INTRODUCTION

A number of antineoplastic drugs are used to combat with different types of cancer which have also shown to be mutagenic in various test systems. Various antineoplastic drugs such as cisplatin, cyclophosphamide, Tamoxifbn Genecitabine and Paclitaxel etc have shown to be clastogenic effects in various test systems (Garrone et al., 1993; Takeda et al., 2001; Padmalatha Rai and Vijaylakhmi, 2001; Boffetta et al., 2007; Padmanabham et al., 2008).

Adriamycin, one of most commonly used anthracyclin is effective in malignant lymphomas, the drug is particularly beneficial in a wide range of pediatric and adult sarcomas. It has been shown that chemotherapist agents including anthracyclins cause gene mutation, chromosomal aberrations rearrangements and aneuploids in somatic cells as well as an increased frequency of secondary treatment related tumor in human cancer survivors (Sandoval et al., 1993; Povirk and shuker, 1994; Ben Yehuda et al., 1996). Further a significant increase was reported in patients involved in cytostatic treatment (chamber et al., 1984). Because of the extensive and increasing use of adriamycin in successfully therapy regimes, an understanding of the mutagenic properties are important. Hence an attempt was made to study the potential mutagenic effect of adriemycin in mice system.

MATERIALS AND METHODS

Eight week old healthy laboratory bred Swiss albino mice (Mus musculus) weighing 25 ± 3g of were maintained under standard laboratory conditions at temperature 22°C relative humidity 50±10% and 12 h photo period commercial pellet diet (Hindustan Lever India) and deionised water were provided by labium.

In the present study the various split doses of Adriamycin (4, 8, 16mg/kg bw) was injected intraperitoneally to the animals for four consecutive days and the animals were killed after 72hr of administration of the test chemical. The treatment for 48 hr was kept to allow bone marrow cells to complete the two cell cycles. The control and treated group of animals were scarified after 6 hr of the last treatment by cervical dislocation. The bone marrow was flushed out into clean glass petri dishes with hypertonic solution (0.56% KCl) to get a homogeneous cell suspension. It was then collected in clean centrifuge tubes and incubated at 37ºC for 45 minutes. Four slides were prepared from control and three groups of experimental animals. The staining was done within 24 hr of preparation according to the method of Preston et al., (1987). The slides were screened for 250 well spread metaphases per animal to examine the presence of various types of chromosomal aberrations like gaps breaks, fragment, chromatid separations and polyploids in control and treated group of animals. The data was analysed using Chi-Square test.

RESULTS

The data on the genotoxic effects of adriamycine evaluated from bone marrow cells of mice after 24, 48, 72 hrs of administration of the drug was furnished in (Table 1 and 2), These include changes in different type of chromosomal aberrations.

The frequency of chromosomal aberration in mice treated with various doses of adriamycin 4, 8 and 16 mg/kg body weight were 6.4, 10.4 and 16.4 % at 24 hrs of administration
respectively when compared to controls (2.4%) (Table 1). The aberrations consisted mostly gaps, breaks, fragments, exchanges and chromatid separation. Gaps were significantly high at 24 hrs. Fragments in the treated mice were more compared to controls. Exchanges were observed at only 16 mg/kg weight. No polyploidy was observed (Table 2). The data was analyzed statistically using $\chi^2$. The results were found to be significant (p<0.05).

At 48 hrs of administration for the various doses of Adriamycin with 4, 8 and 16 mg/kg body weight the frequency of chromosomal aberration in the treated group of mice were 8, 14 and 20 % respectively when compared to control animals 3.2% (Table 1). At 48 hrs of the drug administration the frequencies of the gaps, breaks, fragments, chromatid separation and exchanges were increased in the mice when compared to that of the controls (Table 2). There were no Polyploids. The data was analysed statistically using $\chi^2$. The results were found to be significant (p<0.05).

At 72hrs of administratation of drug the total frequencies (%) of chromosomal aberration in the treated mice were increased to 12.4, 16 and 18 % respectively when compared to controls 3.2 %.

The doxorubicin induced a significant increase (p < 0.01) the frequency of chromosome abnormalities, these results being consistent with those reported (Anderson et al., 1998).

The present results are accordance that Larramendy et al., (1980), the frequency of chromatid-type aberrations exhibited a direct-correlation with the dose in mice treated for 6h but not for 12 h. On the other hand, chromosome-type aberrations detected 12 hrs after injection were directly correlated with the dose of adriamycin, the genotoxic effects of the metacentric-like chromosomes induced by adriamycin arise either from translocations involving entire chromosomes arms or from

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>24 hrs Normal Metaphases Scored (%)</th>
<th>Abnormal Metaphases Scored (%)</th>
<th>48 hrs Normal Metaphases Scored (%)</th>
<th>Abnormal Metaphases Scored (%)</th>
<th>72 hrs Normal Metaphases Scored (%)</th>
<th>Abnormal Metaphases Scored (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>244 (97.60) 6 (2.40)</td>
<td>242 (96.80) 8 (3.20)</td>
<td>242 (96.80) 8 (3.20)</td>
<td>242 (96.80) 8 (3.20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 mg/kg</td>
<td>234 (93.60) 16 (6.40)</td>
<td>229 (91.60) 21 (8.40)</td>
<td>219 (87.60) 31 (12.40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 mg/kg</td>
<td>224 (89.60) 26 (10.40)</td>
<td>214 (85.60) 36 (14.40)</td>
<td>209 (83.60) 41 (16.40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 mg/kg</td>
<td>209 (83.60) 41 (16.40)</td>
<td>207 (82.80) 43 (17.20)</td>
<td>202 (80.80) 48 (19.20)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 The values in parentheses are percentages.

Table 1: Frequency of chromosomal aberrations recorded in somatic cells of mice after treatment with various doses of adriamycin for 24, 48 and 72 hrs interval

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Duration of Treatment (hrs)</th>
<th>Gaps</th>
<th>Breaks</th>
<th>Fragments</th>
<th>Exchanges</th>
<th>Numerical aberrations</th>
<th>Chromatid separations</th>
<th>Total no. of Aberrations(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs</td>
<td>Control I</td>
<td>4(8.00)</td>
<td>1(2.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>1(2.00)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5(10.00)</td>
<td>2(4.00)</td>
<td>1(2.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>1(2.00)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7(14.00)</td>
<td>1(2.00)</td>
<td>3(6.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>2(4.00)</td>
<td>6(12.00)</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>9(18.00)</td>
<td>1(2.00)</td>
<td>4(8.00)</td>
<td>3(6.00)</td>
<td>0(0.00)</td>
<td>1(2.00)</td>
<td>9(18.00)</td>
</tr>
<tr>
<td>48 hrs</td>
<td>Control II</td>
<td>4(8.00)</td>
<td>1(2.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6(12.00)</td>
<td>2(4.00)</td>
<td>3(6.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>1(2.00)</td>
<td>5(10.00)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8(16.00)</td>
<td>3(6.00)</td>
<td>4(8.00)</td>
<td>2(4.00)</td>
<td>0(0.00)</td>
<td>2(4.00)</td>
<td>8(16.00)</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>10(20.00)</td>
<td>5(10.00)</td>
<td>6(12.00)</td>
<td>2(4.00)</td>
<td>0(0.00)</td>
<td>2(4.00)</td>
<td>11(22.00)</td>
</tr>
<tr>
<td>72 hrs</td>
<td>Control III</td>
<td>4(8.00)</td>
<td>1(2.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td>1(2.00)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9(18.00)</td>
<td>2(4.00)</td>
<td>3(6.00)</td>
<td>1(2.00)</td>
<td>0(0.00)</td>
<td>1(2.00)</td>
<td>7(14.00)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>11(22.00)</td>
<td>5(10.00)</td>
<td>6(12.00)</td>
<td>2(4.00)</td>
<td>0(0.00)</td>
<td>1(2.00)</td>
<td>11(22.00)</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>17(34.00)</td>
<td>7(14.00)</td>
<td>8(16.00)</td>
<td>3(6.00)</td>
<td>0(0.00)</td>
<td>1(2.00)</td>
<td>15(30.00)</td>
</tr>
</tbody>
</table>

Gaps and polyploids are not included in total aberrations. The values in the parenthesis are percentages.

Table 2: Classification of various types of chromosomal aberrations in somatic cells of mice analysed after 24, 48 and 72 hrs treatment with various doses of adriamycin.
aberrations of the exchange type between 2 short arms of acrocentric chromosomes.

Similar results were reported by Au and Hsu (1980) the genotoxic effects of adriamycin on somatic cells were studied in mice treated with single injections of 3, 12 or 24 mg/kg of the drug. From 1 to 5 days post-injection, chromosome aberrations were observed in bone-marrow cells the frequency of chromosome breakages peaked at 5 h or 1 day for the bone marrow Univalent formation was increased overall.

The results of present studies are comparable to Venkatesh et al., (2007) the effect of various concentrations of doxorubicin (DOX)-induced genotoxic effects in mice bone marrow was studied. Treatment of mice with different concentrations of DOX resulted in a dose-dependent elevation in the frequency of micronucleated polychromatic (MNCE) as well as normochromatic (MNCE) erythrocytes in mouse bone marrow. The similar results were reported by aydemir and Bilallug, (2004) to evaluate the effects of chromosomal aberrations induced by Doxorubicin (DXR) in bone marrow cells of Wistar rats.

The present results are accordance that Mehmet Dogan Gülkac (2004) induction of chromosomal aberrations (CA) in rat bone marrow cells by injecting DXR (90 mg/kg body wt). Animals treated with single dose of DXR presented a statistically significant increase in total number of CA. The present results are coincided with Amany A. Tohamy et al., (2003) the induction of chromosomal aberrations in the bone marrow cells of mice treated with cyclophosphamide (CP) (2.5 mg/kg bw, i.p.) adriamycin (ADR) (12 mg/kg bw, i.p.) and cis-diaminedichloroplatinum-II (cisplatin) (5 mg/kg bw, i.p.) investigated. Their was increased number of cells with structural chromosomal aberrations scored after the treatment in bone marrow cells.

The present results are accordance to Prahalathan et al., (2003), investigated the ADR-induced clastogenicity and apoptosis in the bone marrow of rats. The animals were randomly divided into eight groups consisting of six rats each. Five groups were administered ADR (20 mg/kg body weight, i.v.) to induce genotoxicity; The effects of adriamycin were monitored by DNA strand breaks, chromosomal aberrations, micronucleus assay and apoptotic studies in the bone marrow cells of rats after 24 h following single dose of ADR treatment. ADR treatment caused significant clastogenicity and apoptosis in rat bone marrow cells.

Wistar rat cells treated with DXR in vivo. The animals were treated by gavage for micronucleus assay (MN) and chromosome preparations. Control groups received a single dose of DXR, rat bone marrow cells developed significantly fewer MN and chromosomal aberrations than those treated with DXR alone. In contradictory Marvin Meistrich et al., (1990) failed to observed increases in chromosomal aberration in the ADR-treated mice at the 6 mg or 8 mg/kg doses. The genotoxic effect of the anticancer drugs such as 5-flouracil, cyclophosphamide, cisplatin in animal model has published elsewhere by Rao (2006). A significant increase in the frequency of chromosomal aberration in somatic cells of mice were reported by anthelmintic drugs (Rudrama Devi and Reddy, 1995), antiasthamatic drugs (Kameswari et al., 1991), heavy metal such as lead (Rudrama devi and Reddy, 1988), chromium (Kiran et al., 1999), cadmium chloride (Rajitha and Rudrama devi, 1999) and Cyclophosphamide and fluorouracil (Rao et al., 2006, Shobha Rani et al., 2006).

REFERENCES


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