Antigenotoxic Effects of *Brassica oleracea* L. Var. *Italica* Aqueous Seed Extract on *Allium cepa* Root Chromosomal Aberration Assay

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Abstract: The herbicide cilluron containing cyhalofop-butyl (10%) is an active ingredient. Cyhalofop-butyl has low oral, dermal and inhalation toxicity. In the present study the antigenotoxic effects of *Brassica oleracea* L. var. *italica* was evaluated using *Allium cepa* root chromosomal aberration assay against cilluron. Three types of treatments were given to roots in pre-treatment roots were first treated with different concentrations (0.5, 1, 2, 4, 8 and 16 mg/ml) of extract for 3 hours followed by cilluron (0.8%). In post-treatment roots were first treated with cilluron followed by different concentrations of extract for 3 hours. In simultaneous treatment roots were treated with different concentrations of extract and cilluron simultaneously for 3 hours. The treatment of roots with distilled water and 0.8% cilluron served as negative and positive control. All the three types of treatments of extract showed dose dependent decrease in number of chromosomal aberrations. These results showed that *Brassica oleracea* L. var. *italica* aqueous seed extract have antigenotoxic effects.

Key words: Cilluron % *Brassica Oleracea* % *Allium Cepa* % Chromosomal Aberration

INTRODUCTION

To increase the agricultural production, pesticides are some of the compounds most frequently released into the environment. Nowadays more than 1500 chemicals are registered for use in formulation of pesticides and there are approximately 600 pesticides active ingredient in market [1-2]. Despite the beneficial effects associated with the use of pesticides, many of these chemicals also pose hazards to human life and environment [3]. In spite of the inconvenience, it is not possible to reduce the pesticides without reducing crop yield [4]. Alternative methods need long period to be effective and the results are not completely satisfactory, therefore, release of pesticides into the environment is continuously increasing day by day. For this reason, it is necessary to extend the evaluation of the genotoxicity of the chemicals. The genotoxic effects of many pesticides have been evaluated by several researchers through various test systems [5-6].

Various genotoxic assays are known over the past years can be used to study the genotoxicity and antigenotoxicity in a variety of methods. Since the results of various studies using *Allium cepa* fit best in test battery composed of prokaryotes and eukaryotes in vitro and *in vivo*. The *A. cepa* root chromosomal aberration assay was simple and reliable as the method where chromosomal aberrations were recorded in all types of mitotic cells [7].

In this study, *Allium cepa* root chromosomal aberration assay was used in the evaluation of antigenotoxic activity of water extract of seeds of broccoli against the genotoxicity of herbicide cilluron. Cilluron containing cyhalofop-butyl (10%) is an active ingredient. Cyhalofop-butyl is an aryloxyphenoxy propionate herbicide for post emergence control of barnyard grasses and silver top grass in rice. Cyhalofop-butyl has low oral, dermal and inhalation toxicity. It is known to induce liver and kidney abnormalities in experimental animals.

The medicinal plants have assumed increasing importance as a very useful therapeutic alternative in the programs of primary health care. Plants have been utilized as medicines from thousands of years [8]. The World Health Organization (WHO) has recognized, the importance of herbal medicine, suggesting that it is a viable alternative and important also for people in developing countries, since its cost is decreased. A large number of natural products referred as chemo preventive agents that offer a greater effect against cancer through a
range of mechanisms including antioxidant, antimutagenic activity, enzyme modulation, gene expression, apoptosis etc. [9-11]

Cruciferous vegetables particularly the members of Brassica genus such as broccoli, cauliflower, cabbage are rich source of glucosinolates which on enzymatic degradation breakdown into various hydrolytic products like isothiocyanates, nitriles etc. Among all, isothiocyanates are protective against the most common cancer types such as lung, prostrate, breast and colon cancers [12-14]. Broccoli contains a wide range of phytochemicals, including glucosinolates, flavonols and carotenoids. It also contains high concentrations of selenium and glucosinolates, particularly glucoraphanin and isothiocyanate sulforaphane [15] which have anticarcinogenic properties [16]. Brassica oleracea L. var. italica gave protection against cancer [17] and heart disease [18]. Antioxidant activity of broccoli on cooked food and juices has also been reported [19, 20]. From the epidemiological data it was suggested that diet rich in Brassica vegetables such as Broccoli (Brassica oleracea var. italica) are associated with a lower risk of lung and colorectal cancer. 56% of the case controlled studies demonstrate a strong relationship between increased broccoli consumption and the protection against cancer [21-23].

In this study, we report antigenotoxic effects of aqueous extract of seeds of broccoli on cell division in root tips against ciluron herbicide using Allium cepa root chromosomal aberration assay.

MATERIALS AND METHODS

Test Organism: Equal sized bulbs of a commercial variety of Allium cepa L. were taken from the local market. The outer scales of the bulbs were carefully removed and the brownish bottom plates were scrapped away without destroying the root primordia.

Test Chemicals: Ciluron (Cyhalofop butyl) herbicide was taken from local market Hall Gate, Amritsar, Punjab. Orcein, glacial acetic acid, HCl and other chemicals were bought from Qualigens Fine Chemicals, Mumbai, India, Thomas Baker (Chemicals) PVT, Limited, Mumbai, India.

Seeds Collection and Extraction: Certified seeds of Brassica oleracea L. var. italica (Broccoli) were procured from Chaudary Sarvan Kumar Agriculture University, Palampur, Himachal Pradesh, India. They were grinded to a fine powder. The finely ground powder of seeds were defatted with hexane to remove fatty oil etc. After filtration, seed meal was air dried for overnight and extracted with double amount of water by placing the mixture on shaker for two nights. Solution was filtered with whatmann’s filter paper. Water was distilled-off to concentrate the water extract and the extract was dried by evaporating on water bath. Different concentrations were prepared by dissolving per mg of each extract first in dimethyl sulfoxide (DMSO) and then equal amount of water was added to have final composition of mg/ml. Afterwards different concentrations were prepared (0.5, 1, 2, 4, 8 and 16 mg/ml) from the stock solution.

Allium cepa Root Growth Test/ Determination of EC₅₀: Clean and healthy onion bulbs were set up and allowed to germinate in distilled water for 24 hours, where after the homogenously rooted bulbs were selected for control and different concentrations (2, 1, 0.8, 0.6, 0.4, 0.2 and 0.1% ) of ciluron herbicide for 96 hours. During the experiment, the test solutions were changed after every 24 hours. The root lengths from the control and experimental sets were measured (lengths of ten roots from each bulb) at the end of exposure time. In this experiment Effective Concentration (EC₅₀) refers to be the effective concentration which indicates the 50% root growth inhibition.

Antigenotoxic Effect of Aqueous Seed Extract: Antigenotoxic effects of aqueous extract of seeds of Broccoli were estimated by analyzing genotoxicity induced by ciluron herbicide in root tip meristematic cells of Allium cepa. The loose outer scales were removed and the brown roots were scrapped to expose the primordia. Onion bulbs were grown on coupling jars containing distilled water until roots grew up to the average length of 0.5-1 cm. Three modes of treatment were given to roots. In pre-treatment roots were first treated with different concentrations (0.5, 1, 2, 4, 8 and 16 mg/ml) of extract for 3 hours followed by ciluron treatment (0.8%, 3 hours). In post-treatment, roots were first treated with ciluron followed by different concentrations of extract for three hours. In simultaneous treatment roots tips were treated with ciluron (0.8%) and different concentration of extract simultaneously for 3 hours. The treatment of roots with 0.8% of ciluron and distilled water served as positive and negative control. After treatment, the bulbs were washed under tap water and root tips were removed from the bulb, fixed in Farmer’s fluid (glacial acetic acid and ethyl alcohol 1:3) for 24 hours. The slides were prepared by hydrolyzing the root tips in 1N HCl with intermittent heating for 1
minute and then squashed in aceto-orcein and 1N HCl (9:1). The tip of root was cut and placed on slide, covered with cover slip and squashed by match stick and sealed with DPX. Each slide was examined under the microscope and photographed using Nikon camera. Different kinds of chromosomal aberrations were scored. About 600 dividing cells from 9-10 root tips were scored for each treatment. The percentage inhibition (PI) of chromosomal aberrations was calculated as follows: $PI = \frac{a-b}{a-c} \times 100$ where, $a$ is the number of aberrant cells induced by positive control (0.8% ciluron), $b$ is the number of aberrant cells induced by seed extract and ciluron (0.8%) and $c$ is the number of aberrant cells induced by negative control (distilled water).

**Statistical Analysis:** The experimental data is presented as mean±SE of triplicate experiment. The linear relationship between dose and effects of aqueous extract was obtained by simple regression and correlation analysis.

**RESULTS**

The average roots length for control and treatment groups is given in Fig. 1. The effect of aqueous extract of broccoli which was applied before, after and simultaneously ciluron treatment on root length is significant when compared with positive and negative controls. The measures of average root length in negative and positive control are 3.3 cm and 2 cm. However it is clear from the result that roots length increase as the concentration of extract increase from 0.5 to 16 mg/ml (Fig. 1).

Firstly the genotoxic effect of different concentrations (0.1, 0.2, 0.4, 0.6, 0.8 and 1%) of ciluron was evaluated. Among different concentrations, 0.8% concentration of ciluron was the effective concentration (EC$_{50}$) and was further used in antigenotoxic studies. Ciluron induced various types of chromosomal aberrations partitioned into physiological aberrations (17.6%) like c-mitosis, delayed anaphase, laggards etc.

Fig 1: Average roots length in control and different concentrations of aqueous extract of Brassica oleracea L. var. italica. NC= Negative control (distilled water); PC= Positive Control (ciluron: 0.8 %)

<table>
<thead>
<tr>
<th>Type of chromosomal aberrations</th>
<th>No. of Aberrant cells’</th>
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<th>No. of Aberrant cells’</th>
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<tr>
<td>Physiological aberrations (PA)</td>
<td></td>
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<tr>
<td>C-mitosis</td>
<td>5</td>
<td>39</td>
<td>21</td>
<td>23</td>
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<tr>
<td>Delayed anaphase</td>
<td>3</td>
<td>21</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Laggards</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Stickiness</td>
<td>2</td>
<td>15</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Vagrant/s</td>
<td>2</td>
<td>6</td>
<td>17</td>
<td>3</td>
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<tr>
<td>Mutipolarity</td>
<td>-</td>
<td>17</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Total PA</td>
<td>13</td>
<td>101</td>
<td>56</td>
<td>61</td>
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<th>Clastogenic aberrations (CA)</th>
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<tr>
<td>Chromatin bridge/s</td>
<td>4</td>
<td>8</td>
<td>6</td>
<td>7</td>
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<tr>
<td>Chromosomal break/s</td>
<td>1</td>
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<td>4</td>
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<td>Total CA</td>
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<td>10</td>
<td>9</td>
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<td>Total aberrant cells (PA+CA)</td>
<td>18</td>
<td>114</td>
<td>66</td>
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*a= out of 600 cells examined; A= Pre-, B= Post-and C= Simultaneous-treatment
and clastogenic aberrations (2.3%) like chromatid bridge/s, chromosomal break/s (Fig. 2). It induces more physiological aberrations than clastogenic aberrations. C-mitosis followed by delayed anaphase was the most frequent kind of aberrations found in dividing cells. The effects of pre-, post- and simultaneous treatment of aqueous extract of seeds of broccoli resulted in a dose dependent decrease in chromosomal aberrations (Table 1). All the three types of treatment of extract were found to be equally effective. Post and simultaneous treatments of aqueous extract of broccoli were found to be very effective and resulted in a significant decrease in clastogenic aberrations (100% at the highest dose tested). On the other hand pre-treatment of extract cause significant reduction in physiological aberrations (98.9% at the highest dose tested). Among the three treatments
Fig. 3: Relationship between different concentrations (0.5, 1, 2, 4, 8 and 16 mg/ml) of water extract of *B. oleracea* L. var. *italica* seeds and percent inhibition of genotoxic effects induced by ciluron herbicide (0.8%) in *Allium cepa* root chromosomal aberration assay.

Simultaneous treatment showed maximum percentage inhibition (98.9%) followed by pre-treatment (97.9%) and post-treatment (95.8%). The linear regression analysis method of determining the P value ($R^2$) indicates that the percent inhibition of chromosomal aberration was dose dependent and positively correlated (Fig. 3).

**DISCUSSION**

In this study, the effect of different concentrations of the aqueous extract of Broccoli seeds were observed on genotoxicity of ciluron employing *Allium cepa* root chromosomal aberration assay. In this study, ciluron was observed to cause dose dependent decrease in root length and increase different types of chromosomal aberrations with increase in concentrations.

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 structural alterations in ploidy, chromosomal aberrations and sister chromatid exchanges (SCE) [24-26]. *Brassica oleracea* L. var. *italica* (broccoli) is one of the major agricultural products widely considered to contain high level of phytochemicals including glucosinolates, flavonoids, vitamins and minerals [27, 28]. Many studies have indicated that consumption of broccoli is inversely related with the occurrence of cancer in human. The health benefits of vegetables in preventing cancer and cardiovascular diseases are mostly attributed to the quality of antioxidative components. The main antioxidative components present in broccoli are flavonoids and vitamins. Broccoli sprouts have also been reported to inhibit cancer growth *in vitro* and *in vivo* to extend their protective role against cancer [29]. Dietary broccoli sprouts and their component glucosinolates and isothiocyanates induce phase II enzymes and afford protection against chemically induced tumours in rodents [30, 31].

*Allium cepa* root chromosomal aberration assay is being used to determine the antigenotoxicity of different plant extracts *Terminalia chebula* [32], *Ocimum sanctum* L. leaf [33], *Plantago lanceolata* [34], *Curcumin* [35], *Aegle marmelos* [36], bitter leaf [37], *Brassica juncea* [7].
The water extract of *Brassica oleracea* L. var. *italica* seeds clearly showed a dose dependent protective effect against genotoxic effect of ciluron herbicide. Percentage inhibition of clastogenic aberrations of this extract was found to be higher as compared to physiological aberration. The present study suggests that aqueous extract of broccoli possess antigenotoxic nature. Further studies are required to characterise these antigenotoxic agents for their further use in research.

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REFERENCES