Impact of Chrysanthemum Indicum on Genotoxicity and Hepatic and Kidney Function in Anticancer Drug Adriamycin Exposed Mice

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ABSTRACT

The present study was undertaken to evaluate the impact of Chrysanthemum indicum (CI) on genotoxicity and kidney and hepatic function in the anticancer drug adriamycin (ADR) exposed mice using cytogenetic analysis, DNA fragmentation and biochemical alterations. Thirty Swiss albino male mice were divided into 6 groups (5 animals each) as follows: 1) Control group (negative control); 2) Adriamycin (0.5 mg as a positive control); 3) Chrysanthemum indicum (LD, low dose, 0.5 mg); 4) Chrysanthemum (HD, high dose, 5 mg); 5) ADR + low dose of Chrysanthemum indicum (0.5 mg); 6) ADR + high dose of Chrysanthemum indicum (5 mg) for 45 days. The results of the present study revealed that ADR induced high frequency of chromosomal aberration in both somatic and germ cells and DNA damage. However, the treatment with Chrysanthemum indicum significantly reduced the frequent of these parameters as compared to the control. The biochemical findings indicate that ADR treatment induce biochemical changes in liver and kidney function, however the treatment with ADR in combination with Chrysanthemum indicum ameliorate these changes especially with the high dose of Chrysanthemum. In conclusion, the data suggest that the complimentary use of Chrysanthemum indicum with ADR treatment will be beneficial to reduce the adverse effect of ADR in cancer chemotherapy, such as the increased incidence of undesirable mutagenic and biochemical side effects.

INTRODUCTION

Adriamycin (ADR, CAS no. 25316-40-9) is a benzanthroquinone drug, which is useful in the treatment of several types of human malignancies [1]. It is cytotoxic and mutagenic in both bacterial and mammalian systems. One type of interaction with the DNA is associated with the production of reactive free radicals [2], but its cytotoxic activity has been related to its interaction with nuclear topoisomerase II [3].

Adriamycin is known to be a cell cycle specific agent. ADR was shown to produce an increase in DNA strand breakage and in the percentage of abnormal frequencies of chromosomal damage in the FISH and conventional chromosomal aberration assays [4]. As well in vivo and in vitro studies in mouse and in different cell lines have shown that ADR (doxorubicin) increases the frequency of micronuclei [5].

Adriamycin (ADR) induced toxicities including renal [6, 7] and hepatic [8, 9] injuries had been determined in experimental studies. The mechanisms of ANT-related toxicities are not yet fully understood. It may be because of lipid peroxidation and the generation of free radicals by ANT-iron complexes.

Chrysanthemum indicum L. (CI), a Compositae plant, is a traditional Chinese medicine and medicinal plant distributed widely in China. Volatile oil and flavonoids are believed to be the main active components in Chrysanthemum. The most abundant and biologically active components flavonoids, in the form of glycoside derivatives, are more polar than volatile oil and hence are readily dissolved in water. Another group is terpenoids, which are present in the volatile oil. The antioxidant properties of flavonoids extracted from chrysanthemum could have been responsible for its broad pharmacological effects. It was found that its water extract showed significant antioxidant activities, suggesting that the extract may reduce lipid peroxidation and play a role in protecting against damages to the cell [10].

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The water extract of *Chrysanthemum* also possessed direct inhibitory effects on various free radicals [11]. The significant correlation between phenolic compounds and antioxidant activity indicates that the flavonoids may contribute directly to the antioxidant activity of the extract. The flavonoids can also be absorbed into the cell membrane and hence protect the cells from the damages of reactive oxygen species (ROS) [12]. Therefore, the present study was undertaken to evaluate the impact of *Chrysanthemum indicum* on genotoxicity, DNA damage and biochemical changes in anticancer drug adriamycin exposed mice.

MATERIALS AND METHODS

Animals:

Swiss albino male mice were purchased from the animal colony of the Animal House of the National Research Center, Dokki, Cairo, Egypt and were kept for 1 week for acclimatization and supplied with feed and water *ad libitum*. Animals were divided into 6 groups (10 animals/group) as follow: 1) control group; 2) Adriamycin (ADR, 0.5 mg/kg bw); 3) *Chrysanthemum* extract (LD, low dose 0.5 mg/kg bw); 4) *Chrysanthemum* extract (HD, high dose 5 mg/kg bw); 5) *Chrysanthemum* extract (LD + ADR, 0.5mg/kg); 6) *Chrysanthemum* extract (HD + ADR, 0.5mg/kg). The treatment period was 45 days and mice were sacrificed 24 hours after the last dose and subjected to cytogenetic analysis in both somatic and germ cells, DNA fragmentation and biochemical analysis.

Chemicals:

Adriamycin (ADR), CAS Number: 25316-40-9 was purchased from Sigma-Aldrich (3050 Spruce Street, Saint Louis, MO 63103, USA).

Chrysanthemum extract preparation:

Samples of *C. indicum* L. (Cl) were prepared from the corolla of the *Chrysanthemum* purchased commercially and pulverised in liquid nitrogen. Extract was obtained from (500 g) via extraction with 4 L of water at room temperature over the course of 3 days. The resulting solution was centrifuged and filtered and the supernatant removed, evaporated, and freeze-dried under a vacuum. The residue (100 mg) was then dissolved in 1 ml of water [13].

Chromosomal aberrations analysis:

For somatic cells, mice were sacrificed 24 h after administration of the last treatment for chromosome aberration analysis. Cytogenetic analysis was performed on tibia bone marrow cells according to the recommendations of [14], with slight modifications. Fifty good metaphases containing 40 chromosomes were examined per animal to score different types of aberrations (gaps, breaks, fragments, deletions and endomitosis).

For spermatocyte cells, chromosomal preparation were made according to the air-drying method [15]. Fifty primary spermatocytes/mouse at diakinesis-metaphase I were scored. Abnormalities recorded included univalents (x–y univalent, autosomal univalent, chains, rings and N±1).

Determination of malondialdehyde (MDA) level:

Liver was homogenized and the supernatant was chemically treated and centrifuged at 10000 rpm for 3 min for quantitative measurement of lipid peroxidase malondialdehyde (MDA) according to the method of [16].

Determination of liver and kidney function:

Blood samples from animals were collected in gel-activated tubes for the assessment of specific liver markers. The gel-activated tubes were allowed to clot, then centrifuged at 3400 rpm for 10 min at 4°C. The serum samples were collected for measuring liver markers, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein. The markers were assayed with a spectro-photometer [17]. Other biochemical parameters have been measured including metabolites (creatinine, uric acid, total protein). These biochemical parameters were determined using an Olympus AU400 analyzer (Olympus SA, Rungis, France) with kits.

Statistical analysis:

Data are expressed as mean ± SE. Statistical analyses were performed by one-way analysis of variance (ANOVA) with Dunnett’s multiple comparison of means test. Different small superscript letters indicate significant correlation at P value of 0.05 or less.

RESULTS AND DISCUSSION

The results of the present study (Table 1) revealed that the frequency of structural chromosomal aberrations in somatic cells (gaps, break, fragment, deletion and endomitosis) increased significantly with the treatment of ADR compared to control group. However, the treatment with *Chrysanthemum indicum* (Cl) decreased these
parameters especially with the high dose (5mg) compared to ADR treated group only. The same findings were found in the frequency of numerical variation (N±1 and total numerical variation).

Table 1: Mean percentages ± S.E. of chromosomal aberration in male mice bone marrow cells treated with Adriamycin and/or Chrysanthemum indicum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Structural aberrations</th>
<th>Total structural aberrations</th>
<th>Numerical variation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gap</td>
<td>Break</td>
<td>Chromosome gap</td>
<td>Fragment</td>
</tr>
<tr>
<td>Control</td>
<td>0.50 ± 0.24 a</td>
<td>0.00 ± 0.00 a</td>
<td>0.00 ± 0.00 a</td>
<td>0.00 ± 0.00 a</td>
</tr>
<tr>
<td>ADR</td>
<td>7.06 ± 0.58d</td>
<td>3.40 ± 0.37e</td>
<td>2.06 ± 0.12c</td>
<td>3.02 ± 0.24 b</td>
</tr>
<tr>
<td>LD(0.5mg)</td>
<td>0.40 ± 0.24 a</td>
<td>0.20 ± 0.12 b</td>
<td>0.24 b</td>
<td>0.24 b</td>
</tr>
<tr>
<td>HD(5mg)</td>
<td>0.60 ± 0.24 a</td>
<td>0.60 ± 0.12 b</td>
<td>0.60 ± 0.12 b</td>
<td>0.40 ± 0.12 b</td>
</tr>
<tr>
<td>LD+ADR</td>
<td>1.80 ± 0.58d</td>
<td>2.60 ± 0.37e</td>
<td>3.60 ± 0.80d</td>
<td>3.0 ± 0.20 d</td>
</tr>
<tr>
<td>HD+ADR</td>
<td>1.60 ± 0.48d</td>
<td>1.80 ± 0.24 c</td>
<td>2.0 ± 0.12 b</td>
<td>1.80 ± 0.20 d</td>
</tr>
</tbody>
</table>

N.B: ADR: Adriamycin; LD: low dose of Chrysanthemum extract; HD: high dose of Chrysanthemum extract.

Different small superscript letters are differ significantly:

Concerning the effect of ADR and/or Chrysanthemum treatment on the frequency of chromosomal aberration in male spermatocyte cells (Table 2), its clear that ADR treatment increased the frequency of both structural (x-y univalent, Autosomal univalent, chain and ring as well total structural aberrations) and numerical (N±1 as well total numerical variation) chromosomal aberrations compared to control group. However, the treatment with Chrysanthemum decreased significantly these parameters specially with the high dose of Chrysanthemum compared to ADR treated group only. The same findings were found in the frequency of numerical variation (N±1 and total numerical variation).

These results coincide with the results of [4] who found that the percentage of abnormal frequencies of chromosomal damage in the FISH and conventional chromosomal aberration assays. In vivo and in vitro studies in mouse and in different cell lines have shown that ADR (doxorubicin) increases the frequency of micronuclei as well [5] which confirm our results.

DNA fragmentation and malondialdehyde (MDA) level:

The results concerning DNA fragmentation (Table 3) revealed that there was a significant difference between the ADR and control group (44.88 ± 0.39 vs.5.60 ± 1.16). However the treatment with Chrysanthemum decreased significantly this parameter especially with the high dose of Chrysanthemum compared to ADR treated group only. The mutagenic effect of ADR may due its interaction with the DNA which associated with the production of reactive free radicals [2]. As well, MDA level was found to increase significantly in the ADR treated group compared to control group (51.82 ± 0.37 vs. 2.10 ± 0.45). However, the treatment with Chrysanthemum decreased significantly this effect especially with the high dose of Chrysanthemum compared to ADR treated group only. That coincide with [18] who found that Chrysanthemum had a protective effect against the ADR-induced increase of MDA level. The inhibition of ADR-induced clastogenicity and lipid peroxidation by Chrysanthemum may be attributed to its antioxidant action.

Table 2: Mean percentages of chromosomal aberration in male spermatocyte cells treated with Adriamycin and/or Chrysanthemum indicum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of examined cells</th>
<th>Structural aberrations</th>
<th>Total aberration</th>
<th>Numerical variation</th>
<th>Total numerical variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No %</td>
<td>No %</td>
<td>No %</td>
<td>No %</td>
<td>No %</td>
</tr>
<tr>
<td>Control</td>
<td>250</td>
<td>0 ± 0.4</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>ADR</td>
<td>250</td>
<td>1 ± 0.4</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>LD+ADR</td>
<td>250</td>
<td>1 ± 0.4</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>HD+ADR</td>
<td>250</td>
<td>2 ± 0.4</td>
<td>1 ± 0.4</td>
<td>1 ± 0.4</td>
<td>1 ± 0.4</td>
</tr>
</tbody>
</table>

N.B: ADR: Adriamycin; LD: low dose of Chrysanthemum extract; HD: high dose of Chrysanthemum extract.

Different small superscript letters are differ significantly:

Determination of liver and kidney function:

Biochemical changes were also highly observed in ADR treated group in relation to the highly significant elevation of ALT and AST levels compared to control group (Table 4). The same findings were also found concerning to creatinine and uric acid. However, the treatment with Chrysanthemum in combination with ADR decreased significantly these parameters especially with the high dose of Chrysanthemum compared to ADR
treated group. The total protein level was found to decrease significantly in ADR treated group compared to control, however the treatment with *Chrysanthemum* in combination with ADR caused a significant increase in this parameter especially with the high dose of *Chrysanthemum* compared to ADR treated group.

Table 3: Mean parentages of DNA Fragmentation and Malondialdehyde (MDA) in male mice cells treated with adriamycin and/or *Chrysanthemum indicum*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DNA Fragmentation</th>
<th>Malondialdehyde (MDA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.60 ± 1.16a</td>
<td>2.10 ± 0.45b</td>
</tr>
<tr>
<td>ADR</td>
<td>44.88 ± 0.39c</td>
<td>51.82 ± 0.37b</td>
</tr>
<tr>
<td>LD(0.5mg CI)</td>
<td>7.04 ± 0.94c</td>
<td>4.06 ± 0.49c</td>
</tr>
<tr>
<td>HD (5mg CI)</td>
<td>8.24 ± 0.98c</td>
<td>5.45 ± 0.22c</td>
</tr>
<tr>
<td>LD+ADR</td>
<td>31.82 ± 0.58c</td>
<td>39.62 ± 0.49c</td>
</tr>
<tr>
<td>HD+ADR</td>
<td>19.70 ± 0.94c</td>
<td>21.24 ± 0.56c</td>
</tr>
</tbody>
</table>

N.B: ADR: Adriamycin; LD: low dose of *Chrysanthemum* extract; HD: high dose of *Chrysanthemum* extract.

Our findings matched those of [6, 7, 19] who found that Adriamycin (ADR) induced toxicities in renal and with [8,9] in hepatic function in experimental studies. The mechanisms of ADR (ANT-related toxicities) may be because of lipid peroxidation and the generation of free radicals by ANT-iron complexes.

However, the hepato-and renal protective effects of *Chrysanthemum indicum* significantly reduced biochemical changes which coincide with [17, 20] and with [19], in liver and kidney function, respectively. The same findings were reported by [21] Jeong *et al.*, who found that Cif significantly reduced the levels of GOT (60.1%, $P = 0.000$) and GPT (64.5%, $P = 0.000$) compared with the vehicle control group. The antioxidant properties of flavonoid compounds in *Chrysanthemum* could have been responsible for its broad pharmacological effects. It was found that its water extract showed significant antioxidant activities, suggesting that the extract may reduce lipid peroxidation and play a role in protecting against damages to the cell membrane [10,19].

Table 4: Effect of adriamycin and/or *Chrysanthemum indicum* treatment on hepatic (ALT, AST levels) and kidney (creatinine, uric acid and protein levels) function.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (u/l)</th>
<th>AST (u/l)</th>
<th>Creatinine (U)</th>
<th>Uric acid (mM/L)</th>
<th>Total Protein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.59 ± 0.24a</td>
<td>43.20 ± 0.22a</td>
<td>0.45 ± 0.22a</td>
<td>2.40 ± 0.24a</td>
<td>9.85 ± 0.41a</td>
</tr>
<tr>
<td>ADR</td>
<td>64.40 ± 0.24a</td>
<td>70.20 ± 0.58a</td>
<td>0.67 ± 0.28a</td>
<td>9.85 ± 0.41a</td>
<td>4.83 ± 0.40a</td>
</tr>
<tr>
<td>LD(0.5mg CI)</td>
<td>29.60 ± 0.67a</td>
<td>45.0 ± 0.31a</td>
<td>0.51 ± 0.22a</td>
<td>3.00 ± 0.00a</td>
<td>9.25 ± 0.33a</td>
</tr>
<tr>
<td>HD(5mg CI)</td>
<td>31.20 ± 0.37a</td>
<td>46.00 ± 0.31a</td>
<td>0.53 ± 0.22a</td>
<td>3.59 ± 0.39a</td>
<td>8.05 ± 0.55a</td>
</tr>
<tr>
<td>LD + ADR</td>
<td>52.40 ± 1.02a</td>
<td>62.20 ± 0.37a</td>
<td>1.99 ± 0.004a</td>
<td>6.24 ± 0.34a</td>
<td>6.85 ± 0.41a</td>
</tr>
<tr>
<td>HD + ADR</td>
<td>41.80 ± 0.37a</td>
<td>50.60 ± 0.50a</td>
<td>1.05 ± 0.05a</td>
<td>7.85 ± 0.41a</td>
<td>7.25 ± 0.33a</td>
</tr>
</tbody>
</table>

N.B: ADR: Adriamycin; LD: low dose of *Chrysanthemum* extract; HD: high dose of *Chrysanthemum* extract.

Different small superscript letters are differ significantly.

In conclusion, it has been reported that *Chrysanthemum indicum* has an antioxidant molecule having a high scavenging activity towards ROS [22-25], its antioxidant property is likely to be associated with the mechanism underlying the protective effect of *Chrysanthemum indicum* against ADR-induced mutagenicity and DNA or chromosomal damage. This idea is supported by our present observations that the *Chrysanthemum* administered with ADR treatment gave effective protection against the production of DNA and biochemical damage, and the effect was also dependent on the dose of *Chrysanthemum*. Thus, our experiments suggest that treatment with *Chrysanthemum indicum* is effective for reducing the mutagenic and biochemical effects of ADR treatment in chemotherapeutic program.

REFERENCES


