The present study was designed to evaluate the genotoxicity of albendazole (ABZ) in mice using the micronucleus test. Mice were treated by gavage with 500, 1,000, and 1,500 mg of ABZ kg\(^{-1}\) b.w., which corresponds to 1/6, 1/3, and 1/2 of the oral LD\(_{50}\) of ABZ. In the second part of the study, the possible protective role of vitamin C (vit. C) was investigated against the genotoxic effect of ABZ. The mice received 200 mg of vit C kg\(^{-1}\) b.w. simultaneously with ABZ. Bone marrow samples were taken 48 h after the treatment. ABZ induced a statistically significant increase (P<0.001) in the percentage of micronucleated polychromatic erythrocytes (MNPCE) in the 1,000 and 1,500 mg of ABZ kg\(^{-1}\) b.w. treatment groups when compared with the negative control. On the other hand, the percentage of induced MNPCE was reduced at various levels in all ABZ treated groups simultaneously treated with an oral administration of vit. C. There were no statistically significant differences in the MNPCE frequency of these groups. The results of the study indicated that ABZ is a potential genotoxic agent, and that no protective effects of vit. C were observed against the genotoxicity of ABZ.

Key words: mice, albendazole, vitamin C, micronucleus, genotoxicity.
oral lethal dose (LD\textsubscript{50}) was >3,000 mg/kg for mice (6). Vit. C showed in vivo and in vitro antimutagenic effects against the mutagenicity of chemotherapeutic agents, such as cyclophosphamide (CYP), ethyl methane sulphonate, cisplatin, and mitomycin C (10, 11, 16, 26, 39). Despite these effects; however, it has also been shown to enhance the cytogenetic damage caused by thiotepa or l-ethionine, and the cytotoxicity of mitomycin C in human lymphocytes in vitro (19, 21). Experimental in vivo and in vitro studies have yielded conflicting results, suggesting that vit. C can be either genotoxic or antigenotoxic as a repair stimulant, depending on the organisms and species studied.

Considering the lack of published data on the genotoxicity of ABZ relating to in vivo genotoxicity in particular, the present study was designed to evaluate the genotoxicity of short term ABZ exposure at high concentrations and to clarify whether this drug can cause chromosomal damages in the bone marrow polychromatic erythrocytes of treated mice. In the second part of the study, the role of vit. C in the form of L-ascorbic acid was investigated in order to determine if vit. C enhances or ameliorates the genotoxic effect induced by ABZ.

**Material and Methods**

**Chemicals.** Albendazole 98% (methyl 5-propylthio-2-benzimidazole-carbamate) and cyclophosphamide monohydrate 97% were obtained from Acros Organics (Belgium). Ascorbic acid and foetal calf serum were purchased from Amresco and Biological Industries (Israel), respectively. Giemsa and May-Grünwald stains were purchased from Merck (Germany).

**Animals.** CD-1 strain male mice, aged 6-8 weeks and weighing 20-25 g, obtained from Pendik Veterinary Control and Research Institute (Turkey), were used. The animals were housed in polypropylene cages and acclimatised for two weeks in the animal house of the department, maintained at 25 ±2°C and humidity 50% ±5% with a 12 h light/dark period. Feed and water were provided ad libitum. The experimental protocol was approved by the Istanbul University Veterinary Faculty Ethic Committee.

**Dose selection.** It was reported that the ABZ oral lethal dose (LD\textsubscript{50}) was >3,000 mg/kg for mice (6). Therefore, the highest dose was determined as 1,500 mg/kg b.w., 50% of the LD\textsubscript{50}. ABZ was dissolved in dimethylsulphoxide (DMSO). CYP known to cause micronucleus was used for the positive control group due to its known clastogenic and mutagenic activity (18, 34). According to many authors, who have studied the antimutagenic effects of ascorbic acid in vivo, the treatment protocols that gave the best results in terms of the reduction of chromosome damage were those in which vit. C was administered in simultaneous treatment with the clastogenic agent. Therefore, the dose of vit. C was selected on the basis of the literature data, which was previously reported to give the highest protective effects (2, 17). CYP and vit. C were dissolved in physiological saline and distilled water, respectively.

**Experimental design.** A total of 72 male mice were used in different control and treatment groups of the micronucleus test (eight mice in each group). The experimental treatment groups were (1) untreated control DMSO; (2) CYP 100 mg/kg b.w.; (3) ABZ 500 mg/kg b.w.; (4) ABZ 1,000 mg/kg b.w.; (5) ABZ 1,500 mg/kg b.w.; (6) ABZ 500 mg/kg b.w. + vit. C 200 mg/kg b.w.; (7) ABZ 1,000 mg/kg b.w. + vit. C 200 mg/kg b.w.; (8) ABZ 1,500 mg/kg b.w. + vit. C 200 mg/kg b.w.; (9) CYP 100 mg/kg b.w. + vit. C 200 mg/kg b.w. All chemicals were administered orally. In each case, the animals were killed 48 h after the treatment.

**Micronucleous test.** The genotoxic effects were evaluated in the mouse bone marrow by the micronucleus test, according to Schmid’s method and OECD guidelines (29, 35). All slides were coded and examined under an Olympus CAMEDIA C7070 light microscope at 1,000x magnification. MN criteria as described by Schmid was also used. In addition, to determine the cytotoxic effects of ABZ, the percentage of PCEs was calculated on the basis of the ratio of PCEs to normochromatic erythrocytes (NCEs) by counting 1,000 erythrocytes (35).

**Statistical analysis.** The statistical analysis was performed using the SPSS 11.5 statistical program. Statistical comparisons among several groups were made by a non-parametric analysis of variance (Kruskal-Wallis test) and between two groups using the Mann-Whitney U test. In both cases a difference was considered as statistically significant when P<0.05.

**Results**

No clinical signs of toxicity were seen in any of the ABZ and simultaneously vit. C treated groups. The results from the MN analysis are shown in Table 1. Animals treated with ABZ alone at the highest doses (1,000 and 1,500 mg/kg b.w.) induced statistically significant increase (P<0.001) in the incidence of MN PCE when compared with the negative control group DMSO, although there was no significant difference between these ABZ treated groups. Mice treated with the ABZ and vit. C combination simultaneously at a dose level of 200 mg/kg b.w. showed a slight decrease in the number of MN PCE without any statistical significance with respect to the ABZ treated groups.

The micronucleous assay revealed a significant increase (P<0.001) in the frequency of MN PCE cells following CYP administration as compared to the negative control. The data confirmed the sensitivity of the experimental protocol followed in the detection of genotoxic effects.
Table 1
Effect of ABZ and vit. C on the frequency of MNPCE in mouse bone marrow cells

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Dose (mg/kg)</th>
<th>MNPCE/2000PCE (mean ±SE)</th>
<th>PCE/NCE (%) (mean ±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (DMSO)</td>
<td>-</td>
<td>6.00 ±0.46</td>
<td>0.87 ±0.01</td>
</tr>
<tr>
<td>CYP</td>
<td>100</td>
<td>47.13 ±1.55</td>
<td>0.55 ±0.01</td>
</tr>
<tr>
<td>ABZ</td>
<td>500</td>
<td>7.00 ±0.46</td>
<td>0.83 ±0.01</td>
</tr>
<tr>
<td>ABZ</td>
<td>1,000</td>
<td>15.38 ±0.68</td>
<td>0.73 ±0.01</td>
</tr>
<tr>
<td>ABZ</td>
<td>1,500</td>
<td>17.38 ±0.89</td>
<td>0.71 ±0.01</td>
</tr>
<tr>
<td>ABZ + vit. C</td>
<td>500+200</td>
<td>6.75 ±0.53</td>
<td>0.79 ±0.02</td>
</tr>
<tr>
<td>ABZ + vit. C</td>
<td>1,000+200</td>
<td>13.5 ±0.85</td>
<td>0.77 ±0.01</td>
</tr>
<tr>
<td>ABZ + vit. C</td>
<td>1,500+200</td>
<td>15.13 ±1.52</td>
<td>0.73 ±0.01</td>
</tr>
<tr>
<td>CYP + vit. C</td>
<td>100+200</td>
<td>30.88 ±1.53</td>
<td>0.68 ±0.02</td>
</tr>
</tbody>
</table>

MNPCE - micronucleated polychromatic erythrocytes, NCE - normochromatic erythrocytes.
Results show means ±SE of between eight mice in each group.
a,b,c,d,e: in each column, groups with different letter superscripts indicate a significant difference in MNPCE number (P<0.05).

Furthermore, CYP plus vit. C showed a significant reduction (P<0.001) in MN frequency, and it was determined that the ascorbic acid showed an antimutagenic effect against CYP mutagenicity.

Regarding the cytotoxicity, the treatment with ABZ at 1,000 and 1,500 mg/kg b.w. doses induced a significant decrease (P<0.001) in the number of PCE/NCE when compared with the negative control group. The animals treated with ABZ (1,000 and 1,500 mg/kg b.w.) and simultaneously with vit. C showed a slight increase in PCE/NCE frequency, but without any statistical significance with respect to the ABZ treated groups. On the other hand, CYP plus vit. C showed a significant increase (P<0.001) in the PCE/NCE frequency when compared with the CYP treated group.

Discussion

The benzimidazoles are potent anti-parasitic agents, and their mode of action is thought to result from their inhibition of microtubule functions (25). The anthelmintic activity of benzimidazole 2-carbamates has been related to their selective antimitotic activity, due to the preferential binding of these agents to helminthic tubulin over mammalian tubulin (22). The ability of benzimidazoles to act as mitotic spindle poisons leads to the potential risk of aneuploidy induction in exposed cells. ABZ inhibits tubulin polymerisation, thus resulting in improper microtubule formation, the disturbance of the mitotic apparatus (spindle, kinetochores), and leads to MN formation, either by chromosome fragments or lagging chromosomes that are not incorporated into daughter nucleus at the time of cell division in the erythropoietic blast cells, and changes in the incidence of MNPCEs are considered to reflect chromosomal damage (1, 7, 14).

From the results of the presented study, it is evident that the ABZ treatment increased the frequency of MN where there was a positive correlation between the increased drug concentration and the induction of MN in the bone marrow cells of mice. While the lowest dose of ABZ (500 mg/kg b.w) was unable to induce MN significantly, the two highest concentrations (1,000 and 1,500 mg/kg b.w.) significantly increased the number of MN. Furthermore, the frequency of MNPCE induced at the highest dose of ABZ was approximately three times higher than control value. The numeric increase in MNPCE between the two highest doses of ABZ was probably due to the absorption of the drug. It was reported that ABZ is poorly absorbed and rapidly metabolised, and the deleterious effects of ABZ in exposed individuals may depend on the concentrations reached during the treatment period and on the tubulin binding capacity of the drug (22, 23). Therefore, in the period of 48 h, the highest dose (1,500 mg/kg b.w.) of ABZ probably can not be absorbed well and could not show a statistically significant higher MN frequency than the lower dose (1,000 mg/kg b.w.) in a dose-dependent manner. MN formation may be due to the fact that this drug is a benzimidazole derivative, which acts as an inhibitor for the tubulin polymerisation that is in charge of vital processes, including the motility and nutrient uptake in the parasite and chromosomal segregation at mitosis in exposed cells (20).

The effects of the drug ABZ reported in the study concurred with the results of previous reports about the aneugenic and mutagenic effects of ABZ (30-32). Ramirez et al. (31, 32) showed that ABZ induced MN and produced non-disjunction events in human lymphocytes treated in vitro, and suggested that the mechanism is capable of inducing aneuploidy and the final consequence of MN formation, based on the
containing MN in the bone marrows of ABZ treated CYP is an alkylating agent and it reacts with the electron vit. C decreased the MN fr equency induced by CYP. promutagen (16, 28).

bioactivation by blocking the oxidation processes, or by DNA and other cell targets; inhibiting the promutagen species, free radicals, or electrophiles, which damage by chemicals in different ways: by scavenging harmful species, free radicals, or electrophiles, which damage DNA and other cell targets; inhibiting the promutagen bioactivation by blocking the oxidation processes, or by reacting with the electrophilic metabolite(s) of a promutagen (16, 28).

In the present study it is worth mentioning that vit. C acted as an antimutagenic agent in bone marrow cells when administered with clastogenic agents (39). Vit.C decreased the frequency of micronucleated cells induced by the potent chemotherapeutic agents such as cisplatin, cyclophosphamide, mitomycin-C, and bleomycin in bone marrows of mice (11, 26). It was suggested that vit. C may reduce the mutation induction of bleomycin in bone marrows of mice (11, 26). It was suggested that vit. C may reduce the mutation induction of bleomycin in bone marrows of mice (11, 26).

It was reported that Mn formed during cell replication as a result of disruption of the mitotic spindle. Recently, Oztas et al. (30) have investigated the genotoxic effect of ABZ in pediatric patients with hepatic hydatid disease. The authors demonstrated that ABZ treatment increased the frequency of sister chromatid exchange and MN, and they concluded that ABZ might be genotoxic to human lymphocytes in vivo. Therefore, we suggested that the increase in the frequency of PCEs containing MN in the bone marrows of ABZ treated mice could occur due to the changes at the stage of cell proliferation and differentiation, and that ABZ may potentially be a genotoxic agent.

In conclusion, ABZ is a frequently used chemotherapeutic agent for the treatment of several helmintiases in veterinary and human medicine, and our observations have demonstrated that ABZ has a genotoxic effect on mice erythocytes, and that the administrated dose of vit.C has not shown a protective effect against this genotoxic activity under the present experimental conditions.

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References


