

Full Length Research Paper

Antigenotoxic effects of Indian mustard *Brassica juncea* (L.) Czern aqueous seeds extract against mercury (Hg) induced genotoxicity

Sonia Sharma and Adarsh Pal Vig*

Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar-143005 Punjab, India.

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The present study evaluates the antigenotoxic effects of *B. juncea* (L.) Czern. (Indian mustard) aqueous seed extract on the *Allium cepa* L. (common onion) root tip cells. The experiment was carried at three stages: (1) onion bulbs were treated with 0.75 ppm mercury for 3 h, and then treated with different concentrations (0.1, 0.25, 0.50, 0.75, and 1.0%) of aqueous extract for another 3 h. (2) onion bulbs were treated with different concentrations of extract for 3 h, and then treated with 0.75 ppm concentration of mercury. (3) Onion bulbs were treated with different concentrations of extract and 0.75 ppm concentration of mercury simultaneously for 3 h. Increase in root growth and mitotic index and decrease in chromosomal aberrations were observed in root tip cells treated with aqueous extract of seeds, after before and simultaneously with mercury treatment as compared to control. The dose dependent effects suggest that aqueous extract of Indian mustard has antigenotoxic potential against mercury induced genotoxicity.

Key words: Indian mustard, antigenotoxic, chromosomal aberrations, mercury.

INTRODUCTION

Disposal of various hazardous wastes, due to the rapid industrialization and urbanization, has increased environmental pollution over the past few decades (Babu and Maheswari, 2006). Among them, heavy metals play a major role in causing several problems, particularly mutagenicity and carcinogenicity (Nagao, 1978). Heavy metals have been recognized as a major pollutant for both aquatic and terrestrial organisms because of their directly accumulating nature in their body or by indirectly through food chain (Memon et al., 2001; Akinola and Ekiyoyo, 2006; Obasohan et al., 2006). Among heavy metals, mercury (Hg) which is a known mutagen and extensively used as a heavy metal, cause various types of problems in plants and animals (Renzoni et al., 1998; Boening, 2000; Florea and Busselberg, 2006). It can

cause nerve, brain and kidney damage, lung and eye irritation, vomiting, diarrhea and can damage DNA and chromosomes. Against this background, interest has generated worldwide to screen the natural products capable of neutralizing the genotoxic effects of various chemicals present in the environment. Recent research has confirmed that natural occurring substances present in plants, have protective effects against various environmental mutagen (Kada et al., 1978; Lai et al., 1980; Edenharder et al., 2003). Nowadays, many plant parts like *Phyllanthus* (aamla) fruit extract, *Ocimum sanctum* (tulsi) leaf extract etc. have been used to decrease the toxicity level created by different chemical agents (Madhavi et al., 2007; Pillai and Damodharan, 2007). Indian mustard which belongs to *Cruciferae* family is one of the most common medicinal plant used to cure several diseases all over the world. It is known for the anti-carcinogenic and anti-proliferative activities. *Brassica* vegetables are regarded for their nutritional value. They

*Corresponding author. E-mail: dr.adarshpalvig@gmail.com.

provide high amount of vitamin C, soluble fibers and contain multiple nutrients like sulforaphane and selenium, etc. with anti cancer properties (Hu, 2003; Lampe, 2003; Willcox et al., 2003). Liquid preparation of Indian mustard is used as an antiseptic against tumor of carcinoma and throat.

The purpose of the present study is to investigate the anti-genotoxic effects of aqueous extract of seeds of Indian mustard using *Allium cepa* root chromosomal aberration assay because currently, there is no published data on the anti-genotoxic effect of this plant. Due to the lack of information about their anti-genotoxicity, it is important to evaluate the effects of complex mixture on genetic alterations. *Allium* test, a standardized test for cyto-genotoxicity monitoring (Fiskesjo, 1985) was used as a control test in various studies. Furthermore, the *Allium* test was simple, not so expensive, easy to apply, and just as reliable as the method where aberrations were recorded in all types of mitotic cells. The *Allium* test combines two test targets: toxicity and genotoxicity. *A. cepa* assay shows good correlation with mammalian test systems (Fiskesjo, 1993; Grant, 1994; Cordell, 1995; Yi and Meng, 2003). Therefore, we used *A. cepa* root chromosomal aberration assay as a test material in this study.

MATERIALS AND METHODS

Chemicals

Mercury (mercuric chloride) 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, or s d FINE-CHEM. LIMITED, Mumbai, India.

Collection of seeds of *Brassica juncea* (L.) Czern

The plant utilized in this study was *B. juncea* (Indian mustard). Seeds of Indian mustard were procured from the Department of Plant Breeding, Punjab Agriculture University (PAU), Ludhiana, India.

Preparation of the aqueous extract

Seeds of Indian mustard were cleaned, air-dried and thoroughly ground to a fine powder. The seed powder (250 g) was first extracted with hexane (500 ml) to remove oil in soxhlet apparatus for 110-115 h approx. The filtered seed meal was air dried for overnight and extracted with 500 ml of water by placing the mixture on shaker for about two nights.

Thereafter, the extract was filtered through a filter paper to remove particulate matter and the extract was dried by evaporating on water bath. Firstly the extract was solubilized in DMSO and then distilled water was added to make a total volume of 200 ml (stock). Fresh concentrations were prepared daily for each experiment.

Allium test

To evaluate anti-genotoxic effects of *B. juncea* aqueous extract, we

used *Allium* test. The incubation of roots was carried out in three different treatments under the same conditions.

Treatments 1: Equal-sized bulbs of common onion (*A. cepa*) were purchased from the local market. Before use, the loose outer scales and dry bottom plates were carefully removed without destroying the root primordials. A series of 8 cleaned onion bulbs were placed on coupling jars, each filled with tap water for 2 days. On the third day onion bulbs with the poorest growth in each set were discharged and the other onions were transferred to treatment jars filled with 0.75 ppm concentration of mercury for 3 h. After the mercury treatment, roots were washed and then treated with different concentrations (1.0, 0.75, 0.5, 0.25 and 0.1%) of Indian mustard seeds extract for 3 h. After the treatment, the root tips were kept in fixative and then processed for the preparation of microscope slides. Tap water was used as negative control and 0.75 ppm mercury was used as positive control and handled alike for all the experiment.

Treatments 2: A series of 8 cleaned onion bulbs were placed on top of coupling jars each filled with tap water for 2 days. On the third day, bulbs with the poorest growth in each set were discharged and other onions were transferred on treatment jars filled with different concentrations (1.0, 0.75, 0.5, 0.25 and 0.1%) of Indian mustard seeds aqueous extract. After extract treatment onion bulbs were washed and treated with 0.75 ppm mercury for 3 h and again roots were washed.

Treatments 3: The effects of both aqueous extract and mercury treatment were investigated. For this purpose, a series of 8 cleaned onion bulbs were transferred to coupling jars filled with concentrations (1.0, 0.75, 0.5, 0.25 and 0.1%) of extract and 0.75 ppm of mercury simultaneously. Further procedure was same as that of treatment 1.

Assay procedure

At the end of the treatment period, the length of roots of each onion bulb with the best growth at each concentration was measured (in cm) using thread and scale. After the completion of 3 h treatment, the root tips of each bulb were cut and fixed in fixative (ethanol: glacial acetic acid (3:1, v/v)). After fixation, the roots were hydrolyzed in 1 part of 1N HCl for 1 minute and squashed in aceto-orcein and 1N HCl (9:1) after intermittent heating for 3-5 min (Sharma and Vig, 2012). After removing well-stained root tips, they were immersed in a drop of 45% acetic acid on a clean slide, squashed under a cover slip with match stick and sealed with DPX and examined microscopically (Nikon fluorescent microscope and camera).

Three slides were examined per onion and six onions in each group. The mitotic index (MI) was determined by counting the number of dividing cells among the total number of cells scored per slide. Different chromosomal aberrations were characterized and percent chromosomal aberration frequency with and without extract in cells were calculated.

Statistical analysis of data

Mitotic index (MI) was calculated by scoring dividing cells. The experimental data is presented as mean \pm SE of triplicate experiment. For the determination of the significance among the mean values, two-way ANOVA was applied ($p < 0.05$). The linear relationship between dose and effect of aqueous extract was obtained by simple regression and correlation analysis.

RESULTS

In this study, the anti-genotoxic effects of different

Table 1. Average roots number and roots length in control and different concentrations of aqueous extract of *B. juncea*.

Concentrations	Roots number (Mean \pm SE)	Roots length (Mean \pm SE)
NC	12.67 \pm 1.76	3.30 \pm 0.33
PC	5.00 \pm 1.52	2.00 \pm 0.55
0.1 %	10.67 \pm 0.66	2.60 \pm 0.66
0.25%	13.00 \pm 1.00*	3.30 \pm 0.33*
0.50%	14.00 \pm 1.15*	3.60 \pm 0.33*
0.75%	15.00 \pm 1.52*	4.30 \pm 0.33*
1.0%	19.33 \pm 0.88*	4.60 \pm 0.33*

NC= Negative control (distilled water); PC= Positive Control (Hg: 0.75 ppm); *p< 0.05 in Two way ANOVA.

Table 2. Mitotic index values in control and different concentrations of aqueous extract of Indian mustard.

Concentrations	Total number of analyzed cells	Number of dividing cells	Mitotic index
NC	1253	400	31.9
PC	1856	400	21.5
0.1%	1232	400	32.4
0.25%	1136	400	35.2 [*]
0.50%	1024	400	39.1 [*]
0.75%	1015	400	39.4 [*]
1.0%	994	400	40.24 [*]

NC= Negative control (distilled water); PC= Positive Control (Hg: 0.75 ppm); * p< 0.05 in Two way ANOVA.

concentrations of aqueous extract of seeds of Indian mustard were evaluated against the genotoxicity of mercury employing *A. cepa* root chromosomal aberration assay.

Macroscopic effects: The experiment was performed in controlled conditions; we observed significant differences between negative and positive control, and treatment groups. The results of the roots length and root numbers, with parameters used in testing for general antitoxicity are given in Table 1. Roots length increased as the concentration of Indian mustard seed extract increased from 2.6 cm (0.1%) to 4.6 cm (1.0%) and 100% roots growth was observed in control (distilled water). The average roots length in negative and positive control was 3.3 and 2.0 cm, respectively. The average root number per onion bulb also increased with the concentration of extract from 10.67 (0.1%) to 19.33 (1.0%). Increase in roots length and number in extract treatment groups was dose dependent and statistically significant (p<0.05).

Microscopic effects: There was a rapid increase in the mitotic index with increasing concentration (0.1 to 1.0%) of the Indian mustard aqueous extract as compared with the negative control and it was positively correlated to roots length that increased with increasing concentration of the aqueous extract. The results of the microscopic effects are summarized in Tables 2, 3 and 4. The mitotic index (MI) values for each concentration are given in

Table 2. The mitotic index value of positive and negative control was found to be 21.5 and 31.9. The values of MI increased from 32.4 at 0.1% to 40.24 at 1.0% concentrations of extract. Increase of MI (number of dividing cells) values explained non-cytotoxicity of Indian mustard aqueous extract in plant test system.

Different kinds of chromosomal aberrations induced by mercury were apportioned into physiological (c-mitosis, stickiness, vagrant chromosome/s, delayed anaphase/s, laggard chromosome/s) and clastogenic (chromatin bridge/s, ring chromosome/s and chromosomal break/s) aberrations are counted by microscopic observations for each slide (Figure 1) and the effect was found to be dose dependent. The effects of aqueous extract on chromosomes of *A. cepa* are presented in Tables 3 and 4.

All the three types of treatments showed dose dependent protective effect against mercury induced chromosomal aberrations (Table 3). At the highest dose (1%) of aqueous extract tested, simultaneous treatment showed maximum percentage inhibition (101.1%) followed by post treatment (100.0%) and pre treatment (98.9%), (Table 4). The linear relationship between the percent inhibition of chromosomal aberrations and different concentrations (0.1, 0.25, 0.50, 0.75 and 1%) of aqueous extract of seeds of *B. juncea* was obtained by regression and correlation analysis (Figure 2) and

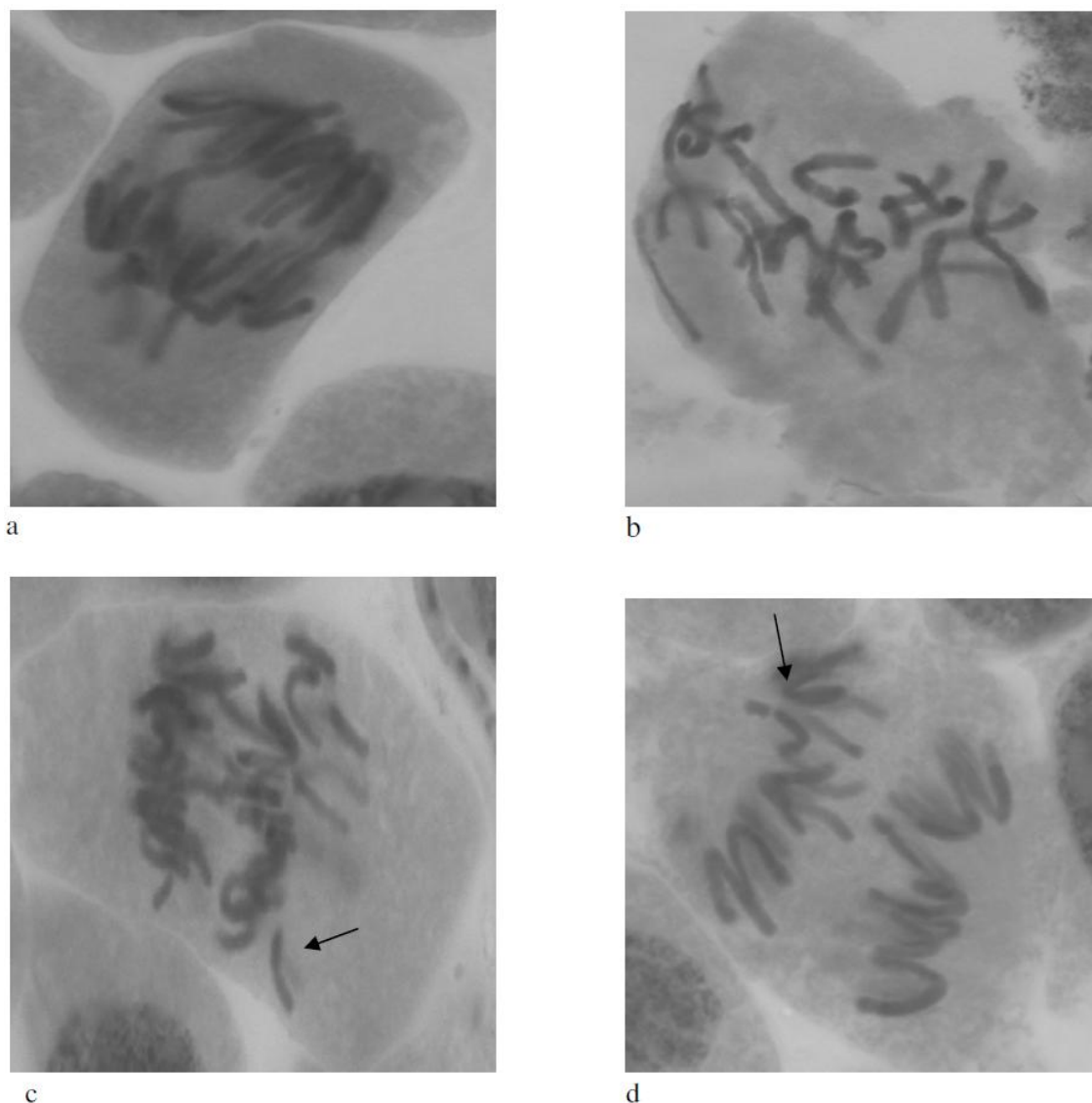


Figure 1. Chromosomal aberrations in mercury exposed root meristem cells of *Allium cepa*. (a) Delayed anaphase, (b) C-mitosis, (c) Vagrant chromosomes and (d) Chromosomal break(s).

showed the dose dependent effect. The reduction in percentage of chromosomal aberrations in extract treated groups before, after and simultaneous mercury treatment showed that substances in Indian mustard aqueous extract have anti-genotoxic effects.

DISCUSSION

Traditional medicines (phytotherapy) are commonly used to treat many diseases, besides modern medicines. People prefer to use herbal product because synthetic drugs can cause different side effects, so about 80% of the world's population uses medicinal plants (Jovtchev et al., 2002; Sultan and Çelik, 2009). Dietary constituents

suppress the genotoxic damage induced by genotoxins/mutagens through various intra and extra cellular mechanisms. Therefore scientific concerns are on the significance of natural compounds. Recently, we also reported anti-genotoxic effects of methanol extract of *B. juncea* in *A. cepa* L. (Sharma et al., 2010). In the present study, we investigated anti-genotoxic potential of *B. juncea* seeds aqueous extract against the genotoxicity induced by mercury. In view of the fact that the aerial parts of this plant are widely used but due to the lack of information about their antigenotoxic nature, it is important to evaluate its effects on genetic material of cells. Data on the effects of the aqueous extract on root growth and root number of *A. cepa* showed that there was concentration-dependent increase in both (Table1)

Table3. Effect of pre-, post- and simultaneous- treatments of aqueous extract of *B. juncea* seeds on genotoxic effects induced by mercury in root tip cells of *Allium cepa*.

Type of chromosomal aberrations	Negative control	Positive control	0.1%			0.25%			0.50%			0.75%			1.0%		
	Aberrant cells ^a	Aberrant cells ^a	Aberrant cells ^a			Aberrant cells ^a			Aberrant cells ^a			Aberrant cells ^a			Aberrant cells ^a		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Physiological aberrations (PA)																	
C-mitosis	4	38	15	8	10	11	7	8	8	5	6	5	3	4	3	1	2
Delayed anaphase	4	24	11	18	12	10	11	10	8	5	8	6	2	6	5	1	2
Laggard/s	1	14	6	10	5	4	6	3	3	4	2	1	3	1	-	-	-
Stickiness	-	19	7	9	4	6	6	3	4	4	2	3	3	1	2	1	1
Vagrant/s	2	10	7	12	8	5	10	6	3	6	4	-	4	3	1	2	2
Total PA	11	105	46	57	39	36	40	30	26	24	22	15	15	15	11	5	7
Clastogenic aberrations (CA)																	
Chromatin bridge/s	-	45	15	10	10	10	9	9	8	8	7	6	5	5	3	3	3
Chromosomal break/s	2	55	8	9	8	8	7	6	6	5	5	4	4	3	2	3	2
Ring chromosomes	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total CA	3	103	23	19	18	18	16	15	14	13	12	10	9	8	5	6	5
(PA+CA)	14	208	69	76	57	54	56	45	50	37	34	25	24	23	16	11	12

NC: Negative control (distilled water), PC: Positive control (Hg-0.75 ppm) a= out of 400 cells examined; A= Pre-, B= Post- and C= Simultaneous- treatment.

Table 4. Effect of pre-, post- and simultaneous- treatments of aqueous extract of *B. juncea* seeds on percentage inhibition of genotoxic effects induced by mercury in root tip cells of *Allium cepa*.

Types of aberrant cells	Negative control ^c	Positive control ^a	Aqueous extract														
			0.1%			0.25%			0.50%			0.75%			1.0%		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
No. of cells with PA ^b	11	105	46	57	39	36	40	30	26	24	22	15	15	15	11	5	7
PI of PA			62.7	51.1	70.2	73.4	69.1	79.7	84	86.1	88.3	95.7	95.7	95.7	100	106.3	104.3
PI of CA			80	84	85	85	87	88	89	90	91	93	94	95	98	97	98
(PA+CA)	14	208	69	76	57	54	56	45	50	37	34	25	24	23	16	11	12
PI of (PA+CA)			71.6	68	77.8	79.3	78.3	84.1	81.5	88.2	89.6	94.3	94.8	95.4	98.9	100	101.1

A= Pre-, B= Post- and C= Simultaneous-treatment, PI= Percentage inhibition, PA=Physiological aberrations, CA= Clastogenic aberrations, TA= Total aberrations, **PI** = a-b/a-c x 100 where a= number of aberrant cells induced by positive control, b= number of aberrant cells induced by seed extract and mercury, c= number of aberrant cells induced by negative control.

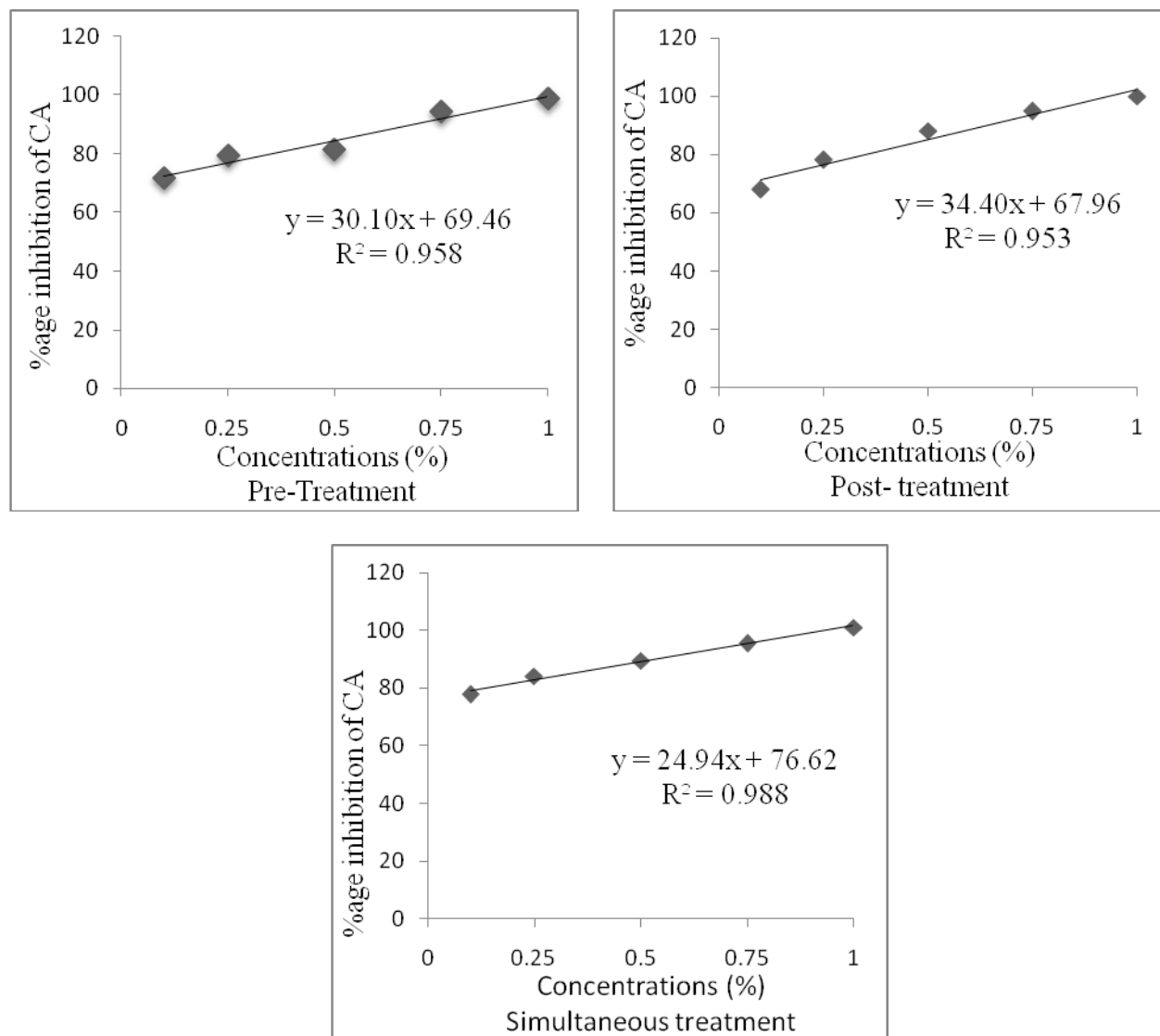


Figure 2. Relationship between different concentrations (0.1, 0.25, 0.50, 0.75 and 1.0 %) of aqueous extract of *B. juncea* seeds and percent inhibition of genotoxic effects induced by mercury (0.75 ppm) in *Allium cepa* root chromosomal aberration assay.

and has inhibitory and mitodepressive effect on *A. cepa* root tip cells. There is a linear relationship between macroscopic and microscopic parameters for all concentrations of all treatments. Aqueous extract of seeds of Indian mustard showed increase in mitotic index at all the concentrations significantly. Indian mustard extract contain a complex mixture of natural substances and preliminary phytochemistry studies revealed the presence of glucosinolates, sinigrin, and many enzymes like myrosin and sinapine.

Van poppel et al. (1999) found the protective effect of Indian mustard against cancer due to the relatively high concentration of glucosinolates and their hydrolytic products like Isothiocyanates and indoles. The important

glucosinolates found in *Brassica* vegetables are methionine-derived glucosinolates (Mithen et al., 2003). Therefore, these compounds could be possibly responsible for the observed antigenotoxic effects. Chromosomal aberrations are structural changes in chromosomes, resulting from a break or exchange of chromosomal material. Most of chromosomal aberrations observed in cells are lethal, but there are many aberrations cause genetic effects, either somatic or inherited (Swierenga et al., 1991; Akinboro and Bakare, 2007). Chromosomal break/s, chromatin bridge/s and c-mitosis were major type of aberrations observed in *A. cepa* cells and showed a significant decrease in their percentage after all three types of extract treatments.

Among different heavy metals, mercury (Hg) is a known mutagen due to its toxic nature and great versatility of uses in a wide variety of chemicals, many of which are proven or suspected carcinogens. In the present investigation Indian mustard aqueous extract exhibits antigenotoxic potential against mercury induced damage in a dose dependent manner. Therefore, our results showed that, aqueous extract of Indian mustard has protective effect on *A. cepa* root tip meristematic cells against chromosomal aberrations induced by mercury. The increase in mitotic index (number of dividing cells) and decrease in chromosomal aberrations showed the antigenotoxic nature of that substances present in *B. juncea* seeds aqueous extract (Table 2). These results are in accordance with the literature data. The various plants extracts, studied have been reported to contain lipids, alkaloids, glucosinolate, and a number of other antioxidant phytochemicals (Rodrigo et al., 1992; Sharaf et al., 2000; Germano et al., 2002).

The results of this study suggest that, *B. juncea* seeds aqueous extract has antigenotoxic potential against chromosomal aberrations induced by mercury in *A. cepa* in a concentration dependent manner, and it is very effective at highest concentrations (0.75 and 1.0%). However, the mechanism by which it acts remains to be investigated in different test system and further studies are necessary. Studying with 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.

Conclusion

On the basis of our results, we conclude that aqueous extract of *B. juncea* (Indian mustard) seeds has antigenotoxic potential against mercury in *A. cepa* in a dose dependent manner. Considering the great versatility of uses of Indian mustard, public awareness regarding its antigenotoxic value is very important. However, for this further studies are necessary with other test systems to strengthen these findings.

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