The Herbal Preparation Abana Protects against Radiation-induced Micronuclei in Mouse Bone Marrow

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ABSTRACT

The induction of micronuclei was studied in mouse bone marrow treated or not with 5, 10 and 20 mg/kg b. wt. of Abana (a herbal preparation) before exposure to 0-3 Gy of $\gamma$-radiation. Whole-body irradiation of mice resulted in a dose-dependent increase in the frequency of micronuclei. Treatment of mice with various doses of Abana before exposure to different doses of $\gamma$-rays resulted in a significant reduction of the micronucleus frequency at all exposure doses. The highest decline in the frequencies of micronuclei was observed after administration of 20 mg/kg Abana, where the frequency of micronuclei was approximately 4-fold less than that of the concurrent control. The PCE/NCE ratio was significantly higher in the drug-treated group compared to DDW + irradiated control and it was almost restored to normal level after administration of 20 mg/kg Abana. Our results demonstrate that Abana protects mice against radiation-induced micronucleus formation and radiation-induced decline in cell proliferation.

Key words: Micronucleus; Abana; Mouse; Bone marrow; Radiation protection; PCE/NCE ratio

1. INTRODUCTION

Since the pioneering work of Patt et al. [1] demonstrating that cysteine protected mice and rats against radiation-induced sickness and mortality, several compounds have been tested for their radio-protective capacity [2]. The major drawback of synthetic compounds has been that they are highly toxic at the optimum protective dose. Therefore, it is desirable to use other materials, which are less toxic and can offer high protection. Herbal drugs offer an alternative to the synthetic compounds which are either non-toxic or less toxic. Herbal drugs have recently drawn the attention of investigators. A Chinese herbal preparation from ginseng has been found to protect C3H mice against the lethal effects of $\gamma$-radiation [3]. Another herbal preparation, Liv.52, has also been reported to protect mice against radiation-induced micronucleus formation and chromosomal aberrations [4,5].

Abana, a herbal preparation, has been clinically used as a cardioprotective drug. It was found to reduce hypertension [6-8] and other cardiovascular diseases in man [9-12]. Abana has been reported to downregulate $\beta$-receptors of adrenaline [13]. It has also been reported to inhibit platelet aggregation [14]. Therefore, it was of interest to evaluate the radioprotective effect of Abana on radiation-induced micronucleus formation in mouse bone marrow exposed to different doses of $\gamma$-radiation.
2. MATERIALS AND METHODS
Six to 8 week-old male Swiss albino mice weighing 32 ± 2 g were selected from an inbred colony maintained under standard conditions.

2.1. Composition of Abana
Abana is a mixture of several plants, viz. *Terminalia arjuna*, *Withania somnifera*, *Tinospora cordifolia*, *Nepeta hindostana*, *Terminalia chebula*, *Phyllanthus emblica*, *Eclipta alba*, *Glycyrrhiza glabra*, *Asparagus racemosus*, *Boerhaavia diffusa*, *Convolvulus pluricaulis*, *Ocimum sanctum* and *Nardostachys jatamansi*.

2.2. Preparation of drug
The drug Abana was supplied by The Himalaya Drug Co., Bombay (India), in the powdered form as a free gift. The powder was extracted in 50% ethanol and the extract was dried and used. Alcoholic extract of Abana (20 mg) was dissolved in 10 ml of sterile distilled water immediately before administration and was diluted according to the required dose.

2.3. Selection of route of administration
To select the best route of administration, the animals were administered with 0 and 5 mg/kg b.wt. of Abana either orally or intraperitoneally before exposure to 2 Gy γ-radiation.

2.4. Selection of optimum dose
To select the optimum dose of Abana, the animals were administered intraperitoneally with 0, 1.25, 2.5, 5, 10 and 20 mg/kg b. wt. of Abana extract before exposure to 2 Gy γ-radiation.

2.5. Effect of various doses of Abana on radiation-induced micronuclei formation
2.5.1. Mode of administration
An amount of 0.01 ml/g b. wt. of distilled water or drug solution was administered intraperitoneally consecutively for 5 days.

2.5.2. Experimental protocol
The animals were divided into the following groups:
*DDW + irradiated group*. The animals of this group received 0.01 ml/g b. wt. of sterile double-distilled water (DDW).

*Abana + irradiated group*. The animals of this group were injected with 5, 10 and 20 mg/kg b. wt. of Abana extract.

2.5.3. Exposure dose
The animals of both groups were exposed to 0, 1, 2 and 3 Gy of ⁶⁰Co γ-radiation after 1 h of DDW or drug administration on the 5th day.
2.5.4. Irradiation
The prostate and immobilized animals (achieved by inserting cotton plugs in the restrainer) were whole-body exposed to different doses of $^{60}$Co $\gamma$-radiation (Gammatron, Siemens, Germany) in a specially designed well-ventilated acrylic box. A batch of 5 animals was irradiated each time at a dose rate of 1 Gy/min at a source-to-animal distance (mid point) of 44 cm.

Five animals per dose point from each group were killed 24 h after exposure to $\gamma$-radiation. The micronuclei were prepared according to the method of Schmid [15] with certain modifications described by Jagetia and Jacob [16]. Briefly, the femurs of each animal were dissected out and the bone marrow was flushed out into Dulbecco’s modified Eagle’s medium (DMEM) separately. The suspension was centrifuged. A few drops of fetal calf serum (FCS) were added and the pellet was mixed thoroughly. Smears were drawn onto precleaned coded slides using a drop of the resultant suspension in FCS. The slides were air dried and fixed in absolute methanol. The results were confirmed by repetition of the experiment.

The slides were stained with 0.125% acridine orange (BDH, England, Gurr Cat. No. 34001 9704640E) in Sorensen’s buffer (pH 6.8). The slides were washed twice in Sorensen’s buffer. The slides mounted in Sorensen’s buffer were observed under a fluorescent microscope (Carl Zeiss Photomicroscope III, Germany) using a 40 x Neofluar objective. A minimum of 2000 polychromatonic erythrocytes (PCE) and 2000 normochromatic erythrocytes (NCE) were counted for the presence of micronuclei for each animal. A total of not less than 10000 PCE or NCE was counted for each drug dose. Data regarding the polychromatic and normochromatic erythrocyte ratio (PCE/NCE ratio) were also collected, where a minimum of 4000 erythrocytes per animal were scored.

The statistical significance of the differences observed between DDW + irradiated control and Abana + irradiated groups was calculated using the Mann-Whitney $U$-test. The data were fitted to the linear quadratic ($Y = C + \alpha D + \beta D^2$) equation to describe the dose response, if any, where $C$ is the control micronucleus frequency, $D$ is the radiation dose and $\alpha$ and $\beta$ are the constants.

3. RESULTS
The results are expressed as micronuclei (MPCE and MNCE) per 1000 ± SEM and PCE/NCE ratio ± SEM in Table 1 and Fig. 1.

3.1. Route of administration
The micronuclei frequencies in orally and intraperitoneally (i.p.) administered mice did not differ significantly from each other (29.60 ± 0.11) for i.p. and 28.15 ± 0.24 for oral route, respectively), that is i.p. and oral routes were equally effective; however, further studies were carried out using i.p. administration.
Table 1: Effect of Abana on the formation of radiation-induced micronuclei in mouse bone marrow exposed to different doses of γ-radiation

<table>
<thead>
<tr>
<th>Exposure dose (Gy)</th>
<th>Treatment</th>
<th>MPCE per 1000 ± SEM</th>
<th>MNCE per 1000 ± SEM</th>
<th>P/N ratio ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>One</td>
<td>Two</td>
<td>Three</td>
</tr>
<tr>
<td>0</td>
<td>DDW + irradiation</td>
<td>2.41</td>
<td>± 0.12</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>5 mg Abana + irradiation</td>
<td>2.67</td>
<td>± 0.06</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>10 mg Abana + irradiation</td>
<td>1.32</td>
<td>± 0.09</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>20 mg Abana + irradiation</td>
<td>0.65</td>
<td>± 0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>1</td>
<td>DDW + irradiation</td>
<td>17.53</td>
<td>± 0.10</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>5 mg Abana + irradiation</td>
<td>9.24</td>
<td>± 0.13</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>10 mg Abana + irradiation</td>
<td>8.87</td>
<td>± 0.04</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>20 mg Abana + irradiation</td>
<td>4.55</td>
<td>± 0.11</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>DDW + irradiation</td>
<td>44.91</td>
<td>± 0.31</td>
<td>4.65</td>
</tr>
<tr>
<td></td>
<td>5 mg Abana + irradiation</td>
<td>28.53</td>
<td>± 0.11</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>10 mg Abana + irradiation</td>
<td>18.20</td>
<td>± 0.58</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>20 mg Abana + irradiation</td>
<td>9.81</td>
<td>± 0.11</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>DDW + irradiation</td>
<td>51.10</td>
<td>± 0.30</td>
<td>8.21</td>
</tr>
<tr>
<td></td>
<td>5 mg Abana + irradiation</td>
<td>31.11</td>
<td>± 0.23</td>
<td>4.56</td>
</tr>
<tr>
<td></td>
<td>10 mg Abana + irradiation</td>
<td>28.65</td>
<td>± 0.08</td>
<td>2.66</td>
</tr>
<tr>
<td></td>
<td>20 mg Abana + irradiation</td>
<td>19.34</td>
<td>± 0.15</td>
<td>0.51</td>
</tr>
</tbody>
</table>

*p<0.004; *p<0.01 and NS. DDW + irradiated group compared with DDW + sham-irradiated group (0 Gy). Abana + irradiated group compared with DDW + irradiated group.

3.1.1. Selection of optimum dose

To select the optimum dose, 5 mice per dose point were administered with 0, 1.25, 2.5, 5, 10 and 20 mg /kg b. wt. of Abana before exposure to 2 Gy γ-radiation. The increasing dose of Abana resulted in a dose-dependent decline in the frequencies of micronuclei and the maximum decline was observed for 20 mg/kg Abana + irradiated group. The micronuclei frequencies (MPCE) were 5.5-fold lower in the 20 mg Abana + irradiated group than that of concurrent DDW + irradiated control (Table 2).
3.2. Effect of various doses of Abana on radiation-induced micronuclei formation

The frequency of MPCE increased in a dose-dependent manner (Fig. 1a) after exposure to 0-3 Gy of \( \gamma \)-radiation and this increase in the MPCE frequency was significantly higher than the DDW + sham-irradiated controls (0 Gy). The irradiation resulted in the induction of PCE bearing two and three micronuclei. The frequency of PCE bearing two micronuclei also increased in a dose-dependent manner (Table 1). The dose-response relationship was linear quadratic (Table 3). Similarly, the frequency of MNCE also increased with the increasing dose of radiation (Fig. 1b) and the dose-effect relationship was linear quadratic (Table 3).

The treatment of mice with 5, 10 and 20 mg/kg b.wt. of Abana reduced the frequencies of micronuclei (MPCE) significantly (Table 1). The total MPCE were 2, 1.8 and 1.7 fold less in the 5 mg Abana + irradiated group after exposure to 1, 2 and 3 Gy of radiation, respectively, than in the respective irradiated controls. An increase in the Abana dose to 10 mg resulted in a further decline in the frequencies of micronuclei at all the exposure doses which was approximately 2-fold lower in the 10 mg Abana + irradiated group, when compared to the concurrent DDW + irradiated controls (Table 1). With the further increase in the drug dose to 20 mg/kg, there was an approximate 4-fold depletion in the frequency of micronuclei in the Abana + irradiated group, when compared to the DDW + irradiated group. The MPCE frequency of 1 Gy DDW + irradiated group and 3 Gy Abana (20 mg) + irradiated group was almost similar (Table 1). Similarly, the frequencies of PCE bearing two micronuclei were drastically reduced in the Abana + irradiated group compared to the DDW + irradiated group and after administration of 20 mg Abana, the PCE bearing two micronuclei were altogether absent for 1 and 2 Gy (Table 1). The administration of 10 and 20 mg/kg Abana even reduced the spontaneous frequencies of micronuclei significantly and this reduction was higher in the latter dose than the former (Table 1). The dose-response relationship was linear quadratic (Table 3).
Table 2: Effect of various doses of Abana on the formation of radiation-induced micronuclei in mouse bone marrow exposed to 2 Gy of γ-radiation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Frequency of MPCE per 1000 ± SEM</th>
<th>MNCE per 1000 ± SEM</th>
<th>P/N ratio ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One</td>
<td>Two</td>
<td>Total</td>
</tr>
<tr>
<td>DDW + irradiation</td>
<td>44.91 ± 0.31^a</td>
<td>4.65 ± 0.33^a</td>
<td>54.22 ± 0.39^a</td>
</tr>
<tr>
<td>1.25 mg Abana + irradiation</td>
<td>34.71 ± 0.19^a</td>
<td>1.07 ± 0.11^a</td>
<td>35.98 ± 0.13^a</td>
</tr>
<tr>
<td>2.5 mg Abana + irradiation</td>
<td>31.04 ± 0.28^a</td>
<td>0.71 ± 0.11^a</td>
<td>31.75 ± 0.33^a</td>
</tr>
<tr>
<td>5.0 mg Abana + irradiation</td>
<td>28.53 ± 0.11^c</td>
<td>0.53 ± 0.10^c</td>
<td>29.60 ± 0.11^c</td>
</tr>
<tr>
<td>10.0 mg Abana + irradiation</td>
<td>18.20 ± 0.58^a</td>
<td>0.28 ± 0.03^a</td>
<td>18.91 ± 0.57^a</td>
</tr>
<tr>
<td>20.0 mg Abana + irradiation</td>
<td>9.81 ± 0.11^c</td>
<td>0.00 ± 0.00</td>
<td>9.81 ± 0.11^c</td>
</tr>
</tbody>
</table>

The frequency of MNCE was significantly lower in the Abana + irradiated group, when compared to the DDW + irradiated group at all the doses of Abana by a factor of 1.2, 2 and 4 for 5, 10 and 20 mg/kg Abana, respectively (Table 1). The dose response was linear quadratic (Table 3).

The treatment of mice with Abana arrested the radiation-induced decline in the PCE/NCE ratio (Fig. 1c) and this increase in the PCE/NCE ratio in Abana + irradiated group was significantly higher than that of DDW + irradiated controls (Table 1). In the case of 20 mg/kg Abana-treated animals, the PCE/NCE ratio was almost restored to normal level for all radiation doses (Table 1). The dose response for both DDW + irradiated and Abana + irradiated groups was linear quadratic (Table 3).

4. DISCUSSION
Abana, a non-toxic herbal preparation, commonly used to treat cardiac disorders and stress-related diseases has been evaluated for its radioprotective activity in mouse bone marrow by micronucleus analysis. Since the radiation also causes stress, we envisioned that Abana may also protect against the radiation-induced damage.

The administration of Abana at a dose of 10 or 20 mg/kg even reduced the spontaneous frequency of micronuclei. Treatment of mice with various doses of Abana before irradiation resulted in a significant decline in the frequency of MPCE and MNCE. The reduction in the

\[\text{Table 3: Co-efficient of correlation after various treatments}\]

<table>
<thead>
<tr>
<th>Treatments</th>
<th>α-values</th>
<th>β-values</th>
<th>γ-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPCE</td>
<td>MNCE</td>
<td>P/N ratio</td>
</tr>
<tr>
<td>DDW + irradiation</td>
<td>23.84 ± 13.44</td>
<td>2.24 ± 0.64</td>
<td>-0.13 ± 0.08</td>
</tr>
<tr>
<td>5 mg Abana + irradiation</td>
<td>10.25 ± 8.23</td>
<td>1.48 ± 0.93</td>
<td>-0.14 ± 0.07</td>
</tr>
<tr>
<td>10 mg Abana + irradiation</td>
<td>6.36 ± 0.05</td>
<td>1.10 ± 0.12</td>
<td>-0.13 ± 0.02</td>
</tr>
<tr>
<td>20 mg Abana + irradiation</td>
<td>1.51 ± 1.40</td>
<td>0.43 ± 0.10</td>
<td>-0.13 ± 0.04</td>
</tr>
</tbody>
</table>

\[\text{Table 3: Coefficient of correlation after various treatments}\]

The frequency of MNCE was significantly lower in the Abana + irradiated group, when compared to the DDW + irradiated group at all the doses of Abana by a factor of 1.2, 2 and 4 for 5, 10 and 20 mg/kg Abana, respectively (Table 1). The dose response was linear quadratic (Table 3).

The treatment of mice with Abana arrested the radiation-induced decline in the PCE/NCE ratio (Fig. 1c) and this increase in the PCE/NCE ratio in Abana + irradiated group was significantly higher than that of DDW + irradiated controls (Table 1). In the case of 20 mg/kg Abana-treated animals, the PCE/NCE ratio was almost restored to normal level for all radiation doses (Table 1). The dose response for both DDW + irradiated and Abana + irradiated groups was linear quadratic (Table 3).

4. DISCUSSION
Abana, a non-toxic herbal preparation, commonly used to treat cardiac disorders and stress-related diseases has been evaluated for its radioprotective activity in mouse bone marrow by micronucleus analysis. Since the radiation also causes stress, we envisioned that Abana may also protect against the radiation-induced damage.

The administration of Abana at a dose of 10 or 20 mg/kg even reduced the spontaneous frequency of micronuclei. Treatment of mice with various doses of Abana before irradiation resulted in a significant decline in the frequency of MPCE and MNCE. The reduction in the
micronuclei frequencies was dose-dependent in the Abana + irradiated group, when compared to the concurrent DDW + irradiated controls. The highest decline was observed after the administration of 20 mg/kg Abana, where the micronuclei frequencies were approximately 4-fold lower than that of DDW + irradiated control group. Similarly, the frequency of MPCE bearing two and three micronuclei were significantly less in the Abana = Irradiated group and they were altogether absent after administration of 0 and 20 mg/kg Abana. Except for 3 Gy where the frequencies of PCE bearing two micronuclei were 9-fold lower (20 mg), than that of concurrent DDW + irradiated control. Jagetia and Ganapathi [4.5] have reported a significant decline in the frequency of micronuclei and chromosomal aberrations in mice treated with another herbal preparation, Liv.52. A Chinese herbal preparation from Panax ginseng an Spirulina platensis has also been reported to protect mice against radiation-induced decline in survival [3] and micronucleus-induction [17].

Treatment of mice with Abana inhibited the radiation-induced decline in cell proliferation as is evidenced by the arrest in the decline in PCE/NCE ratio by Abana. This inhibition in the radiation-induced decline in PCE/NCE ratio was dose-dependent. The administration of 20 mg/kg Abana before irradiation inhibited the radiation-induced decline in the PCE/NCE ratio and almost normal levels were restored for all exposure doses. Earlier findings from our laboratory have reported that Liv.52 treatment protected mice bone marrow against the radiation-induced decline in PCE/NCE ratio [18].

The exact mechanism of action of Abana is not known; however, it may scavenge free radicals produced by radiation and thus inhibit radiation-induced damage to the cellular DNA. It may also reduce lipid peroxidation resulting in the reduction of radiation-induced damage. Irradiation inhibits cell proliferation, therefore the PCE/NCE ratio declines and pretreatment of mouse with Abana protects against radiation-induced inhibition of cell proliferation as well as micronucleus induction.

One important observation emerging from this investigation is that Abana pretreatment resulted in a significant decline in the spontaneous frequencies of micronuclei (10 and 20 mg) affording protection against the natural wear and tear of the genome and the radioprotective effect increased with the increasing dose of Abana. The treatment of mice with Abana suppressed the radiation-induced micronucleus formation significantly in a dose-dependent manner and deserves attention as a radioprotector.

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