Int J Pharm Bio Sci 2014 Jan; 5(1): (B) 165 - 170

Research Article **Ecotoxicology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

CYTOTOXIC AND CHROMOTOXIC EFFECTS OF CRUDE EXTRACT OF BOTTLE GOURD IN *Vicia faba* ROOT MERITEM CELLS

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ABSTRACT

In the present investigation cytotoxic and chromotoxic effects of crude extract of bottle gourd was evaluated by employing *Vicia faba* root meristem assay. Roots of *Vicia faba* were treated with 100, 250 and 500 g/L solution of crude extract at room temperature in dark for 6, 12, 18 and 24 h. Cytotoxicity and chromotoxicity were expressed in terms of depression in mitotic activity and frequency of abnormality in dividing cells in different phases of mitotic cycle respectively. Treatment of roots with the crude extract resulted in concentration and period of treatment dependent depression in mitotic index and increase in the frequency of abnormal cells in root tips. The result shows that crude extract of bottle gourd induced cytotoxic and chromotoxic effects in the root tip cells which may be due to the presence of residues of pesticides.

KEYWORD: Cytotoxicity, Chromotoxicity, Bottle gourd, Crude extract, *VIcia faba,*

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INTRODUCTION

In the recent past contamination of food commodities by pesticide have been extensively highlighted in the media including research journals and attracted wider debate and sharp focus among the interested groups. Indiscriminate and excessive application of synthetic pesticides damaged not only the environment and agriculture but also has entered into the food chain thereby affecting human health. In our country, farmers use about 6000 tonnes of active ingredients of different pesticides to control pests of vegetables and fruits[1] in which vegetables alone consume nearly 14% of the total pesticides. Contamination of vegetables with pesticides residue has been reported worldwide including India. Surveys carried out by different institutions spread throughout the nation indicated that 50-70% of total vegetables are contaminated with pesticide residues[2]. The people stand the risk of exposure to lethal pesticides present in the vegetables they consume. Presence of pesticides residue in fresh vegetables, both above and below the MRLs, have been reported by Dethe et al.[3] and Reddy et al.[4]. Sasi and Sanghi[5] observed presence of high levels of malathion. DDE, a metabolite of DDT, BHC, dimethoate, endosulfan and ethion in freshly collected vegetables from Kanpur. Leafy vegetables like spinach, fenugreek, mustard seem to be most affected. Radish also showed high levels of contamination. Samples of vegetables collected from Jaipur city were found contaminated with Organochlorine pesticides (OCP). OCP residue levels in majority of samples were above the maximum acceptable daily intake (ADI) prescribed by WHO[6]. In a study Kumari Beena et. al.[7] tested samples of vegetables from Hisar, Haryana and found 100% contamination with low but measurable amounts of pesticide residues. In the samples tested residue levels of organophosphorous insecticides were highest followed by carbamates, synthetic pyrethroids and organochlorines. About 32% of the samples showed contamination with organophosphorous and carbamate insecticides above their respective MRL values. Mukherjee[8] analysed several vegetable samples from Delhi and found that all the samples were contaminated with pesticides, but only 31% of the samples contained pesticides above the prescribed tolerance limit. A total of 182 samples of six vegetables collected from different agricultural fields of central Aravalli region of Rajasthan revealed that 40.11% of total samples were contaminated with different pesticide residues, among which 35.62% of total contaminated samples exceeded the maximum residual limits (MRLs) values[9]. Mukherjee et. al.[10] reported presence of residues of Organochlorine pesticide in some vegetables from West Bengal but the residue levels were below maximum residue limits (MRLs) indicating minimal risk to the consumers. Bankar et. al. [11] reported presence of 15 pesticides from different chemical groups in vegetable samples collected from different places in Uttar Pradesh.. Pesticide residues present in fruit and vegetables represent a risk for human exposure. The mutagenic and carcinogenic action of pesticides on experimental animals is well known. This research therefore sought to evaluate the cytotoxic and chromotoxic effects of the aqueous extracts of bottle gourd obtained from an open market using *Vicia faba* root meristem assay.

MATERIALS AND METHODS

Bottle gourd (Lagenaria siceraria, Family Cucurbitaceae), collected from local market and brought to the laboratory for further processing. The samples were washed several times with distilled water until no foreign material remained and air dried for two hours at room temperature. The air dried samples were cut into small pieces and mixed thoroughly. 100, 250 and 500 g of each sample were weighed and blended in 1 L of distilled water until homogenous formulations were obtained. The resultant formulations were filtered with a piece of muslin cloth and the filtrates were used for treatment. 50 seedlings with newly emerged roots 1–2 cm in length were selected for treatment. 20 seedlings were transferred into container (10 cm tall, 30–20 cm diameter) containing one of the solutions viz. 100 g/L, 250 g/L and 500 g/L of extract of bottle gourd and treated for 6, 12, 18 and 24

hours. The negative and positive control groups were suspended over the distilled water and solution containing 0.2 % EMS for the same periods respectively. The solutions of test compounds were put in containers covered with aluminium paper with holes which permitted the roots to be exposed. The experiments were set in the dark. Primary roots from each treatment group were harvested based on uniformity of size after the end of treatment periods. The roots were rinsed in distilled water to remove traces of adhering treatment solution.

The roots were treated with 0.002M 8-hydroxyquinoline for 24 hours at 15°C. After treatment, the roots were washed in distilled water thoroughly and then fixed in freshly prepared fixative (glacial acetic acid and ethanol 1:3 v/v). After 24 hours, the tips were washed and transfered to 70% ethanol and stored in refrigerator for cytological preparation. The solutions were freshly prepared before use. For slide preparation and microscopic examination, the root tips were hydrolyzed in 1 N HCl at 60°C for 10 min. After hydrolysis the roots were stained in 1.5% acetocarmine. 1 mm of the mitotic zone from well-stained root tips were cut on a clean slide and squashed under a cover glass in a drop of acetic acid (45%). The slides were scored under a light microscope for number of dividing and non-dividing cells and number of cells showing abnormal mitosis. Mitotic Index (MI%) was determined by counting the number of mitotic cells among the total amount of scored cells per seedlings. 1000 cells from each root tip and a total of 5000 cells were screened for dividing and non-dividing cells. Mean mitotic index in control, positive control and treated root tips was determined by the following formula:

M.I. (%) =No. of dividing cells/Total number of cells scored X100

Twenty five microscopic fields in each slide, selected randomly, were screened carefully for total number of cells showing abnormal mitosis. The mean percentage of aberrant cells was calculated for each treatment.

RESULTS AND DISCUSSION

The results obtained on the effect of treatment of roots of *Vicia faba* with crude aqueous extracts of Bottle gourd on mitotic index are presented in Tables 1. The data shows that treatment of roots for 6, 12, 18 and 24 h with 100, 250 and 500 g/L crude aqueous extract produced inhibitory effect on mitotic activity. However, statistically significant effects of treatment were seen after 12 h (at 500 g/L concentration), 18 h (at 250 and 500g/L concentrations) and 24 h of treatment. Two way ANOVA test (Table 2) revealed statistically highly significant variations both between concentrations of crude aqueous extract and treatment durations. Mitodepressive action of the crude extract of bottle gourd may be reason of the reduction of mitotic index as observed here. Inhibition of mitotic activities is often used for tracing cytotoxic substances. The lowering of mitotic index might be due to the inhibition of DNA synthesis at S-phase or a blocking in the G2-phase of the cell cycle thus preventing the cell from entering mitosis. Inhibition of the enzyme DNA polymerase, which is necessary for the synthesis of DNA as well as other enzymes directly involved with spindle production, assembly or orientation, may be the reasons for antimitotic effect of the crude extract.

Table 1
Effect of crude aqueous extract of bottle gourd on the cell division in the root meristem of Vicia faba L.

Concentration	Period of treatment	Number of cells observed	Number of actively dividing cells	Mitotic Index (MI) % (Mean ± SD)
Control		5000	376	7.52 ± 0.87
100 g/L	6 hrs	5000	279	5.58 ± 1.22
250 g/L		5000	255	5.10 ± 1.18
500 g/L		5000	246	4.92 ± 1.06
100 g/L	12 hrs	5000	258	5.16 ± 1.34
250 g/L		5000	249	4.98 ± 1.11
500 g/L		5000	223	4.46 ± 1.07*
100 g/L	18 hrs	5000	239	4.78 ± 1.04
250 g/L		5000	227	4.54 ± 0.65*
500 g/L		5000	216	4.32 ± 0.97*
100 g/L	24 hrs	5000	223	4.46 ± 0.65*
250 g/L		5000	211	4.22 ± 0.42*
500 g/L		5000	198	3.96 ± 0.54**

, ** Statistically significant at p<0.05 and 0.01 respectively in Student's t-test

Table 2
Results of ANOVA showing significance of differences, if any, between control and treatments

Source of variation	Degree of freedom	Sum of Square	Mean Squares
Between Treatment	3	24.41	8.13
Between Periods	3	1.21	0.40
Random (residual)	9	0.45	0.05
Total	15	26.07	

F = 159.73 = (MS treatment/MS residual) p <0.001
F = 7.918 = (MS period /MS residual) p<0.01

The chromosome damaging ability of crude aqueous extract of bottle gourd was measured in terms of number of abnormally dividing cells and percent abnormal cells and the data collected are shown in Table 3, Treatment of roots with crude aqueous extract for 6, 12, 18 and 24 h resulted in concentration and duration of treatment related increase in the aberration rate.

Table 3
Induction of mitotic abnormality in the root meristem of Vicia faba after treatment with crude aqueous extract of bottle gourd.

Concentration	Period of treatment	Number of cells observed	Number of actively dividing cells	Number of abnormally dividing cells	% Abnormal cells (% that of observed cells)
Control		1496	94	3	0.20
100 g/L	6 h	1444	70	5	0.35
250 g/L		1468	64	7	0.48
500g/L		1412	62	9	0.64*
100 g/L	12 h	1438	65	6	0.42
250 g/L		1446	62	7	0.48
500g/L		1411	56	9	0.64*
100 g/L	18 h	1432	60	7	0.49
250 g/L		1423	57	8	0.56*
500g/L		1404	54	10	0.71**
100 g/L	24 h	1365	56	8	0.59*
250 g/L		1332	53	10	0.75**
500g/L		1311	50	14	1.07**

**** and ** differ significantly from the control in Student's t-test at p<0.05 and P<0.01 respectively.***

Analysis of variance (ANOVA) test (Table 4) revealed statistically highly significant variation between concentrations ($p<0.001$) of crude aqueous extract and periods of treatment ($p<0.01$). Microscopic examination of the squashes of root tips of roots treated with crude extracts of bottle gourd showed that the abnormality occurred in all the phases in the mitotic cycle. Disturbed metaphase and disturbed anaphase. Fragmentation of chromosomes, bridge at anaphase, were most common types of mitotic abnormalities lagging chromosome/chromatid and stickiness of chromosomes were observed only at higher concentration and longer period of treatment.

Table 4
Results of ANOVA showing significance of differences, if any, between control and treatments

Source of variation	Degree of Freedom	Sum of square	Mean Squares
Between Treatment	3	0.6647	0.2216
Between Periods	3	0.1375	0.0458
Random (residual)	9	0.0699	0.0077
Total	15	0.8722	

F = 28.51 = (MS treatment/MS residual) p <0.001
F = 5.900 = (MS period /MS residual) p<0.01

The induction of mitotic abnormalities as observed in the present experiment show the presence of some chromotoxic chemicals in the crude extract of bottle gourd. These chemicals may have disturbed the synthesis of DNA and protein, or the translation of RNA, so that materials relating to the chromosomal movement could not be synthesized and the chromosomal aberration occurred eventually. It is evident that crude extract of bottle gourd more or less affect the structure and integrity of chromosomes as well as also affects the spindle apparatus.

CONCLUSION

The overall results of the present study show that aqueous extract of bottle gourd contain some cytotoxic and chromotoxic compounds that caused inhibition in mitotic activity and induced abnormality in the mitotically dividing cells in the root tip cells. These compounds may be the residues of different pesticides used by farmer in course of cultivation. Although, presence of pesticide residues has not been traced, this, thus considered as limitation of this study. Nevertheless, the results obtained showed that the tested extracts exhibit mitodepressive and chromotoxic effects in *V. faba* cells.

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