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## Evaluation of cytotoxicity of *Terminalia arjuna* (Roxb.) Wight & Arn. and *Moringa oleifera* Lam. in root Meristem cells of *Allium cepa* L

**Bimal Debnath, Chiranjit Paul, Amal Debnath, Debashis Saha**

### Abstract

The bark of *Terminalia arjuna* and *Moringa oleifera* are the two popular folk medicine that have been used for the treatment of different health problem since long back. In the present investigation cytotoxic and genotoxic effect of bark extracts of both the plants in *Allium cepa* root meristem cells were studied. A significant cell cycle inhibition was observed in very lower concentration (0.5-10mg/ml) of the treatments. In all the tested concentration, cytotoxic effects on cell division were also observed. The mitotic abnormalities were recorded as sticky metaphase, clumped metaphases, laggard chromosome, vagrant chromosome, chromosome bridges at anaphase and at telophase. The frequency of mitotic abnormalities was found to be directly associated with the increase in the concentration of aqueous extracts of barks of tested species. The result indicate that the inappropriate doses of these herbal medicine may lead to health complications.

**Keywords:** Cytotoxicity, Root meristem, Aqueous extract, *Terminalia*, *Moringa*

### Introduction

In recent times traditional medicine is regularly used to treat several diseases, besides modern medicine. People prefer to use herbal products because synthetic drugs can cause different side effects, so that about 80% of the world's population uses medicinal plants<sup>[1]</sup>. The growing awareness of the harmful side effects of chemotherapy has made people to explore the time tested remedies from natural resources such as plants. The universality and ability of traditional medicine/medicinal plants are evident in their constant use and dependence, till the present day, by a major portion of the world's population<sup>[2]</sup>. However, the historic role of medicinal herbs in the treatment and prevention of diseases and in the development of pharmacology does not assure their safety for uncontrolled use of an uninformed public. Injuries and even death resulting from misuse, contamination and/or adulteration of medicinal herbs have been reported<sup>[3]</sup>. In spite of the value of the medicinal herbs in the treatment of various kinds of ailment, the unrefined nature of the preparations and the lack of standard prescriptions on dosage constitute a major obstacle in the use of herbs in Medicare. These two weak points on the medicinal herbs can lead to complications in the human system. Cytotoxicity is a most important subject in pharmaceutical studies mainly in the area of cancer research. Low cytotoxicity to healthy cells and high cytotoxicity to cancerous cells is the ultimate goal of many chemotherapy drugs. Many plant extracts and their active principles have been described and utilized as therapeutic agents. There is significant interest in determining the risks that these products may pose to health, because many of these plants contain compounds which are known to cause illness even death in the animals and humans. Thus, an assessment of their cytotoxic and mutagenic potential is necessary to ensure a relatively safe use of medicinal plants<sup>[4]</sup>.

The bark of *Terminalia arjuna* has been used in indigenous system of medicine for the treatment of angina and different cardiovascular disorder. The bark of the tree is also used as a liver tonic and hepatitis reliever. The arjuna bark is also used to maintain healthy cholesterol level in human body.

*Moringa oleifera* is a miracle tree having a wide range of medicinal uses and good sources of nutrition's. Its different parts are used as cardiac and circulatory stimulant and possess antibacterial, antifungal, antiulcer, anti-tumor and cholesterol lowering activity. Therefore, it is used in traditional medicine for the treatment of different health problem like skin complain, gastric ulcer, bronchitis, eye and ear infection, urinary problem and as a powerful cold remedy.

Rural communities of the state believed that *Moringa* fruit vegetables protect them from cowpox.

In all the traditional system, the aqueous extracts of bark of these plants are used in the folk medicine. But there were no reports on cytotoxic and genotoxic effect of aqueous extract of bark in both the plants investigated. Therefore the present work is designed to evaluate the cytotoxic and mutagenic effect of the aqueous bark extract of both the plants in *A. cepa* root meristem cells.

## Materials and Methods

### Collection of plant samples

The fresh bark of *Moringa oleifera* and *Terminalia arjuna* were collected from Tripura University campus. Plants were identified using the Flora of Tripura<sup>[5]</sup>, Flora of Assam<sup>[6]</sup> and e-flora of BSI<sup>[7]</sup>.

### Sample preparation

Barks were cleaned, chopped, dried in a shade under room temperature for six to seven days and then crushed into a coarse powder using electric grinder. The powder was sieved to get fine powder using fine plastic sieve which was used in the experiment.

### Preparation of aqueous extract

Various concentrations (w/v) of aqueous extract were prepared by soaking the bark powder in sterilized deionized water for overnight. Thereafter the extracts were filtered with Whatman No. 1 filter paper and the supernatant was used for the treatment.

### Fixation, hydrolysis, and dyeing

Fresh onions of  $35 \pm 5$ g were collected and the dry roots were removed. These bulbs were placed in tap water for 48h at room temperature for the emergence of roots. The rooted (1 cm in length) onion bulbs were placed on medium-sized beakers filled with different concentrations (5mg/ml, 10mg/ml, 20mg/ml, 40mg/ml, 60mg/ml) of *T. arjuna* and *Moringa oleifera* aqueous extract in such a way that the base of the onion bulbs remain in touch with the surface of the extract. Onion bulbs placed on beaker filled with mineral water served as control. At each concentration of treatment three exposure periods were considered. After the respective time period root tips were collected from each experimental set and then fixed in ethanol: glacial acetic acid (3:1 v/v) for overnight. Thereafter, root tips were stained with aceto-orcein staining technique<sup>[8]</sup> and then squashed on microscopic slides and temporarily sealed with nail polish. For each concentration five slides were prepared and observed under the high power (40X objective) of the compound microscope. In each treatment 15 observations, three from each slide were taken.

### Microscopic investigation

Five preparations were arranged for every group in the mitotic index calculation. Specimens were transferred from dye to a glass slide and a drop of acetic acid (45%) was added. The tip of the root (1-2 mm) was dissected with a needle on the slide into tiny pieces and covered with a cover-glass. A smear was prepared on the slide by pressing the cover glass slightly over the slide with the thumb. Excess liquid was sucked up by a piece

of blotting paper. Slides were scanned to investigate the different stages of mitosis. The data on the effect of aqueous extracts of *T. arjuna* and *M. oleifera* on mitotic index and the active mitotic index was calculated as percentage of dividing cells.

### Statistical Analysis

The results for each variant of treatment in these experiments were represented as an average of five experiments, and each arm was performed in triplicate. The mean values and standard deviation were calculated using Microsoft excel 2007.

### Result and discussion

The toxic effect of aqueous bark extracts of *T. arjuna* and *M. oleifera* were evaluated on *Allium* root meristem cells. The data on the effect of aqueous extracts of bark on the mitotic index and the active mitotic index were tabulated (Table 1 and Table 2). The result of our investigation showed statistically significant inhibition of cell division in both the treatment in different concentration of extract as compared with the mitotic index value to the control (Table 1 & 2). Cytological observation of our investigation in all the treatment (0.5-6%) of aqueous extract highly reduced MI as well as AMI in both the species with the increase of concentration of the treatment. The significant decline of mitotic indices were observed in 0.5 to 2% concentration of the treatment, but further increase in concentration did not show any significant difference in the MI and AMI of the species.

Our observation also revealed that both the bark extract induced cytological alteration and chromosomal aberration like sticky metaphase, Clumped metaphases, laggard chromosome, vagrant chromosome, Chromosome bridges at Anaphase, and Chromosome bridges at telophase (Plate 3). It was found that Frequency of abnormalities increases with the increase of concentration of the treatment (Table 3 & 4). The mutagenic effect of *T. arjuna* was higher than the *M. oleifera* bark extract. However highest number (2.43% in *T. arjuna* and 0.62% in *M. oleifera*) of abnormal cells were recorded in the treatment 60mg/ml concentration.

Most important observation of our experiment was that all the abnormalities were recoded only in mitotic phases. No abnormalities was seen in non-dividing cell except some lesions in the nucleus which is the indication of inhibition of DNA synthesis. Appearance of chromosomal bridge is the results of stickiness of the chromosomes or due to present of dicentric chromosome which is the cause of breakage and reunion of normal chromosome, clumped chromosome was the expression of changes in the physiochemical properties of DNA or protein or both in the chromatin. Laggard chromosome was the result of inhibition of anaphase movement of the chromosome due to disturbance in the microtubule structure. Most of the Chromosomal aberration are lethal and may causes of genetic effect,

Increase abnormalities with the increase of concentration of the extract in the treatment indicates some phytochemicals in this herbal medicine may leads cellular mutation. This observation is reflection of the result of previous researchers reported in their investigation in different medicinal herb<sup>[9, 10]</sup>.

**Table 1:** Effect of aqueous extract of *Terminalia arjuna* bark on mitosis in *A. cepa*.

Conc. (mg/ml)	2 hours			4 hours			6 hours		
	MI	AMI	FMAC	MI	AMI	FMAC	MI	AMI	FMAC
Control	5.88±0.01	2.77±0.02	0	5.76±0.01	2.32±0.02	0	4.51±0.01	1.8±0.02	0
5	3.62±0.02	1.58±0.02	20.51	3.23±0.03	1.44±0.03	23.68	3.03±0.03	1.36±0.02	35.48
10	1.91±0.01	0.67±0.02	24.00	2.41±0.01	1.30±0.12	26.66	1.79±0.01	0.96±0.01	38.88

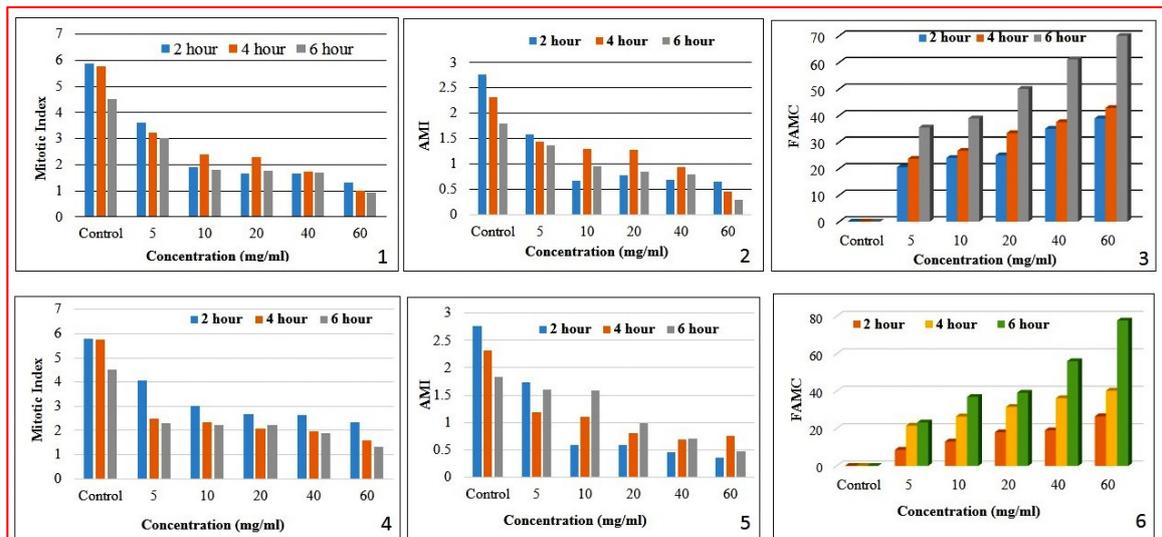
20	1.67±0.01	0.77±0.01	25.00	2.3±0.02	1.28±0.01	33.33	1.76±0.03	0.84±0.02	50.00
40	1.66±0.02	0.68±0.01	35.00	1.75±0.03	0.94±0.02	37.50	1.69±0.01	0.79±0.01	61.11
60	1.32±0.01	0.64±0.03	38.88	1.02±0.01	0.45±0.01	42.85	0.93±0.02	0.28±0.02	70.00

**Note:** Conc. (%) = Concentration in Percentage; TNCO = Total no. of cell observed; TNDC = Total no. of dividing cell; MI = Mitotic index; AMI = Active mitotic index

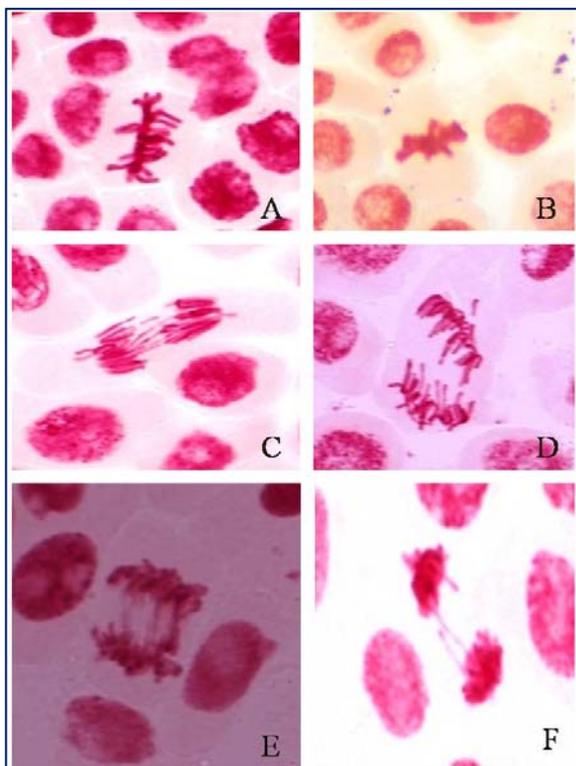
**Table 2:** Effect of aqueous extract of *Moringa oleifera* bark on mitosis in *A. cepa*

Conc. (mg/ml)	2 hours			4 hours			6 hours		
	MI	AMI	FMAC	MI	AMI	FMAC	MI	AMI	FMAC
Control	5.78±0.16	2.76±0.01	0	5.76±0.02	2.32±0.02	0	4.51±0.01	1.84±0.03	0
5	4.07±0.01	1.74±0.02	8.62	2.50±0.30	1.19±0.25	21.53	2.31±0.01	1.6±0.02	23.25
10	3.01±0.01	0.59±0.01	13.04	2.33±0.02	1.11±0.01	26.47	2.24±0.04	1.58±0.04	36.92
20	2.68±0.09	0.58±0.01	18.05	2.06±0.02	0.81±0.01	31.57	2.24±0.01	0.98±0.01	39.13
40	2.63±0.27	0.46±0.12	19.05	1.95±0.01	0.68±0.01	36.26	1.88±0.01	0.71±0.01	56.00
60	2.33±0.01	0.35±0.04	26.56	1.60±0.02	0.76±0.01	40.27	1.31±0.01	0.47±0.02	77.77

**Note:** Conc. (%) = Concentration in Percentage; TNCO = Total no. of cell observed; TNDC = Total no. of dividing cell; MI = Mitotic index; AMI = Active mitotic index



**Fig:** Cytotoxic effect of aqueous bark extract of *T. arjuna* (1, 2 & 3) and *Moringa oleifera* (4, 5 & 6) on *A. cepa* root meristem cells.



**Plate 1:** A- Sticky metaphase B- Clumped metaphase, C- Laggard chromosome, D- Vagrant chromosome in Anaphase, E- Anaphase Bridge, F- Bridge in telophase.

## Conclusion

To measure the cytotoxicity of any chemicals *Allium* root meristem cell bioassay method is well accepted. The cytotoxicity label can be determined by the rate of inhibition of mitotic index. In our experiment mitotic index and active mitotic index were significantly decreased with the increase of treatment concentration up to a certain level in both the plant extract used in the treatment but the chromosomal aberrations were increased with the increase concentration and duration of the treatment. This result is the indication of the presence of cytotoxic compound in the aqueous extract of bark which hinders the cell cycle and leads to chromosomal aberration. Lower rate of mitotic activity may be the result of inhibition of DNA synthesis or blockage in the G2 phase of the cell cycle, inhibiting the cell to enter into divisional phases of both the medicinal plant used in the treatment. Appearance of chromosomal aberration in the different stages of mitosis was the result of blockage of DNA synthesis or spindle formation. Our study with crude drug extract was the basis on the uses of crude drug in the traditional medicine. Since in the aqueous extract of bark of *T. arjuna* and *M. oleifera* contain cytotoxic and genotoxic activity although both the plants have beneficial effect as herbal medicine. This result suggests that in use of inappropriate dose of this herbal medicine can cause health problems. Further investigation is essential to isolate the phytochemicals responsible for cyto-genotoxic effect on the root meristem cell.

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