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Research Article

EVALUATION OF ANTICYTOGENETIC EFFECTS OF *BRASSICA OLERACEAE* L. VAR. *ITALICA* IN *ALLIUM CEPA*.

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ABSTRACT

Objective: The present study evaluate the antigenotoxic effects of ethyl acetate and chloroform seed extracts of *Brassica oleraceae* L. var. *italica* (broccoli) on the *Allium cepa* root chromosomal aberration assay against the genotoxicity induced by ciluron herbicide.

Methods: Roots were given three different modes of treatment. In pre- treatment onion bulbs were firstly treated with different concentrations (0.5, 1, 2, 4, 8 and 16 mg/ml) of extracts for 3 hours, and then treated with 8% concentration of ciluron for another 3 hours. In post treatment, onion were treated with 8% concentration of ciluron and then treated with different concentrations of extract for 3 hours. In simultaneous treatment, onion bulbs were treated with different concentrations of extract and 8% concentration of herbicide simultaneously for 3 hours.

Results: Decrease in chromosomal aberrations frequency were observed in root tip cells treated with ethyl acetate and chloroform extracts of seeds, after, before and simultaneously with herbicide treatment as compared to control.

Conclusion: The dose dependent effects suggest that both the extracts of *Brassica oleraceae* L. var. *italica* have antigenotoxic potential against ciluron herbicide induced genotoxicity.

Keywords: Genotoxicity; *Allium cepa*; Herbicide; *Brassica oleraceae* L var. *italica*.

INTRODUCTION

Synthetic herbicides/pesticides are extensively used worldwide for agricultural programmes due to their higher efficiency, and lower mammalian toxicity than organochlorine insecticides[1]. Herbicides are the substances which are used to kill or to suppress the growth of unwanted plants in the field[2]. The use of herbicides has increased every year without considering their harmful effects on plants, animals and human beings[3]. Several workers have demonstrated that these herbicides are mutagenic/carcinogenic to non target organisms[4,5,6]. The commercial herbicide ciluron contain cyhalofop-butyl as 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 also known to induce liver and kidney abnormalities in experimental organisms[7].

There is a need to protect ourselves from either by avoiding exposure to harmful environmental mutagens that cause various abnormalities like mutation or cancer, or by regular consumption of phytochemicals (antimutagens/anticarcinogens) in the diet which modulate our defence mechanism. Brassicaceae family is considered to be a potent source of various antigenotoxic/ antimutagenic/anticarcinogenic compounds. *Brassica oleracea* L. var. *italica* (broccoli) is one of the important species of brassicaceae family. Broccoli extracts are protective against reactive oxygen species due to the presence of caretenoids, tocopherol, ascorbic acid and flavonoids[8]. The contribution of Brassica vegetables to health improvement has been associated with their antioxidant and antigenotoxic capacity[9,10,11].

Among wide range of bioassays used, higher plant bioassays have obtained very good acceptability in genotoxicity studies such as pesticides[12], antimutagenic[13], drinking water[14] etc. *Allium cepa* root chromosomal aberration assay is one of the well established test system recommended by different organizations for the evaluation of genotoxic potential of different environmental chemicals[15,16].

Keeping in view the widespread use of herbicides in agriculture and its possible genotoxic potential of as coupled with the chemoprotective effects of broccoli, this study was initiated to determine the antigenotoxic potential of this plant against ciluron herbicide in *Allium cepa* root chromosomal aberration assay.

MATERIALS AND METHODS

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 ground 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 ethyl acetate and chloroform by placing the mixture on shaker for two nights. Solutions were filtered with whatmann's filter paper. Solvents were distilled-off to concentrate the ethyl acetate and chloroform extracts to obtain ethyl acetate and chloroform seed extracts. 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.

Test organism and chemicals

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

Evaluation of antigenotoxic activity

Antigenotoxic effects of ethyl acetate and chloroform seed extracts of Broccoli were estimated by analyzing genotoxicity induced by ciluron herbicide in root tip meristematic cells of *Allium cepa.* Onion bulbs were grown on coupling jars containing distilled water until roots grew up to the average length of 0.5-1 cm. Three different 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 extracts 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 concentrations of extracts simultaneously for 3 hours[17].

The treatment of roots with 0.8% of ciluron and distilled water served as positive (highest number of chromosomal aberrations) and negative control respectively. After treatment, the bulbs were washed under tap water and removed root tips were 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 = a-b/a-c x 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).

The linear relationship between dose and effects of ethyl acetate and chloroform extracts were obtained by simple regression and correlation analysis.

RESULTS

In this study the antigenotoxic effects of different concentrations of ethyl acetate and chloroform seed extracts of Broccoli were evaluated against the genotoxicity induced by ciluron herbicide employing *Allium cepa* root chromosomal aberration assay. Effective concentration i.e. 0.8% of ciluron induced various types of chromosomal aberrations includes c-mitosis, vagrant, delayed anaphase, chromatin bridge/s and chromosomal break/s etc. Ethyl acetate seed extract inhibit chromosomal aberrations by 97.9%, 92.7% and 90.6% whereas chloroform extract reduced aberrations by 85.4%, 88.5% and 93.8% [Table 1, 2] during pre-, post- and simultaneous treatments at the highest tested dose of 16mg/ml. The effect of all the modes of treatment of ethyl acetate and chloroform seed extract showed dose dependent decrease in chromosomal aberrations frequency. In ethyl acetate seed extract, among all modes of treatment, pre- treatment showed maximum reduction in chromosomal aberrations and in chloroform extract, simultaneous treatment showed the maximum decrease in the frequency of chromosomal aberrations. 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 [Figure 1].

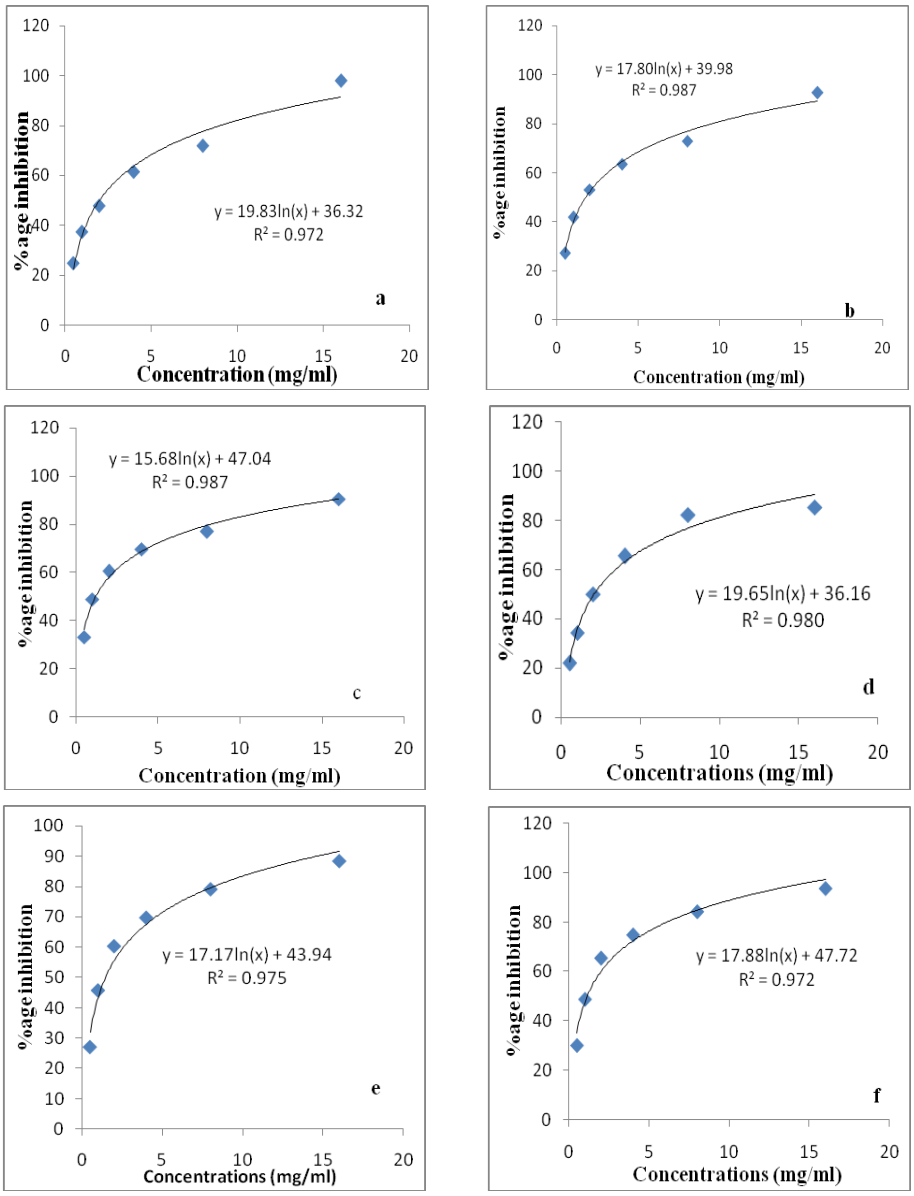

Fig. 1: It shows relationship between different concentrations (0.5, 1, 2, 4, 8 and 16 mg/ml) of ethyl acetate (a,b,c) and chloroform (d,e,f) extracts of *B. oleraceae* L. var. *italica* seeds and percent inhibition of genotoxic effects induced by ciluron herbicide (0.8%) in *Allium cepa* root chromosomal aberration assay.

DISCUSSION

Traditional medicines are commonly used to treat various diseases besides modern medicines because synthetic drugs can cause different side effects[11]. Therefore the scientific concerns are in the significance of natural compounds. Recently we also reported the antigenotoxic effects of different seed extracts of *Brassica juncea* L. Czern using *Allium cepa* root chromosomal aberration assay. In the present study we investigated antigenotoxic effects of ethyl acetate and chloroform extracts of seeds of *Brassica oleraceae* L. var. *italica* (Broccoli) using *Allium* test.

Allium cepa root chromosomal aberration assay shows both physiological aberrations attributable to spindle inhibition (c-mitosis, delayed anaphase, laggard/s, vagrant/s and stickiness) and clastogenic aberrations attributable to direct action on chromosomes (chromosomal break/s and chromatin bridge/s). Therefore *Allium cepa* assay is a well established and standardized test for antimutagenic/ antigenotoxic study of toxic substances[18,19,20]·[21].

Herbicides were not considered as problem causing agents; on the contrary, utilizing these compounds was considered as sign of progress and modernization in increasing agriculture yields. Herbicides are used to inhibit the growth of weeds in agriculture, but in spite of their uses they also induced several mutations and harmful effects on human beings as well as on aquatic ecosystem.

Genotoxicity of ciluron herbicide was determined by using *Allium cepa* root chromosomal aberration assay. The newly emerged roots were treated with different concentrations (0.01, 0.2, 0.4, 0.6, 0.8 and 1.0%) of ciluron herbicide for 3 hours. From the present study it was revealed that ciluron herbicide had greater effect on physiological aberrations, the frequency of physiological aberrations (17.67%) was much more as compared to clastogenic aberrations (2.3%) at the highest dose (1%). Ciluron herbicide increased the percentage of abnormal cells in *Allium cepa*. This increase was significant in all concentrations applied, when compared to the control and was dose dependent. Though maximum chromosomal aberration frequency was observed at 1% but a wider spectrum of aberrations was observed at 0.8% concentration, so this concentration was selected as the optimum concentration to study antigenotoxic effects of different extracts of plant

The effect of all the three types of treatment of ethyl acetate and chloroform extracts on the genotoxicity induced by ciluron herbicide over summarized in Table 1 and 2. Broccoli is one of the major agriculture products widely considered to contain high level of phytochemicals including glucosinolates, flavonoids, vitamins and minerals[22,23]. The health benefits of vegetables in preventing cancer and cardiovascular diseases are mostly attributed to the quality of anti oxidative components. Broccoli sprouts have also been reported to inhibit cancer growth *in vitro* and *in vivo*[24,25,26].

Table 1: It shows the effect of pre-, post- and simultaneous- treatments of ethyl acetate extract of Broccoli seeds on genotoxic effects, induced by ciluron herbicide in root tip cells of *Allium cepa.*

Types of chromosomal aberrations	**Negative control**	**Positive control (ciluron-0.8%)**	**0.5mg/ml**			**1 mg/ml**			**2 mg/ml**			**4mg/ml**			**8mg/ml**			**16mg/ml**		
	No. of Aberrant cells[a]	**No. of Aberrant cells[a]**	**No. of Aberrant cells[a]**			**No. of Aberrant cells[a]**			**No. of Aberrant cells[a]**			**No. of Aberrant cells[a]**			**No. of Aberrant cells[a]**			**No. of Aberrant cells[a]**		
			A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Physiological aberrations (PA)																				
C-mitosis	5	39	29	28	25	26	23	20	23	21	17	20	18	14	15	15	12	5	7	8
Delayed anaphase	3	21	17	19	16	14	16	14	12	14	11	10	13	9	9	9	7	3	4	4
Laggard/s	1	3	2	2	2	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Stickiness	2	15	5	4	3	3	3	2	2	2	2	1	1	1	1	1	1	-	-	1
Vagrant/s	2	6	13	14	11	11	13	9	10	10	8	8	8	7	6	7	6	2	5	4
Mutipolarity	-	17	15	13	16	14	11	13	12	9	11	9	7	10	8	6	8	4	4	5
Total PA	13	101	81	80	73	69	67	59	60	56	49	48	47	41	39	38	34	14	20	22
Clastogenic aberrations (CA)																				
Chromatin bridge/s	4	8	6	6	6	5	5	5	5	4	4	4	4	4	4	4	4	4	3	4
Chromosomal break/s	1	5	3	2	3	4	2	3	3	3	3	3	2	2	2	2	2	2	2	1
Total CA	5	13	9	8	9	9	7	8	8	7	7	7	6	6	6	6	6	6	5	5
Total aberrant cells (PA+CA)	18	114	90	88	82	78	74	67	68	63	56	55	53	47	45	44	40	20	25	27
Percentage inhibition			25	27.1	33.3	37.5	41.7	48.9	47.9	53.1	60.4	61.5	63.5	69.8	71.9	72.9	77.1	97.9	92.7	90.6

a= out of 600 cells examined; A= Pre-, B= Post- and C= Simultaneous- treatments

Table 2: It shows the effect of pre-, post- and simultaneous- treatments of chloroform extract of Broccoli seeds on genotoxic effects induced by ciluron herbicide in root tip cells of *Allium cepa.*

Types of chromosomal aberrations	Negative control	Positive control (ciluron-0.8%)	0.5mg/ml			1 mg/ml			2 mg/ml			4mg/ml			8mg/ml			16mg/ml		
	No. of Aberrant cells[a]	No. of Aberrant cells[a]	No. of Aberrant cells[a]			No. of Aberrant cells[a]			No. of Aberrant cells[a]			No. of Aberrant cells[a]			No. of Aberrant cells[a]			No. of Aberrant cells[a]		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Physiological aberrations (PA)																				
C-mitosis	5	39	31	26	27	29	22	20	25	19	15	19	17	13	12	14	10	11	10	6
Delayed anaphase	3	21	18	19	16	16	15	14	11	10	10	12	8	8	7	6	6	8	5	5
Laggard/s	1	3	2	2	2	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Stickiness	2	15	5	3	3	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1
Vagrant/s	2	6	12	13	13	11	9	10	9	8	8	7	7	7	6	6	5	4	4	3
Mutipolarity	-	17	14	16	14	12	12	11	10	9	8	6	7	6	3	5	5	3	3	4
Total PA	13	101	82	79	75	71	61	58	57	48	43	45	40	35	29	32	27	27	23	19
Clastogenic aberrations (CA)																				
Chromatin bridge/s	4	8	7	6	6	7	5	4	6	4	5	4	4	4	4	4	3	3	3	3
Chromosomal break/s	1	5	4	3	4	3	4	5	3	4	3	2	3	3	2	2	3	2	3	2
Total CA	5	13	11	9	10	10	9	9	9	8	8	6	7	7	6	6	6	5	6	5
Total aberrant cells (PA+CA)	18	114	93	88	85	81	70	67	66	56	51	51	47	42	35	38	33	32	29	24
Percentage inhibition			21.9	27.1	30.2	34.4	45.8	48.9	50	60.4	65.6	65.6	69.8	75	82.3	79.2	84.4	85.4	88.5	93.8

a = out of 600 cells examined; A= Pre-, B= Post- and C= Simultaneous- treatments.

For pre treatment of ethyl acetate extract, the significant decrease in both physiological and clastogenic aberrations was observed. The percentage inhibition of total aberrant cells ranged from 25 to 97.9%. The percentage inhibition of total aberrant cells in post- and simultaneous treatment ranged from 27.1 to 92.7% and 33.3 to 90.6%. For pre treatment of chloroform extract, the percentage inhibition of total aberrated cells ranged from 21.9 to 85.4%. For post and simultaneous treatments, there was a significant reduction in all the aberrations and percentage inhibition of total aberrant cells ranged from 27.1 to 88.5% and 30.2 to 93.8%. So it was observed from the present investigation that both the extracts showed greater potential to inhibit the genotoxicity induced. The results of the present investigation clearly showed genoprotective or cytoprotective potential of *Brassica oleraceae* L. var. *italica* against genotoxicity induced by ciluron herbicide using *Allium cepa* root chromosomal aberration assay.

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