysis Systems (SAS) program (SAS, 2005) ®.

RESULTS AND DISCUSSION

The results of antitumor activity test presented in Table 1 revealed that treatment with CIS and 5-FU combination resulted in significant increase in mean survival time (MST) compared with the negative control group. These results are in agreement with the reported antitumor activity of CIS and 5-FU combination against several types of tumors [4, 27, 28]. The antitumor activity of CIS and 5-FU is thought to be due to inhibition of DNA synthesis. CIS inhibited DNA synthesis by tight binding to guanine residues of the double –stranded DNA while 5-FU inhibited DNA synthesis due to inhibition of thymidylate synthase necessary for formation of d TMP [4]. Table 1 showed that CHL pretreatment resulted in non significant changes in the antitumor actions of CIS and 5-FU combination as indicated by the observed non statistically significant change in MST of the groups pretreated with CHL (groups 6, 7 and 8) compared with MST of the group treated with CIS + 5-FU combination (group 5). These results indicated that CHL pretreatment did not affect the antitumor activity of CIS and 5-FU combination because the mechanism of the antitumor action of CIS and 5-FU are thought to be related mainly to the direct binding of two guanine bases of the DNA strand and inhibition of thymidylate synthase, respectively rather than free radical generation [4].

Table 1: Effect of pretreatment with different doses of chlorophyllin (CHL) on the antitumor activity of cisplatin (6 mg/kg) (CIS) + 5-fluorouracil (10 mg/kg) (5-FU) in mice bearing Ehrlich Ascites Carcinoma (EAC) cells

Groups	Treatment (Dose in mg/kg)	MST (days) Mean±S.E.
1	Negative control	13.1±1.18
2	CHL(2)	13.3±0.92
3	CHL(4)	14.5±0.67
4	CHL(6)	14.7±1.62
5	CIS + 5-FU	39.1±5.18 [a]
6	CHL(2) + CIS + 5-FU	38.6±4.73 [a b c]
7	CHL(4) + CIS + 5-FU	39.1±4.15 [a b c]
8	CHL(6) + CIS + 5-FU	40.3±3.47 [a b c]

[a]: Statistically significant different from the negative control group ($p < 0.05$).

[b]: Statistically significant different from their respective CHL groups ($p < 0.05$).

[c]: non-significant difference from combined treatment with CIS+5-FU group

MST: Meansurvival time.

The results of chromosomal aberrations test (Table 2) confirmed the previously reported non-clastogenic effect of CHL by several previous studies reported by Morales-Ramirez and Garcia-Rodriguez [16], Sarkar *et al*. [29] and Bez *et al*. [30] as indicated by non-significant changes in both the total number of cells with structural aberrations and total structural aberrations as compared with the negative control group. Furthermore, CHL did not cause any significant increase in the induction of micronuclei in polychromatic erythrocytes as compared with negative control group (Table 4). Moreover, Table 4 showed that CHL at dose of 6 mg/kg caused significant reduction in the frequency of micronucleated polychromatic erythrocytes compared with negative control group. The observed decrease in the incidence of

Table 2: Effect of pretreatment with different doses of chlorophyllin (CHL) on the frequency of chromosomal aberrations in bone marrow cells of mice induced by combined treatment with cisplatin (6 mg/kg) (CIS) and 5-fluorouracil (10 mg/kg) (5-FU).

		Number of different types of structural chromosomal aberrations (mean±S.D)					
Groups	Treatment (Dose in mg/kg)	Chromatid breakages	Centric fusion	Ring chromosomes	End to end association	Total structural aberrations (TSA)	Total number of cells with structural aberrations (%)
1	Negative control	5 (1.0±0.71)	0	0	0	5 (1.0±0.71)	5(1.0)
2	CHL(2)	7 (1.4±0.55)	0	0	0	7 (1.4±0.55)	7(1.4)
3	CHL(4)	5(1.0 ±1.22)	0	0	0	5 (1.0±1.22)	5(1.0)
4	CHL(6)	5 (1.0±0.71)	0	1 (0.2±0.45)	0	6 (1.2±1.09)	6(1.2)
5	CIS+5-FU	286 ^{a***}(57.2±9.63)	2 (0.4±0.55)	3 ^{a*}(0.6±0.55)	3 (0.6±0.89)	294 ^{a***}(58.8±10.16)	127 ^{a***}(25.4)
6	CHL(2)+CIS+5-FU	157 ^{b***}(31.4±3.36)	2 (0.4±0.55)	3^{a}(0.6±0.89)	0	162 ^{b***}(32.4±2.30)	87 ^{b***}(17.4)
7	CHL(4)+CIS+5-FU	91 ^{c***}(18.2±4.32)	3* (0.6±0.55)	1^{a}(0.2±0.45)	1(0.2±0.45)	96 ^{c***}(19.2±4.49)	55 ^{c***}(11.0)
8	CHL(6)+CIS+5-FU	32 ^{d**}(6.4±3.36)	0	0	0	32^{d**}(6.4±3.36)	22 ^{d**}(4.4)

Different letters within the same column indicate statistically significant difference (P< 0.05) between compared groups according to Duncan's multiple range test.

*; ** and ***: significantly different from negative control group at P< 0.05, P< 0.01 and P< 0.001, respectively, using student t-test.

Table 3: Effect of pretreatment with different doses of CHL on mitotic activity of bone marrow cells of mice treated with CIS (6 mg/kg) and 5-FU (10 mg/kg) combination

Groups	Treatment (Dose in mg/kg)	Number of examined cells	Number of dividing cells	Mitotic index (MI) mean±S.D
1	Negative control	15000	503	33.53±2.74
2	CHL(2)	15000	646	43.07±1.78 ***
3	CHL(4)	15000	763	50.87±2.31 ***
4	CHL(6)	15000	842	56.13±3.43 ***
5	CIS + 5-FU	15000	375	25.00±1.78 ^{a***}
6	CHL(2)+CIS +5-FU	15000	422	28.13±1.79 ^{b**}
7	CHL(4)+CIS +5-FU	15000	471	31.40±0.86 ^{c}
8	CHL(6)+CIS +5-FU	15000	640	42.67±1.18 ^{d***}

Different letters within the same column indicate statistically significant difference (P< 0.05) between compared groups according to Duncan's multiple range test.

*;** and ***: significantly different from negative control group at P<0.05, P<0.01 and P<0.001, respectively, using student t-test.

Table 4: Effect of pretreatment with different doses of CHL on the frequency of micronuclei in mouse polychromatic erythrocytes (PCEs) induced by CIS (6 mg/kg) and 5-FU (10 mg/kg) combination.

Groups	Treatment (Dose in mg/kg)	Number of MNPCEs/1000 PCEs	%MNPCEs mean±S.D	%PCEs mean±S.D
1	Negative control	3.6	0.36±0.05	49.94±0.46
2	CHL(2)	2.9	0.29±0.04	49.77±0.44
3	CHL(4)	2.5	0.25±0.03	50.13±0.60
4	CHL(6)	2.0	0.20±0.05 **	49.74±0.17
5	CIS+5-FU	29.8	2.98±0.15 ^{a***}	37.05±0.44 ^{a***}
6	CHL(2)+CIS+5-FU	20.2	2.02±0.14 ^{b***}	40.67±0.52 ^{b***}
7	CHL(4)+CIS+5-FU	14.6	1.46±0.54 ^{c**}	46.30±0.21 ^{c***}
8	CHL(6)+CIS+5-FU	6.5	0.65±0.12 ^{d*}	49.99±0.33 ^{d}

Results are expressed as mean±S.D.% MNPCEs: percentage of micronucleated polychromatic erythrocytes,%PCEs: percentage of polychromatic erythrocytes. Different letters within the same column indicate statistically significant difference (P< 0.05) between compared groups according to Duncan's multiple range test.

* ** and ***: significantly different from negative control group at P <0.05, P <0.01 and P <0.001, respectively, using student t-test.

chromosomal damage after CHL treatment confirmed the reported capacity of CHL to decline the basal reactive oxygen species (ROS) levels compared with the control level both *in vitro* and *in vivo* [31]. The results of mitotic activity in Table 3 showed that treatment with CHL alone (2, 4 or 6 mg/kg) caused significant increases in the mitotic indices of bone marrow cells compared with the negative control group. Increases in bone marrow cells mitotic activity after CHL treatment observed in this study may be attributed to the enhancement of bone marrow cells proliferation by CHL [32].

Chromosomal analysis test showed that treatment with CIS and 5-Fu resulted in significant ($p < 0.001$) increases in both the total structural aberrations and the

total number of cells with structural aberrations compared with the negative control group (Table 2). Chromosomal aberrations observed in the present study were only in the form of structural aberrations and chromatid breakages were the main type detected. These results are in agreement with the reported induction of chromosomal aberrations by CIS and 5-FU [8, 33, 34, 35]. In addition results of the micronucleus test (Table 4) confirmed the genotoxicity of CIS and 5-FU combination as manifested by significant increases in the frequencies of micronucleated polychromatic erythrocytes (MNPCEs) compared with negative control group. These results are in agreement with the reported induction of micronuclei after treatment with CIS and 5-FU in different systems [8, 35, 36, 37]. Micronuclei indicate the occurrence of chromosomal damage [38] and arise either from a broken centric or a centric part(s) of chromosomes or from intact whole chromosomes lagging behind at the anaphase stage of cell division, i.e. have failed to be incorporated into one of the daughter nuclei [39]. Agents which break chromosomes (clastogens) or interfere with spindle assembly or function are known to induce micronuclei. Chromosome damaging effects of CIS, 5-FU and their combination has been shown in many studies [8, 36, 37].

The genotoxicity of CIS is correlated with its ability to form intrastrand cross-link between adjacent guanines (GpG) at the N7 position [40]. These cross-links could be processed into single-strand and double-strand breaks during repair [41]. Moreover, cisplatin upon hydrolysis in aqueous solution forms various reactive hydroxyl species [42]. On the other hand, it has been suggested that the DNA double-strand breaks which follow 5-FU treatment accumulate as a consequence of the unbalanced deoxyribonucleotide pools [43]. Furthermore, in 5-FU treated cells, both FdUMP and deoxyuridine triphosphate (dUTP) incorporate into DNA in place of the depleted physiologic TTP. This incorporation into DNA would call into action the excision repair process. This process may result in DNA strand breakage, because DNA repair requires TTP but this substrate is lacking as a result of thymidylate synthase inhibition [44]. In addition to the direct DNA damage induced by anticancer drugs previously described, they can cause DNA damage indirectly through the elevated level of reactive oxygen species (ROS) [45]. Reactive oxygen species (ROS) such as superoxide anions and hydroxyl radicals can damage DNA and form strand breaks of DNA [46]. CIS and 5-FU combination has been show to induce DNA damage by the generation of ROS such as the superoxide anions and hydroxyl radicals [33, 47, 48] and inhibit the activity of antioxidant enzymes.

The results of the current study confirmed the cytotoxic effect of CIS and 5-Fu as indicated by significant reduction in both the mitotic indices and percentage of polychromatic erythrocytes (% PCEs) in bone marrow cells in the group of mice treated with CIS and 5-FU combination as compared with the negative control group (Table 3 and 4, respectively). These results are in agreement with the reported cytotoxic effect of cisplatin [9, 11, 12] and 5-FU [13]. CIS cytotoxicity may be attributed to its ability to form DNA cross-linking which cause cell cycle arrest, inhibition of DNA replication and transcription and eventually apoptosis [49, 50]. Moreover, correlation between the cytotoxicity of CIS and the frequency of interstrand DNA cross-links it forms have been reported [51]. On the other hand, 5-FU is an inhibitor of DNA synthesis acting primarily in S-phase of the cell cycle; it acts on enzymes that are involved in the synthesis of DNA precursor thymidine triphosphate (TTP) and affects the fidelity of DNA replication and repair [52].

The results of the present study revealed that CHL (2, 4 and 6 mg/kg) pretreatment caused significant reduction in total structural aberrations and in the total number of cells with structural chromosomal aberrations (Table 2) as compared with CIS+5-FU group (group 5). Moreover, CHL pretreatment caused significant reduction in the percentage of $MNPCE_S$ as compared with CIS+5-FU group in a dose dependent pattern (Table 4). Regression analysis showed a strong negative dose-dependent correlation (r = -0.960) between CHL pretreatment and total structural aberrations (TSA_S) induced by combined treatment with CIS and 5-FU combination (Fig. 1). In addition, regression analysis revealed a strong negative correlation (r=-0.878) between the percentage of micronucleated polychromatic erythrocytes (% $MNPCE_S$) and pretreatment with different doses of CHL (Fig. 3).

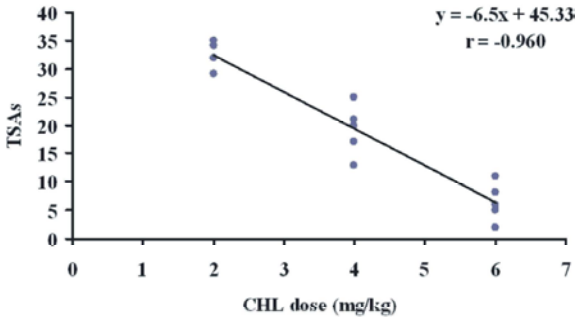

Fig. 1: Regression line and correlation coefficient for total number of structural aberrations (TSAs) induced by CIS and 5-FU combination in different groups of mice pretreated with different doses of CHL.

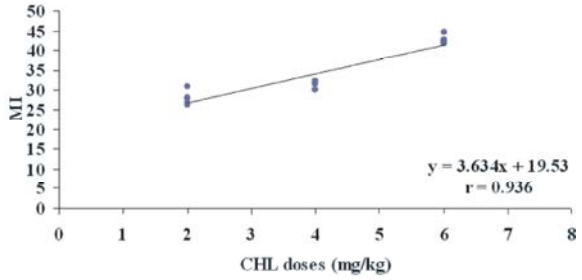

Fig. 2: Regression line and correlation coefficient for mitotic indices (MI) of bone marrow cells of mice treated with different doses of CHL before CIS and 5-FU.

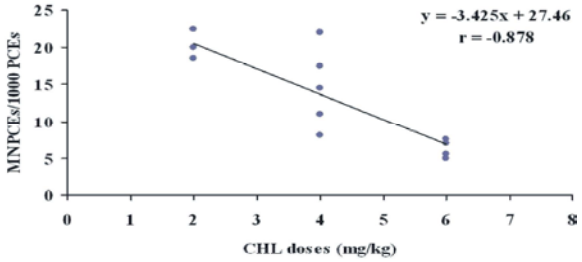

Fig. 3: Regression line and correlation coefficient for micronucleated polychromatic erythrocytes (MNPCEs) per 1000 polychromatic erythrocytes (PCEs) induced by CIS and 5-FU combination in different groups of mice pretreated with different doses of CHL.

These results confirmed the antigenotoxic effects of CHL that have been previously described by Noshy *et al.* [12], Noshy and Ramadan [13], Morales-Ramirez and Garcia-Rodriguez [16], Bez *et al.* [30] Abraham *et al*. [53]. The protective effects of CHL against the effects of mutagens and clastogens may be attributed to its ability to scavenge free radicals. Kumar *et al.* [18] suggested that the protective role of CHL could be arising mainly due to the scavenging of hydroxyl radicals [18]. They also correlated the hydroxyl radical scavenging ability of CHL and the inhibition of DNA strand breaks formation, the protection of CHL given in terms of number of strand breaks/DNA molecule. Moreover, CHL or chlorophyll has a strong adsorbing property against various chemical substances [54]. Several previous reports suggested that CHL and chlorophylls bind genotoxic substances to form complexes and reduce the availability of these mutagens [55]. The formation of a complex with CHL can facilitate degradation of a given mutagen and may induce hinder its migration to target DNA [19]. CHL reduces the toxicant-DNA binding by acting as a desmutagen or interceptor molecule forming reversible complexes with the mutagen/carcinogen [56].

The results of mitotic activity in Table 3 showed that the groups of animals treated with different doses of CHL 1h before CIS and 5-FU administration (groups 6, 7 and 8) restored the mitotic activity of bone marrow cells as indicated by significant increase ($p < 0.05$) in the mitotic indices of bone marrow cells as compared with the group treated with CIS and 5-FU combination (group 5) in a dose-dependent manner. Regression analysis indicated a strong positive correlation (r=0.936) between mitotic index (MI) and pretreatment with CHL different doses (Fig. 2). Moreover, the results of micronucleus assay (Table 4) showed that CHL pretreatment caused a significant increase in the percentage of PCEs indicating the restored of mitotic activity of bone marrow cells that had been suppressed by CIS and 5-FU treatment. These results are in agreement with the reported enhancement of bone marrow proliferation by CHL and the immuno-modulatory effects of CHL [12, 13, 32]. The linear dose-dependent protective effects of CHL and the non-toxic effects of different doses of CHL used in this study reflecting the possibility of using higher doses of CHL in patients treated with cancer chemotherapeutic agents to reduce or prevent the undesirable toxic side effects of anticancer drugs. These results are well correlated with previous studies that emphasized the protective linear dose-dependent effect of CHL [16, 17, 57].

CONCLUSION

CHL supplement during the treatment with CIS and 5-FU based chemotherapy can enhance their therapeutic effectiveness by inhibiting their genotoxicity and cytotoxicity without affecting their antitumor activity.

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