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Cytotoxic effects of Cabbage (*Brassica oleracea* var. *capitata*) Leaves extract on the Root Meristematic Cells of *Vicia faba* L.

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ABSTRACT: Cabbage leaves are consumed as a vegetable all over the world. This member of family Brassicaceae is a popular ingredient of international salad. It is a rich source of vitamins and minerals but its over consumption may hamper human health also. In this study the investigator has tried to find out the possible cytotoxic effects of cabbage leaves aqueous extract on meristematic cells of *Vicia faba* L. Actively growing root tips of *Vicia faba* L. were treated with 100%, 75%, 50%, 25% and 10% concentrations of cabbage leaves extract for 2, 4 and 6 hours. The results obtained showed that the cabbage leaves extract is mitodepressive in nature and induces various types of chromosomal and nuclear aberrations such as multinucleate cells, stickiness at metaphase, disturbed metaphases, scattered metaphases, chromatid separation, polarity abolition, laggards and bridges at anaphases and telophases. It is concluded that cabbage leaves extract not only affect mitotic activity but also shows adverse impact on chromosomal behaviour of cells.

Keywords: Cabbage, Chromosomal and nuclear aberrations, Cytotoxic.

INTRODUCTION

Cabbage is a widely consumed vegetable all over the world. Its botanical name is *Brassica oleracea* var. *capitata* and it is the member of family *Brassicaceae*. It is an important constituent of international salad and consumed raw as well as in cooked form. It is used in several Chinese and Indian dishes as an unavoidable constituent. Because of colour and crispness, it is used as salad in several cuisines. It is a rich source of vitamins and minerals particularly vitamin 'A' and calcium. Now it is well known fact that fresh and raw vegetables are excellent source of minerals, vitamins and other essential dietary constituents. Moreover, raw vegetables provide roughage for the body which is highly helpful in the bowel movement. Though fresh and raw vegetables have an excellent amount of essential dietary constituent but their excessive use may hamper human health also. It has been studied that *Brassica* species and some green leaves contain glucosinolates and fatty acids. Glucosinolates are known to induce thyroid deficiency. Cabbage, cauliflowers and mustard seeds contain goitrogenous compounds. Kassie *et al.* (1996) studied that *Brassica* vegetables contain DNA damaging compounds.

It is well known that raw vegetables used in salad ensure better health without any adverse effect on the body. But when we use them regularly in excessive amount, some side effects or cytotoxic activity may result in future which may cause major disorders in the human body. Till now several vegetables and other plant extracts have been tested for their cytotoxic and mutagenic potentiality. Yadav *et al*. (2001) studied the cytotoxic potentiality of tomato fruit aqueous extract on the root tip cells of *A. cepa* and found that the tomato fruit extract is not only mitodepressive in nature but also produced several chromosomal aberrations. Ali and Celik (2007) evaluated the cytotoxic effects of *Citrus limon* and *Citrus simensis* peel extracts on the root meristematic cells of *Allium cepa*. They found that each test concentration of *Citrus limon* and *Citrus sinensis* ethanolic extracts adversely affected the mitotic index and also produced genotoxic effects like breaks, bridges, stickiness, pole deviation and micronuclei in the dividing cells.

Ali (2010) studied the cytotoxicity of extract of flowers of *Hibiscus rosa-sinensis* on root meristem of *A. cepa* and suggested that flowers contain anti-mitotic substances which inhibit cell division at any stage of the cell cycle. Vaish (2016); Vaish and Saxena (2017) studied the cytotoxic effects of radish (*Raphanus sativus*) root extract on the root meristem of *Allium cepa* and *Vicia faba* respectively and found out that the root extract not only adversely affected mitotic activity in both the plants but also showed significant nuclear and chromosomal aberrations in the dividing cells. Mert and Burun (2020) investigated cytotoxic and genotoxic effects of the *Euphorbia rigida* Bieb. aquous extract, a natural pesticide, and observed adverse effects on mitosis and exhibited cytotoxic and genotoxic effects on the dividing cells. Vaish (2021) studied the mitodepressive and chromotoxic effects of spinach leaves extract on root meristematic cells of *A. cepa* and concluded that the extract is not only mitodepressive but also induced several types of nuclear and chromosomal aberrations like nuclear disintegration, binucleate cells, breaking in chromosomes, chromatid separation, scattered metaphases, stickiness of chromosomes, bridge formation, chromosomal

condensation and disturbed polarity. Velazquez-Vazquez *et al.* (2022) evaluated the genotoxicity and cytotoxicity of ethanol extract of *Sambucus canadensis* on the root tip meristematic cells of *Allium sativum*. They observed a dose dependent decrease in the number of dividing cells and also studied several cellular abnormalities in the root tip meristematic cells of *Allium sativum*. Vaish (2022) evaluated the effect of carrot (*Daucus carota* L.) root extract on meristematic cells of *A. cepa* L. and found that the low concentrations of the root extract showed positive impact on the mitotic activity but high concentrations exhibited mitodepressive nature and induced some chromosomal abnormalities also. Farooquee and Dutta (2023) studied that the raw *Aloe vera* gel extract shows few cytotoxic effects on the root tip cells of both *Allium cepa* and *Allium sativum*. Singh *et al.* (2023) evaluated the cyto-genotoxicity of *Parthenium hysterophorus* plant extract on *Allium cepa* and suggested that extract is capable to produce various structural and functional changes in mitotic cells.In the present study, the author has tested the possible mitodepressive and cytotoxic nature of the aqueous extract of cabbage leaves and possible nuclear and chromosomal aberrations induced by it in the root meristematic cells of *V. faba.*Thus, from this investigation it will come to know whether the fresh cabbage leaf aqueous extract have any cytotoxic or chromotoxic property or not.

MATERIAL AND METHOD

The seeds of *V. faba* were procured from G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The seeds were disinfected by keeping them in a decanted 5% calcium hypo chloride solution for 3-4 minutes before soaking. Seeds were thoroughly washed after this treatment, with 4-5 changes of distilled water and then were soaked for 6-18 hours in distilled water at room temperature. The soaked seeds were then allowed to germinate in sterile moist sand for 3-4 days at the temperature of 24°C. The seedlings produced 3-5 cm. long primary roots during this period. The tips of primary roots were removed to stimulate the growth of lateral secondary roots. The lateral roots developed and attained 1-2 cm. length within 4-5 days which were used for the treatment.

The fresh cabbage heads were directly purchased from the farmers. The leaves of the heads were separated and washed thoroughly then cut into pieces and transferred to electric juicer to obtain the juice of the leaves. The juice filtered and four concentrations (10%, 25%, 50% and 75%) of cabbage leaves extract were prepared by adding fresh distilled water. Pure aqueous extract of cabbage leaves was also used as 100% concentration for the experiment. The root tips of *V. faba* were treated with all these five concentrations of vegetable extract separately for 2, 4 and 6 hours respectively in the environmental chamber at 20°C, following the method of Mousa (1982). The treated root tips were washed with distilled water, cut carefully and fixed in Carnoy's fluid (1:3 acetic-alcohol) for 24 hours and then stored in 70% alcohol for cytological investigation. The treated root tips of *Vicia faba* L. (2n = 12), were first hydrolyzed in 1N HCl for 5 minutes at room temperature and finally washed with 3-5 changes of distilled water. The root tips were then squashed in few drops of 2% aceto-carmine stain for chromosomal studies following the standard acetic-carmine method. The smears were sealed temporarily for the cytological studies. The mitotic index, frequency of aberration and their percentages as triggered by aqueous solutions of vegetables extracts, in each case, were calculated by the method of Mousa (1982); Auti *et al.* (2010). The significant induced aberrations in each case were microphotographed using NIKON microphotographic equipment.

RESULT AND DISCUSSION

It is clear from the Table 1 and Graph 1, that all five concentrations (10%, 25%, 50%, 75% and 100%) of aqueous extract of cabbage leaves extract showed inhibitory effect on mitotic activity in *V. faba* root meristematic cells as compared to control. Mitotic Index (MI) in control sets was 22.34%, which decreased to 20.00% in 10% concentration when the root tips were exposed for 2 hours of treatment. In all other concentrations and durations, a gradual decrease in MI was observed and finally in 100% concentration, the MI decreased to 18.46% when the 6 hours treatment of the aqueous solution of cabbage leaves was provided. It was concluded that the aqueous solution of cabbage leaves is mitodepressive in nature. The cabbage leaves extract induced various nuclear and chromosomal aberrations also in the dividing cells of *V. faba* root. Only multinucleate cells were observed as abnormality during interphase. The higher concentrations (75% and 100%) were found to be potent to cause multinucleate cells. Highest percentage of multinucleate cells was 2.13 observed in 75% concentration, which dropped to 2.05 in 100% concentration. At prophase, no aberration was recorded. At metaphase, four types of aberrations were recorded including disturbed, scattered, sticky metaphases and breaking of chromosomes. Percentage of disturbed metaphases (Fig. a) in 10% concentration was 16.32, which increased up to 20.51 in 50% concentration, but a sudden drop in the percentage was observed in 75% concentration, which again increased up to 23.68 in 100% concentration. Scattered metaphases (Fig. c) were observed only in 50%, 75% and 100% concentrations with a percentage value of 2.56, 4.44 and 5.26 respectively. Stickiness of chromosomes (Fig. b) was observed in all the concentrations showing increasing trend in its percentage value with an exception in 75% concentration where a slight drop was observed in comparison of previous concentration. The highest value of stickiness at metaphase was 34.21% when root tips were treated with 100% concentration. Breaking of chromosomes during metaphase was observed only in 100% concentration with percentage value of 2.63. Bridges, polarity abolition and laggards were the three types of aberrations recorded at anaphase. Bridges were observed in all concentrations and their percentage increased from 3.70 in 10% concentration to 12.0 in 100% concentration. Polarity abolition was observed in higher concentrations only. In 75% concentration, the

percentage of the aberration was 9.67 whereas in 100% concentration, it increased up to 12.0. Laggard was observed only in 50% concentration with a percentage value of 3.84. At telophase, bridge formation (Fig. d) was noticed in all concentrations. In 10% concentration, the percentage of bridge formation was 4.0, which decreased to 3.03 in 25% concentration but in 50% concentration it again increased to 4.0 and then showed an increasing trend up to 100% concentration with highest value of 9.52.It has been concluded that cabbage leaves extract is mitodepressive in nature and shows considerable impact on behavior of chromosomes only when *V. faba* root tips were treated with higher concentrations of the cabbage leaves extract. Several studies indicated that aberrations in chromosomes serve as an indicator of mutation. According to an estimate, dietary factors responsible for 30% of cancer deaths. Plants produce their own toxic chemicals to ward off insects, fungi, bacteria and other harmful organisms. A number of these chemicals are animal carcinogens or are converted to carcinogens by metabolic reactions in the body. We are eating more than 10000 times more of nature's pesticide than of man-made pesticides (Ames, 1984). The adverse effect of cabbage leaves extract may be due to the presence of glucosinolates and/or their breakdown metabolic products which have been previously found to be potent genotoxic in bacterial and mammalian cells. It has been concluded that cruciferous vegetables have DNA damaging constituents (Kassie *et al.,* 1996). Among all, only the higher concentrations (75% and 100%) of cabbage leaves extract induced multinucleate cells in the testing plant (Table 1). Similar aberration was also observed when effect of Hashish (*Cannabis*) was tested on mitosis of *A. cepa* L. The formation of binucleate and multinucleate cells in treated materials may be due to disorders leading to the inhibition of cytokinesis. Multinucleate cell may result from a multipolar telophase, if cytokinesis failed after the telophase (Malallah and Kabarity 1982).

Scattered metaphases were studied only in the cells when root tips were treated with 50%, 75% and 100% concentrations. Uncoordinated and partial loss of chromatid cohesion trigger the entry into the scattered state (Stevens *et al.,* 2011). All the used concentrations of cabbage leaves aqueous extract induced stickiness in root meristematic cells of *V. faba*. Stickiness was also observed by Arindam (2000) in *Hordeum vulgare* treated with carbonaceous effluent of sugar mill. According to Klasterska *et al.* (1976), improper folding of chromosome fibers results in chromosomal stickiness. El-Sadek (1972), considered it as an unspecific physiological disturbance and has been attributed to an action on the proteins of chromosomes. Stickiness is to be the result of physiological effect by radiations or chemicals, which later on may lead to fragmentation of chromosomes from the stress of anaphase movement or in the formation of bridge, when the chromosomes fail to separate (Sudharsan and Reddy 1971).

Bridges were observed in the dividing cells when treated with all the five concentrations of cabbage leaves extract.

Previously, bridges at anaphase were reported in *V. faba* with the treatment of asafetida (Das *et al.,* 1968), Whisky (Gowrishanker *et al.,* 1993). Bridges at anaphase were also observed in meristematic cells of *A. cepa* when treated by tomato extract (Yadav *et al.,* 2001). Evans (1962) suggested that bridges are formed because of difficulty in separation of chromosomes at anaphase due to clumping or adhesion of chromosome. Chromosomal bridges may also be formed due to the stickiness of chromosomes and the subsequent failure of anaphase separation (Abraham and Koshy 1979). Njagi and Gopalan (1981) encountered that the bridges may result in loss of genetic material of the cell. According to Gopalan and Njagi (1984), bridges are produced due to attachment of sister chromatids at the secondary constriction. It results in the fusion of bridges and leads to a high occurrence of single and multiple bridges. Finally, these bridges are broken and the broken ends are withdrawn into the resting nuclei, in which these were generally present as a pear-shaped projection to mark their position.

75% treatment of the aqueous extract of cabbage induced laggard formation in *V. faba*. Adam and Farah (1989) reported laggards in *V. faba* root tips treated with water extract of *Cymbopogon proximus*. Sometimes lagging chromosome interfere with the process of cytokinesis and lead to binucleate cells. Lagging chromosomes are the fragments or the daughter chromosomes left over on equatorial plate after anaphase, which lack centromere and do not connect to any of the poles, but remain in the cytoplasm and later form micronuclei (Somashekar *et al.,* 1985). The chromosome fragments both large and small get incorporated in the daughter nuclei probably due to stickiness, since none of them was observed lying at the equatorial region or between the two anaphase groups of chromosomes at late anaphase stage.

Telophasic bridges were observed in the cells in all the concentrations of cabbage leaves extract. Telophasic bridges were also observed by Prasad and Das (1977) in *V. faba* root tips cells during the study of effect of some growth substances. Soh and Yang (1993) have also observed telophase bridges in *A. cepa* L., when they studied the impact of plant growth regulators on mitotic chromosomes Arindam (2000) has also reported telophasic bridges in *Hordeum vulgare* with the treatment of carbonaceous sugar mill effluent. These bridges might be due to the stickiness at cytochemical level.

Recently, attention is being paid to test the cytotoxic and mutagenic efficacy of medicinal plants, vegetables, fruits, spices and other plant extracts. However, only a fragmentary work is available on vegetables. The aim of present study was to evaluate the cytotoxic effects of cabbage leaves extract on chromosomal behavior during mitotic divisions in root meristem of plants.

Table 1: Mitotic index, frequency of aberrations and their percentage as induced by *Brassica oleracea* L. var. *capitata* on *V. faba* L. (2n = 12).

Concentration	10%				25%				50%				75%				100%				Control			
Duration (in hrs.)	2	4	6	% of abr. (conc. wise)	2	4	6	% of abr. (conc. wise)	2	4	6	% of abr. (conc. wise)	2	4	6	% of abr. (conc. wise)	2	4	6	% of abr. (conc. wise)	2	4	6	
Mitotic index (in %)	20.00	19.85	19.04		19.92	19.62	19.00		19.66	19.07	18.69		19.25	18.80	18.66		19.06	18.82	18.46		22.34	22.34	22.34	
Types of aberrations																					NO ABERRATION			
M.N	-	-	-	-	-	-	-	-	-	-	-	-	-	M.N	M.N	2.13	M.N	M.N	M.N	2.05				
Br. 'M'	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Br. 'M'	-	2.63				
D. 'M'	D. 'M'	D. 'M'	D. 'M'	16.32	D. 'M'	D. 'M'	D. 'M'	17.77	D. 'M'	D. 'M'	D. 'M'	20.51	D. 'M'	D. 'M'	D. 'M'	20.00	D. 'M'	D. 'M'	D. 'M'	23.68				
Sc. 'M'	-	-	-	-	-	-	-	-	Sc. 'M'	-	-	2.56	-	Sc. 'M'	Sc. 'M'	4.44	-	-	Sc. 'M'	5.26				
St. 'M'	St. 'M'	St. 'M'	St. 'M'	18.36	St. 'M'	St. 'M'	St. 'M'	20.00	St. 'M'	St. 'M'	St. 'M'	25.64	St. 'M'	St. 'M'	St. 'M'	24.44	St. 'M'	St. 'M'	St. 'M'	34.21				
Bri 'A'	-	Bri 'A'	-	3.70	Bri 'A'	Bri 'A'	-	4.87	-	Bri 'A'	-	7.69	-	Bri 'A'	Bri 'A'	9.67	Bri 'A'	Bri 'A'	Bri 'A'	12.00				
Lag. 'A'	-	-	-	-	-	-	-	-	-	Lag. 'A'	-	3.84	-	-	-	-	-	-	-	-				
P.A.	-	-	-	-	-	-	-	-	-	-	-	-	-	P.A.	P.A.	9.67	-	P.A.	P.A.	12.00				
Bri. 'T'	-	-	Bri. 'T'	4.00	-	Bri. 'T'	-	3.03	-	Bri. 'T'	-	4.00	-	Bri. 'T'	Bri. 'T'	6.25	-	Bri. 'T'	Bri. 'T'	9.52				

Abbreviations: M.N.- Multinucleate cells, Br. 'M'- Breakage at Metaphase, D. 'M'- Disturbed Metaphase, Sc. 'M'- Scattered Metaphase, St. 'M'- Stickiness at Metaphase, Bri. 'A'- Bridge at Anaphase, Lag. 'A'- Laggards at Anaphase, P.A.- Polarity Abolition, Bri. 'T'- Bridge at Telophase.

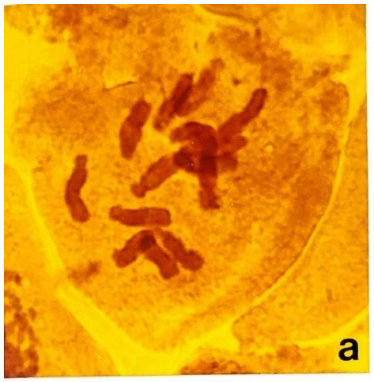


Fig. a. Disturbed metaphase

Fig. b. Scattered metaphase

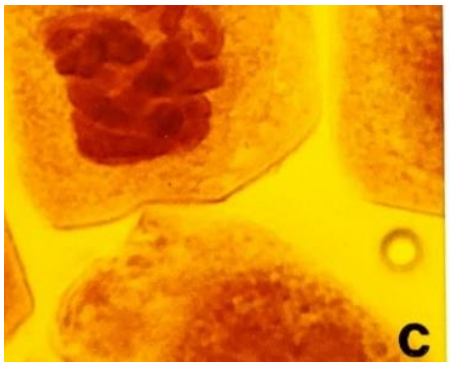


Fig. c. Stickiness at Metaphase

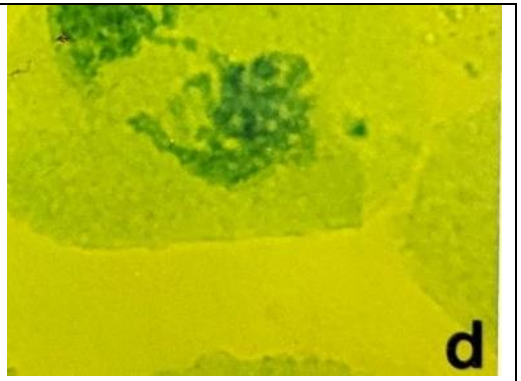
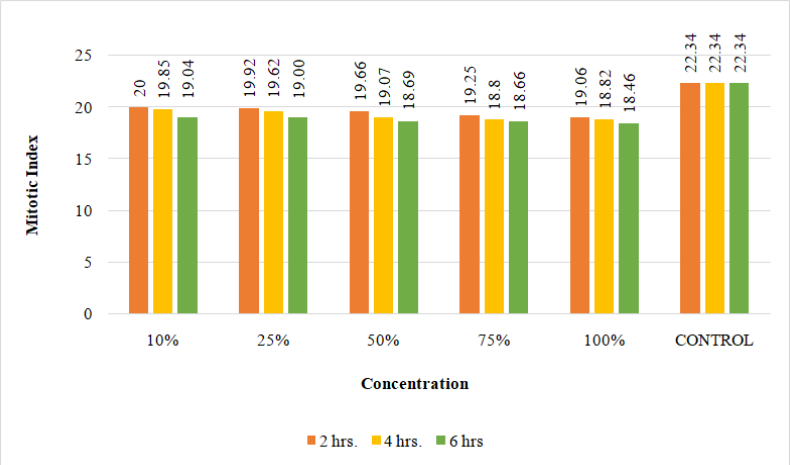


Fig. d. Bridge at Telophase



Graph 1. Mitotic index as induced by *Brassica oleracea* var. capitata on root meristematic cells of *V. faba*.

It was concluded that the cabbage leaves extract is mitodepressive in nature and induced various types of chromosomal and nuclear aberrations. The author has tried his best to test the cytotoxicity of said vegetable extract and to elaborate its possible reasons. The author does not claim himself to be perfect but definitely the present findings will open a new approach for further research.

CONCLUSIONS

The investigator concluded that the cabbage (*Brassica oleracea* var. *capitata)* leaves extract not only inhibits mitotic activity but also induces various nuclear and chromosomal aberrations. From this investigation it can be concluded that the raw cabbage should not be consumed as it causes mitotic inhibition in *Vicia faba* root tip cells. Some researchers also indicated that *Brassica* vegetables contain DNA damaging compounds. The author is of opinion that cabbage leaves must be further investigated for their mitodepressive and mutagenic potential.

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Conflict of Interest. None.

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