Genoprotective potential of *Brassica juncea* (L.) Czern. against mercury-induced genotoxicity in *Allium cepa* L.

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**Abstract:** *Brassica juncea* (L.) Czern. is an important species of the family Brassicaceae with immense medicinal value. The main goal of this study was to investigate the antigenotoxic effects of different concentrations (0.1%-1.0%) of chloroform extract of *B. juncea* seeds on mercury-induced (0.75 ppm) genotoxic effects in root cells of *Allium cepa*. Three different modes of treatment were performed: pretreatment, posttreatment, and simultaneous treatment. For pretreatment, freshly emerged root tips were first treated with different concentrations of seed extract for 3 h and then treated with Hg for 3 h. For posttreatment, the roots were first treated with Hg and then with extract concentrations. For simultaneous treatment, different concentrations of extract were added along with the Hg. The study revealed that chloroform extract was neither toxic nor in possession of genotoxic activity. Treatment with Hg caused a decrease in mitotic index and induction of chromosomal aberrations as compared to the negative control. This effect was reversed by treatment with different concentrations of chloroform extract of seeds of *B. juncea*. Maximum percentage inhibition (95.3%) of genotoxic effects was observed with 1% chloroform extract (post- and simultaneous treatment). This study clearly indicates that the chloroform extract of *B. juncea* seeds has antigenotoxic potential against mercury-induced genotoxicity in a dose-dependent manner by evaluating *A. cepa* root chromosomal aberration assay.

**Key words:** Antigenotoxicity, chloroform extract, *Allium cepa* root chromosomal aberration assay

**Introduction**

Heavy metal contamination of the biosphere has increased for the past few decades (1-3). The majority of metals have the potential to cause mutations and can be referred to as environmental mutagens. Among heavy metals, mercury is a major environmental genotoxin required in various items like barometers, thermometers, pesticides, preservatives, paints, dental fillings, etc. and is also consumed in agricultural industries for seed dressing, etc. (4,5). Hg is also known to induce various clastogenic and mutagenic effects in higher plants. The genotoxic effects of Hg depend on its oxidation state, its concentration, and the duration of exposure. The most noticeable and consistent effect of mercurials is the induction of c-mitosis through disturbance of the spindle activity, resulting in the formation of polyploid and aneuploid cells and c-tumors (5-7). Exposure to inorganic salts of mercury in *A. cepa* reduced the mitotic index in the root tip cells and increased the frequency of chromosomal aberrations, which was directly proportional to the concentrations used and to the duration of exposure (8).

Plants have been utilized as medicines for thousands of years. According to World Health Organization estimates, up to 80% of the world’s population depends on traditional medicinal systems for some aspects of primary health care. The use of
antimutagens/anticarcinogens in everyday life is the most effective procedure for preventing various genetic diseases. Antimutagens/antigenotoxins are the chemical agents that interfere with mutagen-induced DNA damage to protect the cells/organisms from the harmful consequences of exposure to mutagens (9).

A number of bioassays are being used to study the genotoxicity of different environmental chemicals/mixtures (10-16). The same assays have also been used to determine the antigenotoxicity of different chemicals/plant extracts. Among different assays available, the *Allium cepa* root chromosomal aberration assay is widely used to determine genotoxic and antigenotoxic effects of different plant extracts (17-23).

*B. juncea* (Indian mustard), a member of the family Brassicaceae, is a multipurpose plant with immense medicinal value. Seeds of *B. juncea* are used in the treatment of tumors and act as antibiotic agents. *Brassica* vegetables appear to be protective against cancer and heart diseases (24-26). Glucosinolates and other sulfur-containing metabolites present in *Brassica* act as anticancer agents due to their ability to induce detoxification enzymes in mammalian cells and to reduce the rate of tumor development (27,28). Currently, there are no published data on the antigenotoxic effects of Indian mustard seed extract. Therefore, the present investigation was planned to evaluate the antigenotoxic effects of the chloroform extract of seeds of Indian mustard against mercury-induced genotoxicity in *A. cepa* root chromosomal aberration assay.

**Materials and methods**

*Brassica juncea* (L.) Czern.

Certified and disease-free seeds of *Brassica juncea* (Indian mustard) were procured from the Department of Plant Breeding, Punjab Agriculture University, Ludhiana, India.

**Chemicals**

Mercury was purchased from Qualigens Fine Chemicals, Mumbai, India. HCl, orcein, glacial acetic acid, and other chemicals were bought from Thomas Baker (Chemicals) Pvt. Limited, Mumbai, India; Loba Chemic Pvt. Ltd, Mumbai, India; and S D Fine-Chem Limited, Mumbai, India.

**Preparation of the chloroform extract of seed**

Seeds of Indian mustard were washed with water, air-dried, and thoroughly ground to a fine powder. The seed powder (250 g) was first extracted with hexane (500 mL) using a Soxhlet extractor for approximately 110-115 h to remove oil. The seed meal was air-dried overnight and extracted with 500 mL of chloroform by placing the mixture on a shaker for about 2 nights. Chloroform was distilled off to concentrate the chloroform extract. Chloroform extract (2 g) was first solubilized in DMSO (1 mL), and then distilled water was added to make a total volume of 200 mL (stock).

**Toxicity assay**

Healthy onion bulbs were allowed to root in tap water. After 2-3 days, the onion bulbs with freshly emerged roots were placed on coupling jars filled with different concentrations of *B. juncea* seed extract. Among different concentrations (0.1, 0.25, 0.5, 0.75, 1, and 2 ppm) of mercury, we found a concentration of 0.75 ppm to be the EC$_{50}$ value, and so this concentration was used as a negative control in the study. The experiment was done in triplicate. On the fourth day of treatment, onion bulbs with roots were thoroughly washed, and the root length and root number of each onion bulb were measured and compared with the controls.

**Allium cepa root chromosomal aberration assay**

Healthy onion bulbs were allowed to root in tap water contained in coupling jars kept in the dark. Freshly emerged roots (0.5-1.0 cm) were treated with different concentrations of chloroform extract of seeds of *B. juncea* and Hg (0.75 ppm) for 3 h. Three kinds of treatments, pretreatment, posttreatment, and simultaneous treatment, were applied. For pretreatment, the roots were first treated with different concentrations (1.0%, 0.75%, 0.5%, 0.25%, and 0.1%) of seed extract for 3 h followed by Hg treatment (0.75 ppm). For posttreatment, onion bulbs were first treated with Hg (0.75 ppm) and then with different concentrations of the extract for 3 h. For simultaneous treatment, the freshly emerged root tips were exposed to 0.75 ppm Hg and different concentrations of the extract simultaneously.
Genoprotective potential of *Brassica juncea* (L.) Czern. against mercury-induced genotoxicity in *Allium cepa* L.

Table 1. Effect of different concentrations of chloroform extract of *Brassica juncea* seeds on root number and root length of *Allium cepa*.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Root number (Mean ± SE)</th>
<th>Roots length (cm) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>26.33 ± 2.603</td>
<td>4.06 ± 0.636</td>
</tr>
<tr>
<td>PC</td>
<td>12.67 ± 1.764*</td>
<td>1.46 ± 0.521</td>
</tr>
<tr>
<td>0.1%</td>
<td>27.67 ± 1.202</td>
<td>3.06 ± 0.353</td>
</tr>
<tr>
<td>0.25%</td>
<td>28.33 ± 2.603</td>
<td>3.06 ± 0.437</td>
</tr>
<tr>
<td>0.50%</td>
<td>29.67 ± 1.856</td>
<td>3.23 ± 0.498</td>
</tr>
<tr>
<td>0.75%</td>
<td>29.33 ± 1.764</td>
<td>3.33 ± 0.811</td>
</tr>
<tr>
<td>1.0%</td>
<td>31.00 ± 1.155</td>
<td>3.90 ± 0.569</td>
</tr>
</tbody>
</table>

NC: negative control (distilled water); PC: positive control (0.75 ppm Hg). *P < 0.05 in one-way ANOVA. Root number: F-ratio = 10.517*; honestly significant difference (HSD) = 9.3086. Root length: F-ratio = 2.254; HSD = 2.7200.

Respectively, 0.75 ppm Hg and water served as the positive and negative control. The onion bulbs were washed under tap water after each treatment. The root tips from each treated onion bulb were fixed in Farmer’s fluid (glacial acetic acid and ethyl alcohol, 1:3) for 24 h and stored at 4 °C. For chromosomal analysis, the root tips were hydrolyzed in 1 N HCl for 1 min at a fixed temperature of about 60 °C. They were subsequently processed for cytological study by the aceto-orcein squash technique (1 N HCl and aceto-orcein, 1:9). The squash preparation was examined under the microscope for different types of chromosomal aberration assay.

Three slides were examined per onion and 6 onions were used for each treatment. The mitotic index (MI) was determined by counting the number of dividing cells among the total number of cells scored per slide. The percentage inhibition (PI) of chromosomal aberrations was calculated as follows: PI = a – b / a – c × 100, where a is the number of aberrant cells induced by the positive control (0.75 ppm Hg), b is the number of aberrant cells induced by seed extract and mercury (0.75 ppm), and c is the number of aberrant cells induced by the negative control (distilled water).

**Statistical analysis of data**

The mitotic index was calculated by scoring dividing cells. The experimental data are presented as means ± standard errors (SEs) of triplicate experiments. For the determination of the significance among the mean values, one-way ANOVA was applied (P < 0.05).

Table 2. Effect of different concentrations of chloroform extract of seeds of *B. juncea* on mitotic index observed in root tip cells of *Allium cepa*.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Total number of cells analyzed</th>
<th>Number of dividing cells</th>
<th>Mitotic index (% ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>10,118</td>
<td>1200</td>
<td>11.86 ± 0.054</td>
</tr>
<tr>
<td>PC</td>
<td>11,040</td>
<td>1200</td>
<td>10.86 ± 0.657</td>
</tr>
<tr>
<td>0.10%</td>
<td>9843</td>
<td>1200</td>
<td>12.19 ± 0.015</td>
</tr>
<tr>
<td>0.25%</td>
<td>9790</td>
<td>1200</td>
<td>12.25 ± 0.02</td>
</tr>
<tr>
<td>0.50%</td>
<td>9717</td>
<td>1200</td>
<td>12.34 ± 0.009</td>
</tr>
<tr>
<td>0.75%</td>
<td>9641</td>
<td>1200</td>
<td>12.44 ± 0.01</td>
</tr>
<tr>
<td>1.00%</td>
<td>9246</td>
<td>1200</td>
<td>12.97 ± 0.167</td>
</tr>
</tbody>
</table>

NC: negative control (distilled water); PC: positive control (0.75 ppm Hg). *P < 0.05 in one-way ANOVA. Mitotic index: F-ratio = 2.8821*; HSD = 1.708931.
0.05). The linear relationship between the dose and effect of chloroform extract was obtained by simple regression and correlation analysis.

Results
In this study, the effects of different concentrations of the chloroform extract of *B. juncea* seeds were observed on the genotoxicity of mercury employing the *Allium cepa* root chromosomal aberration assay. In our earlier study, mercury was observed to cause a dose-dependent decrease in the mitotic index and trigger different types of chromosomal aberrations (29).

Toxicity assay
The effect of the chloroform extract of *B. juncea* seed extract was observed on the root length and root number of *Allium* bulbs. The results of the root length and root number for the controls (positive and negative) and the treatment groups are shown in Table 1. A dose-dependent increase in root growth and root number was observed in the extract treatment groups compared to untreated control samples.

Mitotic index
Table 2 shows the effect of different concentrations of the chloroform extract of *B. juncea* on the mitotic index in root tip cells of *A. cepa*. A slight but significant increase in the mitotic index was observed following treatment with different concentrations of the extract with respect to the control value. This indicates that the extract was not cytotoxic.

*Allium cepa* root chromosomal aberration assay
The results on the effects of the chloroform extract

![Figure 1. Different types of aberrations induced by mercury (0.75 ppm) in *Allium cepa* root tips: a) c-mitosis, b) delayed anaphase, c) stickiness, d) vagrant chromosomes, e) chromatin bridge, f) chromatin bridge, g) chromosomal breaks, h) chromosomal breaks.](image-url)
of *B. juncea* seeds on different types of chromosomal aberrations induced by mercury in the root tip cells of *A. cepa* are summarized in Table 3. Different kinds of chromosomal aberrations induced by mercury were apportioned into physiological (c-mitosis, stickiness, vagrant chromosome(s), delayed anaphase(s), and laggard chromosome(s)) and clastogenic (chromatin bridge(s), ring chromosome(s), and chromosomal break(s)) aberration categories (Figure 1). Physiological aberrations are attributable to action on the spindle apparatus and clastogenic aberrations are attributable to direct breaking action on chromosomes. The effect of pre-, post-, and simultaneous treatment of chloroform extract showed a dose-dependent decrease in chromosomal aberration frequency (Table 3). All 3 types of treatment were found to be equally effective. The simultaneous treatment of the extract was found to be very effective in decreasing the clastogenic aberrations and posttreatment was found to be effective in decreasing physiological aberrations. For posttreatment of chloroform extract of seeds of *B. juncea*, the percentage inhibition of total aberrant cells ranged from 62.3% for 0.1% to 95.3% for 1.0% of the extract. Similarly, for the simultaneous treatment and the pretreatment, the percentage inhibition of total aberrant cells ranged from 60.8% for 0.1% to 95.3% for 1% and 61.4% for 0.1% to 91.7% for 1% of the extract, respectively (Table 3). The present investigation is a clear indication of the presence of antigenotoxic compounds in *B. juncea* plants.

The linear regression analysis method of determining the P-value ($R^2$) represents the

Table 3. Effects of pre-, post-, and simultaneous treatment of chloroform extract of *B. juncea* seeds on genotoxic effects induced by mercury in root tip cells of *Allium cepa*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentrations (%)</th>
<th>Physiological aberrations</th>
<th>Clastogenic aberrations</th>
<th>Total aberrant cells</th>
<th>Percentage aberrant cells</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cm</td>
<td>Da</td>
<td>Lg</td>
<td>Vg</td>
<td>Sc</td>
<td>Bg</td>
</tr>
<tr>
<td>NC</td>
<td>-</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>PC</td>
<td>-</td>
<td>38</td>
<td>24</td>
<td>14</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>Pre-</td>
<td>0.1</td>
<td>10</td>
<td>7</td>
<td>12</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>12</td>
<td>7</td>
<td>6</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Post-</td>
<td>0.1</td>
<td>21</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>19</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>15</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Simultaneous</td>
<td>0.1</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>11</td>
<td>7</td>
<td>7</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

* Out of 400 cells examined. Cm: c-mitosis, Da: delayed anaphase(s), Lg: laggard chromosome(s), Vg: vagrant chromosome(s), Sc: stickiness, Bg: chromatin bridge(s), Bk: chromosomal break(s), Rg: ring chromosome(s).

Percentage inhibition = $a - b / a - c \times 100$, where $a$ = number of aberrant cells induced by positive control, $b$ = number of aberrant cells induced by seed extract and mercury, and $c$ = number of aberrant cells induced by negative control.
significance of the null hypothesis and indicates that the percentage inhibition of chromosomal aberration was dose-dependent and positively correlated (Figure 2). The reduction in percentage of chromosomal aberrations in the extract-treated groups and pre-, post-, and simultaneous treatments showed that substances in B. juncea chloroform extract have antigenotoxic effects.

Discussion

The secondary metabolites present in plants have been reported to provide a multitude of antimutagenic, antigenotoxic, or anticarcinogenic effects. Plants have been used as an indicator organism in studies on mutagenesis in higher eukaryotes. Plant systems have a variety of well-defined genetic endpoints like alterations in ploidy, chromosomal aberrations, and sister chromatid exchanges (30). Dietary constituents suppress the genotoxic action or damage of xenobiotics through various cellular mechanisms (31,32). The most noticeable effect of mercurials was the induction of c-mitosis and polyplody and aneuploid cells (33,34). De et al. (35) exposed water cabbage for 2 days to mercuric chloride and the dose decreased chlorophyll content, protein, RNA, and dry weight.

In the present study, we investigated dose-dependent antigenotoxic effects of the chloroform extract of B. juncea against Hg-induced chromosomal aberrations in Allium cepa using 3 types of treatments: pre-, post-, and simultaneous treatments. Our results indicating the antigenotoxic potential of B. juncea are in good agreement with those of Rani et al. (18), who observed the antigenotoxic potential of the ether extract of Withania somnifera in root tip cells of Allium cepa.

Results obtained from this study showed that B. juncea chloroform extract at concentrations of 0.75 ppm and 1.0 ppm increased the mitotic index significantly. Mitotic index values of extract-treated
groups after mercury treatment were significantly higher than those of the control groups. The increase in mitotic index (number of dividing cells) showed that substances in the chloroform extract have no cytotoxic effects. These results are in accordance with the literature data on Indian goose berry (36), Terminalia chebula (37), pomegranates and guava (38), T. arjuna (39), Hippophae rhamnoides (40), etc. Allium cepa root chromosomal aberration assays have been used to show the antigenotoxic effects of Withania somnifera (18), Aegle marmelos (41), etc. Seeds of B. juncea have been used as a traditional herb in Chinese medicines against cough, asthma, and swelling pain. Mustard has been used as food and medicines in Ayurveda, as the leaves are considered a vegetable, while seeds are used as condiments and as a source of mustard oil.

On the basis of our results, we conclude that chloroform extract of B. juncea has a protective effect on Allium cepa root meristem cells against the genotoxic effects produced by mercury in a dose-dependent manner, but it is effective at a high dose (1%). However, the mechanism by which it acts remains to be investigated in different test systems and further studies are necessary. Studying crude plants extracts is appropriate because working with crude extracts means working with complex mixtures of active compounds. Some of these compounds can be genotoxic or antigenotoxic.

Considering the great versatility of uses of B. juncea, public awareness regarding its antigenotoxic value is very important. For this, further studies are necessary with different plant and animal test systems to strengthen these findings.

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