Prevention of radiation-induced chromosome damage in mouse bone marrow by aqueous leaf extract of *Chicorium intybus*

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**Abstract**

The radioprotective effect of aqueous leaf extract of *Chicorium intybus* (*Chicory*) against radiation induced chromosomal damage was investigated. Adult Swiss albino mice were exposed to 4 Gy ⁶⁰Co γ-rays. Aqueous extract (50 and 100 mg/kg body wt) was administered orally just after irradiation. Bone marrow protection was evaluated by scoring the different types of individual aberrations and aberrant metaphases, 24hrs after irradiation. Significant reduction in number of aberrant cells and different type of aberrations was observed in treated group compared to irradiated untreated group of animals. The administration of aqueous leaf extract of *Chicory* to the animals showed significant reduction in micronucleus induction. The extract also possessed significant hydroxyl radical scavenging activity. The effectiveness of the drug to prevent chromosomal damage consequent to irradiation along with its high solubility in water, favor its application, in accidental radiation exposures and nuclear accidents.

**Key Words**: Chromosomal aberration, micronucleus, *C. intybus*, aqueous extract.

**Chicorium intybus** sulu yaprak ekstreleri ile fare kemik ilgiinde radyasyon nedenli kromozomal hasarın önlenmesi

**Özet**


**Anahtar Sözcüklər**: Kromozomal anormallik, mikronükleus, *C. intybus*, sulu ekstre
Introduction

Ionizing radiation inflicts deleterious effects to living cells by damaging the vital cellular target, the DNA. The lesions produced in DNA by radiation include intra or inter strand cross-linking and single and double strand breaks. The cellular responses include arrest in cell cycle, progression at cell cycle checkpoints and the induction of DNA repair. Protecting living system from onslaughts of ionizing radiation is of paramount importance in radiation biology. This has particular relevance in nuclear warfare, nuclear accidents and nuclear terrorism. Radiation protection is also important in the radiotherapy of cancer where normal cells have to be protected while cancers are exposed to radiation (Gandhi and Nair, 2004). A large number of compounds natural and synthetic have been evaluated for this purpose (Nair et al., 2001). Hence search for an ideal radioprotector without side effects and toxicity is a compelling urgency.

Chicory, belonging to the Asteraceae family, has high medicinal value and economic importance. It is cultivated for substitution of coffee and for extraction of fructose and inulin syrup. The leaves of Chicory are edible and it is used to make salads. Chicory is known as the friend of liver. The root and leaves contain inulin and levulose that promote liver functioning. It also has eupeptic and appetizing property (Roger, 2001). The plant is used in liver congestion; portal hypertension.

The aqueous extract of the leaves of wild type Chicory was reported to have cardio protective effects (Nayeemunnisa and Kumuda Rani, 2003). The present study was undertaken to check the in vivo radioprotective property of the aqueous extract of Chicory leaves, which remain unexplored.

Materials and methods

Preparation of the extract

The leaves were collected locally, shade dried and powdered. The powder was dissolved in double distilled water and extracted at a temperature of 80°C for 8-10 hrs and was concentrated. The yield was 5%. The concentrated extract was dissolved in double distilled water for the experiments. The preliminary phytochemical analysis of the extract was carried out by thin-layer chromatography (TLC) on silica gel G using n-butanol:acetic acid:water (4:1:5 or 12:3:5) with chloroform:methanol as solvent systems (Wagner, 1984). The spots were examined under UV. The presence of carbohydrates was detected by anthrone (Yemm and Wills, 1954) and phenol sulphuric acid test (Dubois, 1956).

Animals

Male Swiss mice, 8-10 weeks old and weighing 20-25 g, were selected from an inbred group maintained under standard conditions of temperature (25±2°C) and humidity. All the experiments were conducted strictly according to the ethical guidelines decided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division of the Government of India.

Irradiation

Junior Theratron unit (AECL, Ottawa, Canada) with a dose rate of approximately 1Gy/min at 38 cm was used for irradiation. Whole body irradiation was given to unanesthetized mice, which were kept in well-ventilated Perspex boxes.

Experiment

The animals were divided into 5 groups and treated as follows:

i) Double distilled water orally + Sham irradiation (after 1 hr)
ii) Sham irradiation + 100 mg aqueous extract /kg body wt orally (after 5 mins)
iii) Double distilled water + 4 Gy Radiation
iv) Radiation 4 Gy + 50 mg aqueous extract /kg body wt orally
v) Radiation 4 Gy + 100 mg aqueous extract /kg body wt orally

Metaphase preparation

At 22 hr after irradiation all the animals were injected i.p. with 0.025 colchicine and sacrificed 2h later by cervical dislocation. Both femurs were dissected out and metaphase plates were prepared by air-drying method (Uma Devi, 1998). Briefly, bone marrow from the femur was aspirated, washed in saline, treated
hypotonically (0.565% KCl), fixed in 3:1:methanol:acetic acid, spread on clean slides and stained with 4% Giemsa. Chromosomal aberrations were scored under light microscope. A total of 500 metaphase were scored per animal. Different types of aberrations like chromatid breaks, chromosome breaks, fragments, rings and dicentrics as well as cells showing polyploidy and severe damage (SDC, cells with 10 or more aberrations of any type) were scored. When breaks involved both the chromatids it was termed as “chromosome type” aberration, while “chromatid type” aberration involved only one chro-

Table 1. Effect of Chicory leaf extract on the induction of individual aberrations and number of aberrations per cell in mouse bone marrow by whole body γ-irradiation (4Gy).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fragments</th>
<th>Chromosome break</th>
<th>Chromatid break</th>
<th>Rings</th>
<th>Dicentrics</th>
<th>Number of aberrations per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5.0±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.01±0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract alone</td>
<td>4.1±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.008±0.0006&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Radiation alone 4Gy</td>
<td>201±11.1</td>
<td>15.3±3.1</td>
<td>5.2±0.6</td>
<td>15.3±0.4</td>
<td>6.3±0.7</td>
<td>0.46±0.031</td>
</tr>
<tr>
<td>Radiation 4Gy + Extract (50mg/kg body wt)</td>
<td>76.3±6.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.5±1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.2±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Radiation 4Gy + Extract (100mg/kg body wt)</td>
<td>56.4±4.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.3±0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.1±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> p<0.01 compared Radiation alone.
<sup>b</sup> p<0.0001 compared Radiation with 50 mg/kg body wt alone.

Table 2. Effect of Chicory leaf extract on the induction of polyploidy, SDC and pulverization in mouse bone marrow by whole body γ-irradiation (4Gy).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Polyploidy</th>
<th>Pulverised cells</th>
<th>Severe Damaged cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Extract alone</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Radiation alone 4Gy</td>
<td>5.2±0.40</td>
<td>4.31±0.6</td>
<td>14.23±1.7</td>
</tr>
<tr>
<td>Radiation 4Gy + Extract (50mg/kg body wt)</td>
<td>4.1±0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.2±1.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Radiation 4Gy + Extract (100mg/kg body wt)</td>
<td>2.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>4.2±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> p<0.01 compared Radiation alone.
<sup>b</sup> p<0.0001 compared Radiation with 50 mg/kg body wt alone.
matid. If the deleted portion had no apparent relation to a specific chromosome, it was called a fragment (Bender et al., 1998). Data are presented as mean ± (S.E).

**Table 3.** Effect of Chicory leaf extract on micronucleus induction in mouse bone marrow by whole body γ-irradiation (4Gy).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>MPCE/1000</th>
<th>MNCE/1000</th>
<th>P/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>0</td>
<td>1.31±0.07</td>
</tr>
<tr>
<td>Extract alone</td>
<td>0</td>
<td>0</td>
<td>1.0±0.01</td>
</tr>
<tr>
<td>Radiation alone 4Gy</td>
<td>129.6±2.5</td>
<td>24.2±0.6</td>
<td>0.54±0.01</td>
</tr>
<tr>
<td>Radiation 4Gy + Extract (50mg/kg body wt)</td>
<td>74.2±2.1</td>
<td>12.1±1.2</td>
<td>0.69±0.02</td>
</tr>
<tr>
<td>Radiation 4Gy + Extract (100mg/kg body wt)</td>
<td>54.3±1.8</td>
<td>9.1±0.6</td>
<td>0.84±0.01</td>
</tr>
</tbody>
</table>

* p<0.01 compared radiation alone.

**Figure 1:** Hydroxyl radical scavenging activity of Chicory leaf extract at different concentrations.

**Micronucleus assay**

The method of Schmid (1975) was used with some modifications. Bone marrow from the femur was flushed out, vortexed and centrifuged. The pellet was resuspended in a few drops of fetal calf serum. Smears
were made on pre-cleaned, pre-coded, dry slides, air dried and fixed in absolute methanol. The slides were stained with May–Gruenwald stain and observed under light microscope for micronuclei in polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs). A total of 2000 erythrocytes were scored in each animal. The number of PCE and NCE and the frequency of micronucleated NCE and micronucleated PCE were recorded. The data were expressed as the number of MPCE or MNCE per 1000 PCE or NCE respectively. The ratio of PCE to NCE (P/N) ratio was also calculated.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured as described by Elizabeth and Rao (1990). Hydroxyl radical was generated by Fe$^{2+}$ ascorbate–EDTA–H$_2$O$_2$ in TBARS formed was estimated by thiobarbituric acid method of Ohkawa et al. (1979). The hydroxyl radical scavenging activity was determined by comparing absorbance of control with that of treatments (Ajith and Janardhanan, 2002).

Statistical analysis

Data were analysed by students t-test. A value of p < 0.05 was considered to be significant.

Results

Preliminary screening

Preliminary phytochemical analysis of the extract showed the presence of water-soluble terpenes and proteins which produced fluorescence in the presence of UV light. The extract also answered to anthrone and phenol sulphuric acid tests, by giving typical colour reactions indicating the presence of carbohydrates.

Sham treated control showed less than 1% aberrant cells, treatment of extract alone did not induce any significant changes compared to control. Radiation produced a significant increase in the percent aberrant cells. A corresponding increase was found in all the individual aberrations. Treatment with aqueous extract of Chicory leaves after irradiation resulted in very significant decrease in the percent aberrant cells and number of aberrations per cell compared to the group where distilled water was given with radiation. There was a decrease in all types of aberrations, as well as polyploidy and cells with pulverisation (Table 1 and 2).

Whole body radiation resulted in significant increase in MPCE (129.6/1000) and MNCE (24.2/1000) and a significant reduction in the P/N ratio (0.54). Treatment with Chicory extract reduced micronucleus induction from 129 to 74.2 in MPCE and from 24.2 to 12.1 in MNCE at the concentration of 50 mg/kg body wt and from 129 to 54.3 in MPCE and from 24.2 to 9.1 in MNCE at 100mg/kg body wt. The P/N ratio increased from 0.54 ± 0.01 in the irradiated group to 0.69 (50 mg/kg body wt) and 0.84 (100 mg/kg body wt) by administration of the extract (Table 3).

Aqueous extract of Chicory leaves showed significant scavenging activity of hydroxyl radical generated from Fe$^{2+}$ ascorbate–EDTA–H$_2$O$_2$ system. The IC$_{50}$ value of for hydroxyl radical scavenging was 5.3 ± 0.2330 mg/ml (Figure 1). The hydroxyl radical scavenging activity of Chicory extract was found to increase in a concentration dependent manner.

Discussion

The results of our present study demonstrate the radioprotective effect of Chicory leaf extract on radiation induced chromosomal aberrations, whilst extract itself does not have any marked effect on the bone marrow chromosomes. The successful functioning of a cell and the faithful transmission of the genetic information contained in to its progeny depend on the maintenance of the structural integrity of each molecule of DNA. The development of radiation protectors is important not only to enhance the effectiveness of cancer treatment, but also for the study of the underlying mechanisms of radiation cytotoxicity (Hahn et al., 1994). Of all the compounds studied as potential radioprotective agents, aminothiols and their derivatives are still considered among the best. Unfortunately, the available radioprotective substances possess unacceptable toxicity limiting its clinical usefulness. Therefore it is necessary to develop protectors with minimum toxicity while maintaining efficacy (Weiss et al., 1990).

The extract does not have genotoxicity, as it does not increase the induction of micronucleus in control. It minimized radiation induced genomic instability. The P/N ratio is an indicator of the rate of proliferation and a decrease in the ratio at 24hrs post irradiation
is an expression of the known early effects of radiation on the cell cycle, indicating a suppression of erythropoiesis.

The most effective in vivo radioprotectors like plant flavanoids and thiol compounds studied so far are effective when administered before irradiation, as they must be present in the system at the time of irradiation (Hahn et al., 1994). Hence they can be used only when the eventuality of the exposure is known and are not suitable against unplanned exposures, e.g. accidents, spillage, warfare and terrorist attack. In this context, the present finding that Chicory extract offered protection when administered post-irradiation, is of significant importance. Also oral administration of the drug is the most convenient and popular route. In the treatment of humans with conventional radiotherapy, it is more convenient to use a radioprotector, which can be given orally (Uma Devi et al., 1999). Presently amifostine is the only FDA approved radioprotector used clinically. The side effects associated with amifostine like hypotension, nausea, vomiting etc. limits its clinical usefulness (Tannehill and Mehta, 1996).

Earlier studies reveal that administration of wild type aqueous leaf extract of Chicory in mice gave cardiac protection where it rendered the catalase enzyme of the heart biologically more efficient to suppress peroxidative damage (Nayeemunnisa and Kumuda Rani, 2003). The components of the aqueous extract of Chicory leaves are proteins, carbohydrates, polyphenols, and water-soluble terpenes. Free radical scavenging may be a likely mechanism of action as the extract was found to possess significant hydroxyl radical scavenging activity. The experimental results show that Chicory leaf extract afforded in vivo protection against radiation induced cytogenetic damage.

An ideal radioprotector should be free from side effects, should be long acting, less expensive and capable of long term storage without change in action and constitution. However, further investigations are necessary to identify the active component(s) responsible for protection and to study their mechanism of action. As Chicory leaf is used as a leafy vegetable and also used for its therapeutic significance which is freely available in our country, it is worthwhile to conduct detailed studies in order to explore the full potential of this plant in human radiation protection.

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References


Tannehill SP and Mehta MP. Amifostine and radiation lethargy. Past, present and future. Semin Oncol. 23 (Sup.8): 69-77, 1996.


