J. Bio-Sci. 32(2): 71-82, 2024 **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index
DOI:https://doi.org/10.3329/jbs.v32i2.75760

EVALUATION OF CYTO-GENOTOXIC EFFECTS OF *MUCUNA BRACTEATA* DC. EX KURZ LEAF EXTRACTS BY *ALLIUM CEPA*

Muntasir Ahmed Chowdhury and Animesh Biswas*
Department of Botany, University of Chittagong 4331, Chattogram, Bangladesh

Abstract

This study was conducted to evaluate the cyto-genotoxicity of leaf decoctions of *Mucuna bracteata by Allium cepa bioassay*. This kind of study can be used as a baseline monitoring system for plant products before their use. It is an inexpensive, simple, and reliable test to determine the cytotoxic and genotoxic effects of various plant extracts on living organisms. In this experiment, *Allium cepa* bulbs were placed in five concentrations (0.5 mg/mL, 2.5 mg/mL, 5.0 mg/mL, 7.5 mg/mL, and 10.0 mg/mL) of leaf decoction, with tap water as the negative control and 20.0 µL/L EMS (Ethylmethene sulphonate) as the positive control. Each concentration had five replicates. The cyto-genotoxicity assay exhibited a dose-dependent influence on the mitotic index. The mitotic index of the negative control and the positive control were 16.8 ± 0.37% and 6.86 ± 1.71%, respectively. The lowest dose of the extract, 0.5 mg/mL, had the highest mitotic index (14.2 ± 0.73%), while the highest dose, 10.0 mg/mL, had the lowest (9.6 ± 0.40%), and all indices were significantly higher than the positive control. Except for the 10 mg/mL concentration, the mitotic indices of other concentrations were significantly lower than the negative control. Different types of chromosome disorders, namely, disorderly prophase, sticky chromosomes, chromosome bridges, laggard chromosomes, pole deviation, vagrant chromosomes, nuclear filaments with terminal expansion, c-mitosis, micronuclei, and elongated nuclei, were observed. Various changes in root size, growth, and color were revealed by macroscopic examinations. Tumors were formed in roots at the highest doses but not at lower concentrations. Therefore, *M. bracteata* leaf extract showed cytotoxic and genotoxic effects on the *Allium* root meristem.

Key words: Cytogenotoxicity, Mitotic index, Chromosome abnormalities, Baseline monitoring.

Introduction

The genus *Mucuna* belongs to the plant family Fabaceae and contains about 150 species of annual and perennial legumes (Murthy et al. 2015). Species of the *Mucuna* genus have been used in the Indian Subcontinent for many centuries as a remedy in the ancient Ayurvedic system. *Mucuna bracteata* DC. ex Kurz originates from North-East Indian forest areas of the Tripura State. In Bangladesh, it has been reported from Chattogram district and the Chittagong Hill Tracts and is considered a vulnerable species (Ahmed 2009). It is a leguminous climbing shrub. Young parts have grey pubescent. Leaves alternate, trifoliolate, up to 25 cm long. Flowers blackish purple, 3.5 - 4.5 cm long. The seeds are medium in size and are black-brown mosaic in color. The medicinal use of different parts of this plant has also been reported (Kumar et al. 2009, Mai et al. 2009, Sangvikar et al. 2016). Traditionally, the roots of this plant are useful against nephropathy, elephantiasis, dropsy, fever, and delirium, and a leaf decoction is used for ulcers. Seeds are used to treat Parkinson's disease, arthritis, and kidney problems (Kar et al. 2019).

Various parts of this plant are traditionally used to treat various ailments by ethnic communities in the Chittagong Hill Tracts. They apply this plant in crude forms (powder, paste, decoction, etc.) based on their preconceptions and without determining the dosage. The cytotoxicity and genotoxicity of plant extracts can

*Author for correspondence: animesh.biswas@cu.ac.bd

be determined using plants and animal models. Considering its traditional use, an evaluation of the genotoxicity and cytotoxicity of *Mucuna bracteata* leaves should be done by a reliable technique. The *Allium cepa* assay has been accepted for determining the toxicity of phytochemicals (Çavuşoğlu et al. 2016, Owolarafe et al. 2020). The *Allium cepa* test system is a short-run test with many advantages such as ease of use, low cost, and good chromosomal conditions for the study of chromosomal aberrations such as damage or disturbance during cell division (Fiskesjö 1985, Rank 2003). So far, no report is available on the cytotoxicity of *Mucuna bracteata* leaves. In this regard, the objective of the current investigation was to carry out a thorough cytotoxicity test of *Mucuna bracteata* leaf extract.

Materials and Methods

Preparation of aqueous extracts (decoction) of *Mucuna bracteata* leaves

Mucuna bracteata leaves were used as plant material. Samples were collected from Silchari, at Rangamati Hill district in Chattogram. The collected plant sample was carefully examined and authenticated. In the present research, leaf decoction was used in the cyto-genotoxicity assay. After collection, the leaves were rinsed with normal tap water and dried in a ventilated area for seven days. After drying well, the leaves were milled into a fine powder using a kitchen blender. The powder was stored in a zip-lock plastic bag at 4°C until use. In this experiment, a 10% stock solution made from *Mucuna bracteata* leaves was used. Twenty grams of powdered leaves were boiled in 200 mL of distilled water to make the decoction. The boiling procedure was executed in a covered beaker for 10 minutes. Then the solution was cooled until it reached room temperature. Thereafter, the solution was filtered using Whatman No. 1 filter paper. In this way, a 10% stock solution was prepared. The stock solution was then diluted with distilled water to concentrations of 0.5 mg/mL, 2.5 mg/mL, 5.0 mg/mL, 7.5 mg/mL, and 10.0 mg/L using the appropriate dilution formula. The fresh extract was prepared for each experiment. All experiments were carried out at room temperature (25±2°C).

Allium cepa bioassay

The bulbs of common onion (*Allium cepa*) were used for this assay. Onion bulblets are preferable to medium-sized because they fit into 50 mL test tubes. Suitable onions were collected from the local market and stored in a dry and well-aerated area to increase viability. The test continued for five days. Concentrations (0.5, 2.5, 5.0, 7.5 and 10.0 mg/mL) of 10% stock were prepared using a dilution formula. Mum water was taken as negative control and 20 µL/L EMS solution was used as positive control. The test included microscopic and macroscopic evaluation.

Microscopic evaluation

For microscopic evaluation, 5 onion bulbs were suspended in each of the test samples and were controlled for 48 hours. Afterward, the root tips from every replicated bulb were excised and fixed in freshly prepared ethanol: glacial acetic acid (3:1 v/v) solution and kept the vials airtight for 24 hours and then preserved in 70% ethanol. For microscopic observation, preserved root tips were removed from the vials and placed in watch glasses containing 3:1 aceto-alcohol solutions and 1N HCl solution (1:9). Then the entire solution was heated on a spirit lamp for a few seconds to soften the tissue and rinsed 2 to 3 times with distilled water. The root tips were then gently placed on a clean microscopic slide and squashed and stained with 0.2% (N/V) acetocarmine. They were allowed to stain for 5 minutes before carefully placing clean cover slips over them. Excess stains were removed with tissue paper. A total of about 500 cells from each replication were observed at 40× and 100× magnification for different mitotic stages and chromosomal abnormalities using a light microscope. Photomicrographs were taken using a B-350 Optika Microscope with an Optika digital camera fitted with the microscope. Based on the number of aberrant cells per total cell scored at each concentration of each replication, the mitotic index and frequency of chromosomal aberration were computed.

The mitotic index and percent of aberrant cells were determined using the following formula:

$$\text{Mitotic index} = \frac{\text{No. of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\% \text{ aberrant cells} = \frac{\text{Number of Aberrant cells}}{\text{Number of dividing cells}} \times 100$$

Macroscopic evaluation

After 96 hours of treatment, onion root cells exposed to *Mucuna bracteata* leaf extract showed alterations. Root number, root lengths, color of roots, tumor formation, curving, and root size are the parameters observed in this macroscopic evaluation.

Statistical analysis

Statistical analysis was done using the statistical software OPSTAT (14.139.232.166/opstat/). The differences between treatments and control groups were tested by applying one-way ANOVA and DMRT. The level of significance was accepted at $p \leq 0.55$.

Results

Mitotic index (MI) in *Allium* assay

The mitotic index was calculated to assess the rate of mitosis cell divisions at different concentrations of *Mucuna bracteata* leaf. The result showed that, the mitotic index decreased in leaf decoctions in a dose-dependent way (Table 1). The lowest dose, 0.5 mg/mL, had the highest (14.2 ± 0.73%) mitotic index, while the highest dose, 10.0 mg/mL, had the lowest (9.6 ± 0.40%) mitotic index, the tap water (negative control) treated samples showed the highest mitotic index (16.8 ± 0.37%), and while the EMS (positive control) treated samples had the lowest mitotic index (6.86 ±1.71%). The mitotic index of 0.5 mg/mL solution was closer to the mitotic index of negative control. On the other hand, the mitotic index of 10 mg/mL solution was closer to the positive control. The mitotic index of 0.5 mg/mL, 7.5 mg/mL, and 10 mg/mL were significantly different from the positive index but mitotic index of all concentrations was significantly different from the negative control indicating some shorts of cyto-genotoxicity existed in the solution.

Anomalies in the cell division

Microscopic evaluation revealed many forms of chromosomal structures. It is important to have a thorough understanding of the morphology of typical mitotic chromosomes before looking at the treated root tips. Fig. 1 depicts the stages of normal mitotic cell division. The leaf extract-treated cells were inspected after the normal mitotic chromosomes were evaluated. Unusual chromosomal morphologies of various kinds have been observed (Table 1, Fig. 2). Total aberrant cells were highest in the concentration of 2.5 mg/mL and positive control. Negative control had the lowest total of aberrant cells. Among the abnormalities found, an elongated nucleus was the highest in number followed by micronucleus, vagrant chromosomes, disorderly prophase, and Chromosome bridge. Sticky chromosomes and c-mitosis were reported in six cells. Laggard, pole deviation, and nuclear filament were found in a single number.

The data presented in Table 2 depicts a significant reduction in the number of roots at a concentration of 0.5, 5.0, and 7.5 mg/mL solutions. No consistent pattern of increase or decrease in the number of roots at different concentrations was observed. The number of roots was highest at 10.0 mg/mL and lowest at 0.5 mg/mL. The number of roots in all concentrations was significantly lower than the negative control except for 10 mg/mL. No significant difference in root number between the 2.5 mg/mL and the negative control was noted. The length of roots was sequentially reduced and was significantly lower than that of the negative control and higher than that of the positive control. The percent root growth of the control was sequentially reduced from a lower concentration to a higher concentration. The appearance and color of the roots were

Table 1: Cytological effects of treatment with different concentrations of *Mucuna bracteata* aqueous leaf extract on *Allium cepa* root tip cells.

Cont.	No. of Dividing cells	No. of cells at prophase	No. of cells at metaphase	No. of cells at anaphase	No. of cells at telophase	DP*	SC*	CB*	LC*	PD*	VC*	NF*	CM*	MN*	EN*	TAC*	MI*	%AC*
0.5	71±0.63^{b}	52±0.63^{b}	7±0.70^{c}	1±0.54^{cd}	5±0.70^{c}	1±0.31^{b}	1±0.31^{a}	-	-	-	1±0.31^{cd}	-	-	Nil	3±0.44^{c}	6±.89de	14.2±0.73^{b}	8.45±0.25^{cd}
2.5	67±0.44^{c}	38±0.63^{d}	9±0.44^{b}	-	4±0.44^{c}	-	-	-	-	-	3±0.70^{ab}	-	-	5±0.31^{b}	8±0.31^{a}	16±0.70^{a}	13.4±0.24^{bc}	23.88±1.052^{b}
5.0	63±1.67^{d}	42±0.94^{c}	50.70^{d}	2±0.63^{c}	7±0.31^{b}	1±0.31^{b}	-	2±0.54^{a}	-	-	2±0.31^{bc}	1±0.31^{a}	1±0.31^{b}	1±0.31^{d}	1±0.31^{d}	9±1.51^{cd}	12.4±0.40^{bc}	14.27±2.40^{c}
7.5	58±1.14^{e}	43±0.83^{c}	11±0.44^{a}	11±0.44^{a}	11±0.44^{a}	3±0.44^{a}	1±0.31^{a}	-	-	-	-	-	-	7±0.44^{a}	3±0.54^{c}	14±1.14^{ab}	11.6±0.24^{cd}	24.14±1.96^{b}
10.0	48±0.63^{f}	340.54^{e}	3±0.83^{e}	-	-	1±0.31^{b}	-	-	-	1±0.44^{a}	-	-	-	4±0.31^{c}	5±0.31^{b}	11±1.70^{bc}	9.6±0.40^{d}	22.92±1.43^{b}
pc	34±1.67^{g}	171.14^{f}	-	-	2±0.31^{d}	3±0.44^{a}	1±0.44^{a}	2±0.31^{a}	-	-	4±0.70^{a}	-	2±0.54^{a}	-	3±0.54^{c}	15±1.54^{a}	6.86±1.71^{e}	44.12±4.55^{a}
nc	84.6±1.32^{a}	54.40.40^{a}	11±0.44^{a}	4±0.44^{b}	11±0.31^{a}	-	-	1^{b}	1±0.44^{a}	-	2±0.31^{bc}	-	-	-	-	4±0.83^{e}	16.8±0.37^{a}	4.76±0.99^{d}

Mean ±SEM values of five replications are presented. One-way ANOVA was used to analyze the data and Duncan's was used for the multiple range test. All test values with different superscripts in the column are significantly different ($p \leq 0.55$)]. *DP = Disorderly prophase; SC = Sticky chromosome; CB= Chromosome bridge; LC = Laggard chromosome; PD= Pole deviation; VC = Vagrant chromosome; NF = Nuclear filaments with terminal expansion; CM = C mitosis; MN = micronucleus; EN = Elongated nucleus. TCA = Total aberrant cell, MI = Mitotic index, %AC = Percent aberrant cell.

changed when treated with leaf extract. The roots appeared to be brown, white, or dark brown. In the highest doses, tumor formation occurred; however, in the lowest concentrations, such as 0.5 and 2.5 mg/mL, no tumor was produced. With increasing doses, the root size became stunted, and the root apices began to curve at concentrations of 0.5, 2.5 and 7.5 mg/mL.

Table 2: Effects of root growth of *Allium cepa* exposed to different concentrations of *Mucuna bracteata* aqueous leaf extract.

Cont.	No. of roots	Length of root (cm)	% root growth of control	Root colour	Tumor formation	Curving of root apex	Root size
0.5	32.6±1.12c	1.51±0.03b	25.22	White	Absent	Absent	Not stunted
2.5	40.4±1.63ab	0.91±0.05c	19.96	White	Absent	Present (Slightly)	Not stunted
5.0	38.6±1.86ab	0.72±0.07cd	15.79	Brownish white	Absent	Present (Slightly)	Not stunted
7.5	37.4±1.93b	0.51±0.02de	11.18	Brownish	Present (slightly)	Absent	Not stunted
10.0	43±1.20a	0.42±0.02e	9.21	Brown	Present (slightly)	Present (Slightly)	Stunted
pc	18±0.63d	0.390.01e	8.55	Dark brown	Present (slightly)	Present	Stunted
nc	40.61.63ab	4.56±0.23a	100	White	Present	Absent	Not stunted

Mean ±SEM values of five replications are presented. One-way ANOVA was used to analyze the data and Duncan's was used for multiple range test. All test values with different superscripts in the column are significantly different ($p \leq 0.55$).

Macroscopic evaluation

Following a 96 hours treatment, the extract's effects on the root of *Allium cepa* were observed. Root number, root lengths, color of roots, tumor formation, curving, and root size were the morphological features that changed with the increase in concentrations. The percentage root growth of control was calculated to determine the EC_{50} value using the following formula:

$$\% \text{ Root growth control} = \frac{\text{Overall mean root length of test solution}}{\text{Overall mean root length of negetive control}} \times 100$$

Determination of EC_{50} value

The concentrations were placed in the X axis and Root growth (% of control) was placed in the Y axis to find the EC_{50} or half maximal effective concentration value. The EC_{50} value for the *M. bracteata* leaf extract, determined from this graph chart is 1.16 mg/mL (Fig. 3). This indicates that *M. bracteata* leaf extract may exhibit the half-maximal impact at a concentration of 1.16 mg/mL.

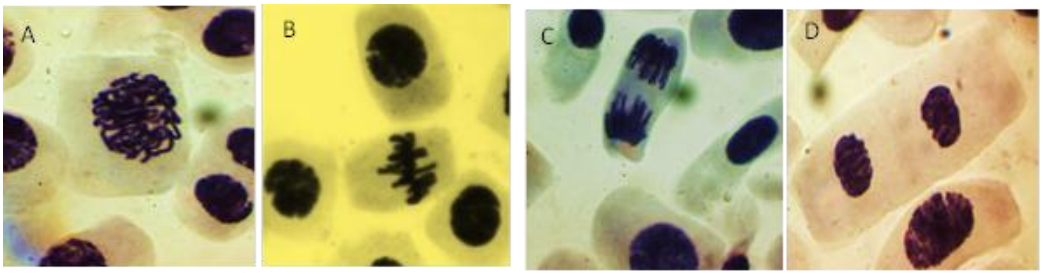

Fig. 1(A-D): Morphology of normal mitotic chromosomes in *Allium* root cells. A. prophase; B. metaphase; C. anaphase; C. telophase.

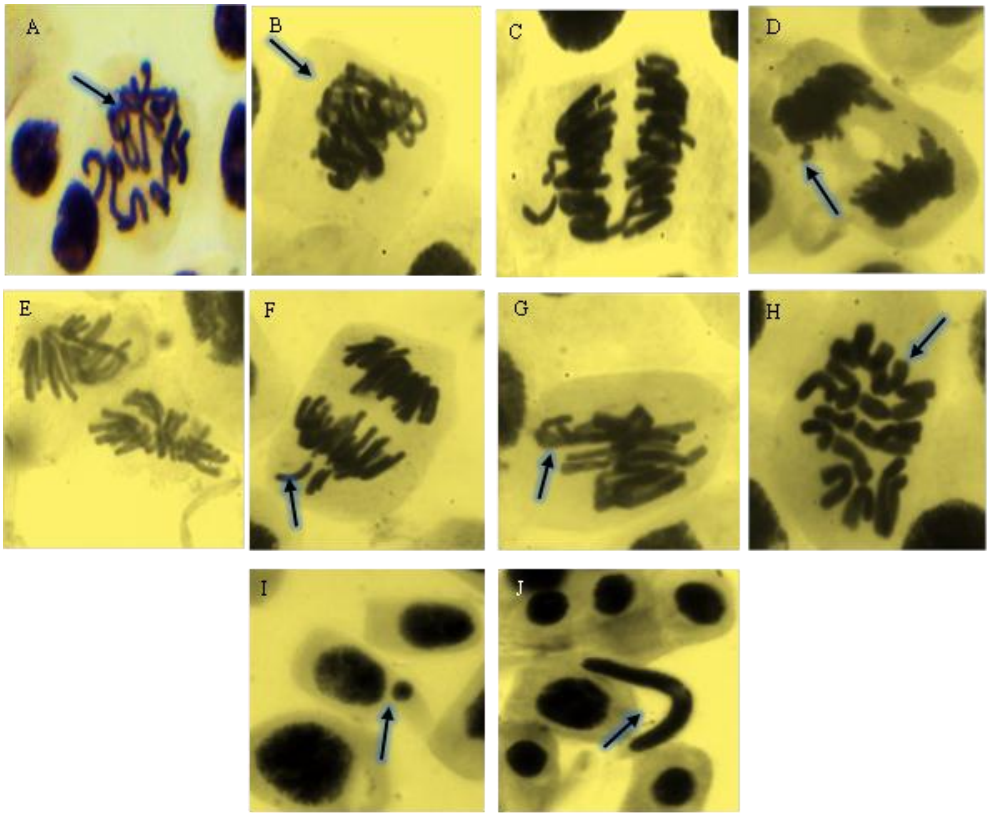

Fig. 2(A-J): Chromosomal and nuclear aberrations in cells treated with different treatment solutions. (A) disorderly prophase, (B) sticky chromosome, (C) chromosome bridge, (D) laggard chromosome, (E) pole deviation, (F) vagrant chromosome, (G) nuclear filaments with terminal expansion, (H) C mitosis, (I) micronucleus, (J) elongated nucleus. (Arrow indicates the specific cell abnormality).

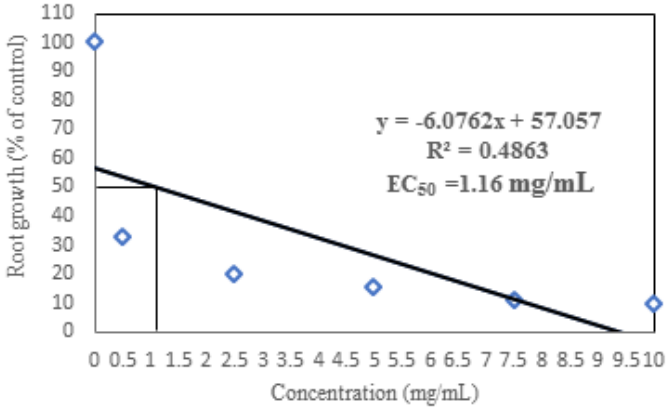

Fig. 3: Calculation of EC_{50} from macroscopic data.

Fig. 4 (A-G): Macroscopic observation (after 96 hours) of *Allium* roots after treatment with Negative control: (A) Mum water, Treatment solutions: (B) 0.5 mg/mL, (C) 2.5 mg/mL, (D)5.0 mg/mL, (E) 7.5 mg/mL, (F) 10.0 mg/mL concentration of 10% stock of *Mucuna bracteata* leaf and Positive control: (G) 20 µl/L EMS solutions.

Discussion

Plant species are significant sources of physiologically active substances, the majority of which have little-known impacts on heritable material. However, given the current widespread use of aqueous extracts in conventional medicine as well as antiseptics, soaps, deodorants, flavors and dentistry products, and essential oils in industry and pharmacy, it would appear that further study into their cytotoxicity and genotoxicity is required. It is worth mentioning that such studies are few and by no means comprehensive in most medicinal plants (Prashar et al. 2004, Reddenna et al. 2017). Sometimes, plant toxins exhibit genotoxicity. Genotoxic substances cause mutations that alter a cell's genetic code and increase the risk of cancer. Permanent genetic alterations can impact the organism's somatic cells or germ cells, which are then handed down to succeeding generations (Grant 1994). For a long time, the *Allium* model has been utilized to evaluate the cytotoxic and genotoxic effects of hazardous compounds due to its ability to interact with mutagenic agents during its cell cycle (Fiskesjo 1997, Timothy et al. 2014, Tank and Thaker 2014, Rahman et al. 2023). Since morphological and cytological changes in onion root tips can be seen at 24-hour intervals, detecting potential treatment effects on mitotic indices in treated onion root tips was made possible by a 48-hour treatment period (Babatunde and Bakare 2006).

Several investigations have demonstrated that the number of dividing cells consistently decreases in the *Allium* test whenever there is a reduction of root growth (Smaka-Kinel et al. 1996, Timothy et al. 2014). The mito-depressive effect on *Allium cepa* cell division is reflected in the decreased number of dividing cells or the mitotic index. All organisms can safely be measured for cytotoxicity using the mitotic index (Adegbite and Sanyaolu 2009). With an increase in extract concentration, a decrease in MI was seen. As previously observed (Taek-Keun et al. 2012), this decrease is linked to disruptions in the cell cycle and an increase in the frequency of chromosomal abnormalities. A break or exchange of chromosomal material can cause chromosomal aberrations. The results of the current investigation revealed a dose-dependent decrease in the mitotic index, indicating that the leaf extract of *Mucuna bracteata* had a depressing effect on mitosis. Leaf decoction at concentrations of 5.0-10.0 mg/mL showed a significant decrease in the mitotic index compared to positive and negative control in *A. cepa* root cells (Table 1). Root growth inhibition with increasing concentrations of plant extracts is supported by previous studies (Gaulden 2007, Gulzar et al. 2016).

The prophase had more cells than any other phase, considering to the phase index. Phase index was affected by concentrations of 0.5, 2.5, 5.0, and 10 mg/mL in contrast to the positive control. Except the anaphase, the concentration of 7.5 mg/mL was comparable to the negative control and had the most cells across all phases. When the cells are unable to advance to the subsequent stages of division, an excess of prophase and metaphase cells is observed. Disorderly prophase, sticky chromosomes, chromosome bridges, lagging chromosomes, pole deviation, vagrant chromosomes, nuclear filaments with terminal expansion, c-mitosis, micronucleus, and elongated nuclei are some of the chromosomal abnormalities recorded in this study.

The DNA condenses into chromosomes during prophase, and fibers start to extend from the centromeres, which connect the two arms, or chromatids, of each chromosome. Disorderly prophase occurs when hazardous compounds from any source come into contact with dividing cells, inhibiting the condensation of DNA to chromosomes and chromatid formation. In all concentrations, except 2.5 mg/mL, the dividing root cells displayed some disordered prophase when *Allium* roots were treated with *Mucuna bracteata* leaf extract. The concentration of 7.5 mg/mL had the maximum number of disordered prophase. Chromosomal clustering throughout any stage of the cell cycle is a sign of chromosome stickiness, which is thought to be caused by a malfunction of one or two types of specialized non-histone proteins involved in chromosome

organization that are required for chromatid separation and segregation (Turkoglu 2007). The altered functioning of these proteins is caused by a mutation in the structural genes coding for them or by the direct action of mutagens (Nefic et al. 2013). Stickiness was caused by increased nucleoprotein production and improper protein-protein interactions as a result of the linking of sub-chromatid chromosomal strands (Engelbert et al. 2017). It is also possible that stickiness was a result of severe disorganization of the spindle apparatus due to the presence of bioactive components of the test extract (Amer and Ali 1986). Roots treated with *Mucuna bracteata* leaf extract displayed sticky chromosomes in concentrations as low as 0.5 mg/mL and as high as 7.5 mg/mL. This implies that the extract might contain compounds that are harmful to nucleoproteins.

Another sort of anomaly that can be seen in the presence of toxic substances is chromosome-bridge or anaphase-bridge. DNA double-strand breaks are related to anaphase bridges. Incorrect fusion events that result in bridging also happen to restore the original chromosomal structure (Amer and Ali 1986). This type of anomaly was only observed in 5.0 mg/mL concentration. Defective microtubules cause chromosomes to lag behind and not fully separate from their corresponding daughter cell (Tank and Thaker 2014). The impairment of microtubules often leads to other mitotic aberrations such as laggard chromosomes resulting from disturbed anaphase-telophase (Levan 1938). Roots treated with *Mucuna bracteata* leaf extract didn't exhibit any signs of chromosomal lag. It was seen only in the negative control. Daughter chromosomes split at the centromere in a polar deviation; instead of looking opposing, they appear next to one another (Tank and Thaker 2014). It was a rare type of anomaly observed in extract-treated root cells. Only 10.0 mg/mL concentration showed the presence of polar deviation of chromosomes. Vagrant chromosomes have a weak C-mitotic effect (Hadimani et al. 2015). Partial chromosomal deserialization is the cause of laggards and vagrants. The lowest concentrations of *Mucuna bracteata* leaf extract i.e. 0.5 mg/mL, 2.5 mg/mL, and 5.0 mg/mL showed the presence of vagrant chromosomes. This indicates the presence of substances in this extract which can create a C-mitotic effect and vagrant chromosomes.

Extract-treated root cells sometimes show some irregular chromosome structure called nuclear filament with terminal expansion. In this type of anomaly, chromosomes didn't follow the normal route, instead, they acted like a separate filament that was intended to move terminally. In extract-treated root cells, only 5.0 mg/mL concentrations show nuclear filaments. C-mitosis causes the formation of inactive spindles and the spreading of chromosomes within the cytoplasm. This situation has been referred to as spindle poisoning or mitotic poisoning (Tank and Thaker 2014). This type of anomaly was rarely found in *Mucuna bracteata* leaf extract-treated root cells. Only 5.0 mg/mL concentration showed a C-mitotic effect. Toxicity sometimes shows micro-sized nuclei. *Mucuna bracteata* leaf extract shows micro-nucleus in all the concentrations except 0.5 mg/mL. The highest no. of micronucleus was encountered in the concentration of 7.5 mg/mL.

Normal cell division exhibits round shaped nuclei whereas high doses of the extract revealed a great number of elongated nuclei. *Mucuna bracteata* leaf extract shows a great number of elongated nuclei which is also an indication of cytotoxicity. All of the concentrations that were treated with the leaf extract exhibited this kind of abnormality. Microscopic examination revealed some chromosomal aberrations that indicate the presence of toxic substances. Macroscopic observation showed different changes in root size, growth, and colour. The data in Table 2 revealed a sequential reduction in root growth. The root length was remarkably reduced in a solution containing 10 mg/mL leaf extract. The number of roots did not follow any predictable pattern. The number of roots was lowest at 0.5 mg/mL and highest at 10.0 mg/mL. *Mucuna bracteata* leaf extract caused roots to take on a brown, white, or dark brown appearance. Tumors form at the highest doses, while none appeared at lower concentrations, such as 0.5 mg/mL and 2.5 mg/mL. At concentrations of 0.5, 2.5, and 7.5 mg/mL, the root apex started to curve and the root size became stunted with increasing doses. The characteristics suggest that higher concentrations of leaf extracts have cyto-genotoxic effects. So far we

know, no published information regarding the cytogenotoxic study of *M. bracteata* is available. There are some reports on the phytochemical and cytotoxic studies on *Mucuna* pruriens (Yadav et al. 2015, Jimoh et al. 2020). They used animal model in their experiments.

Conclusion

According to the findings of this study, *Mucuna bracteata* leaf extract may contain bioactive components that affect mitosis and block mitosis in higher doses by causing spindle and chromosomal damage. The current study on *Mucuna bracteata* leaf extract revealed toxicity on *Allium* roots, with an EC_{50} value of 1.16 mg/mL. Thus, the result from this study should be considered as a warning and also an indication that the tested leaf extract may be a risk to human health.

Ethical issues: Ethical issues are not required for this study.

Conflict of interest: The authors declare no conflict of interest.

Author contributions

Muntasir Ahmed Chowdhury: Experiment setting and data collection. Animesh Biswas: Conceptualization, methodology, writing- original draft preparation, reviewing and editing, statistical analysis.

Acknowledgements

We acknowledge the financial support from the Ministry of Science and Technology.

References

Adegbite AE and Sanyaolu EB (2009). Cytotoxicity testing of aqueous extract of bitter leaf (*Vernonia amygdalina* Del.) using the *Allium cepa* chromosome aberration assay. Scientific Research and Essay 4(11): 1311–1314.

Amer S and Ali EM (1986). Cytological effects of pesticides. XVII Effect of the insecticide dichlorvas on root mitosis of *Vicia faba.* Cytologia 51: 21-25.

Babatunde BB and Bakare AA (2006). Genotoxicity screening of wastewater from Agbara industrial estate, Nigeria evaluated with the *Allium* test. Pollution Research 25(2): 227-234.

Çavuşoğlu D, Tabur S and Çavuşoğlu K (2016). Role of *Ginkgo biloba* L. Leaf Extract on Some Physiological and Cytogenetical Parameters in *Allium cepa* L. Seeds Exposed to Salt Stress. Cytologia 81(2): 207-213.

Engelbert RH, Juul-Kristensen B, Pacey V, de Wandele I, Smeenk S, Woinarosky N, Sabo S, Scheper MC, Russek L and Simmonds JV (2017). The evidence-based rationale for physical therapy treatment of children, adolescents, and adults diagnosed with joint hypermobility syndrome/hypermobile Ehlers Danlos syndrome. American Journal of Medical genetics 175(1): 158-167.

Fiskesjo G (1985). The *Allium* test as a standard in environmental monitoring. Hereditas 102(1): 99-112.

Fiskesjö G (1997). Allium Test for Screening Chemicals: Evaluation of Cytologic Parameters. In W. Wang, J. W. Gorsuch, & J. S. Hughes (Eds.), Plants for Environmental Studies pp. 308-333. Boca Raton, New York: CRC Lewis Publishers.

Gaulden ME (1987). Hypothesis: some mutagens directly alter specific chromosomal proteins (DNA topoisomerase 11 and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. Mutagen 2: 357-365.

Grant WF (1994). The present status of higher plant bioassays for the detection of environmental mutagens. Mutation Research 310: 175-185.

Gulzar A, Siddiqui MB and Bi S (2016). Phenolic acid allelochemicals induced morphological, ultrastructural, and cytological modification on *Cassia sophera* L. and *Allium cepa* L. Protoplasm 253 (5): 1211-1221.

Hadimani GA, Desai SD, Biradar P, Nanjappaiah HM, Hugar S and Bagoji IB (2015). Evaluation of acute oral toxicity and phytoconstituents of methanolic extract of *Mucuna pruriens.* Journal of Pharmaceutical Sciences and Research 7(1): 33-36.

Jimoh MA, Idris OA and Jimoh MO (2020). Cytotoxicity, phytochemical, antiparasitic, screening, and antioxidant activities of *Mucuna pruriens* (Fabaceae). Plants (Basel) 9(9): 1249. doi: 10.3390/plants9091249.

Kar S, Das D, Das A and Datta BK (2019). Ethnomedicinal uses of some legumes in Tripura, India. Pleione 13(2): 258-268.

Khatun BMR (2009). *Mucuna bracteata* DC. Ex Kurz. *In*: Ahmed ZU, Hassan, MA Begum, ZNT, Khondker M, Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA and Haque EU (eds.). Encyclopedia of Flora and Fauna of Bangladesh, Vol. 8, Angiosperms: Dicotyledons (Fabaceae-Lythraceae). Asiatic Society of Bangladesh, pp. 140-142.

Ahmed ZU et al. (2009) Encyclopedia of Flora and Fauna of Bangladesh, Vol. 8. Angiosperms: Dicotyledons (Fabaceae-Lythraceae). Asiatic Society of Bangladesh, Dhaka, 1-478.

Kumar A, Rajput G, Dhatwalia VK and Srivastav G (2009). Phytocontent screening of *Mucuna* seeds and exploit in opposition to pathogenic microbes. Journal of Biological and Environmental Sciences 3(9): 71-76.

Levan A (1938). The effect of colchicine on root mitoses in *Allium*. Hereditas 24: 471-486.

Mai CW, Pakirisamy P, Tay EF, Subramaniam S, Shamsuddin ZH and Pichika MR (2009). Nasopharyngeal carcinoma cell proliferation and apoptosis induced by the standardised ethanolic extracts of *Mucuna bracteata*. Malaysian Journal of Chemistry 11: 143-148.

Murthy SN, Manasi MM, Shirolkar AR, Pawar SD, Sangvikar S and Kulkarni YR (2015). A comparative assessment of pharmacologically active principles and antioxidant activity of commonly occurring *Mucuna* sps. in India. International Journal of Ayurveda and Pharma Research 3 (11): 8-13.

Nefic H, Musanovic J, Metovic A and Kurteshi K (2013). Chromosomal and nuclear alterations in root tip cells of *Allium cepa* L. Induced by alprazolam. Medical Archives (Sarajevo, Bosnia and Herzegovina) 67(6): 388-392.

Olorunfemi DI, Okoloko GE, Bakare AA and Akinboro A (2011). Cytotoxic and genotoxic effects of *Cassava* effluents using the *Allium cepa* assay. Research Journal of Mutagenesis1: 1-9.

Owolarafe TA, Salawu K, Ihegboro GO, Ononamadu CJ, Alhassan AJ and Wudil AM (2020). Investigation of cytotoxicity potential of different extracts of *Ziziphus mauritiana* (Lam.) leaf *Allium cepa* model. Toxicology Reports 7: 816-821.

Prashar A, Locke IC and Evan CS (2004). Cytotoxicity of lavender oil and its major components to human skin cells. Cell Prolif 37(3): 221-229.

Rahman MS, Pritha US, Sarkar MM and Kabir G (2023) Cytological effects of two varieties of *Lathyrus sativus* L. extracts on onion root tip cells. Journal of Bio-Science 31(1): 51-60.

Rank J (2003). The method of *Allium* anaphase–telophase chromosomal aberration assay. Ekologija 1: 38-42.

Reddenna L, Venkatesh P, Kumar KS and Reddy ASK (2017). Genotoxicity. Pharma Tutor 5(9): 23-34.

Sangvikar S, Mhase A, Kumar S, Rao GB and Murthy SN (2016). The velvet bean (*Mucuna* sp.): in Ayurvedic era. World Journal of Pharmacy and Pharmaceutical Sciences 5(4): 583-601.

Smaka-Kinel V, Stegnar P, Lovka M and Toman MJ (1996). The evaluation of waste, surface, and groundwater quality using *Allium* test procedure. Mutation Research 368: 171-179.

Taek-Keun OH, Shinogi Y, Chikushi J, Young-Hwan L and Choi B (2012). Effect of aqueous extract of biochar on germination and seedling growth of lettuce (*Lactuca sativa* L.). Journal of Faculty of Agriculture, Kyushu University 57(1): 55-60.

Tank J and Thaker V (2014). Systemic control of cell division and endoreduplication by NAA and BAP by modulating CDKs in root tip cells of *Allium cepa.* BioMed Research International 5: 1-13.

Timothy O, Idu M, Olorunfemi DI and Ovuakporie-Uvo O (2014). Cytotoxic and genotoxic properties of leaf extract of *Icacina trichantha* Oliv. South African Journal of Botany 91: 71-74.

Turkoglu S (2007). Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. Mutation Research - Genetic Toxicology and Environmental Mutagenesis 626(4): 14-74.

Yadav RK, Singh M, Sarkar S, Maity S and Saha S (2015). Cytotoxicity of different extracts of *Mucuna pruriens* seeds on hepatoma cells but not on normal hepatic cells. Pharmacognosy Communications 5(1): 63-69.

(Manuscript received on 10th June 2024 and revised on 11st July 2024)