DETERMINATION OF CHROMOSOMAL ABERRATIONS OF TUBLI *(Derris elliptica)* ROOT EXTRACT TO *Allium cepa*

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Abstract — Higher plants are recognized as excellent genetic models to detect environmental mutagens and are frequently used in monitoring studies. Among the plant species, *Allium cepa* has been used to evaluate DNA damage, such as chromosome aberrations and disturbances in the mitotic cycle. Nowadays, it has been used to assess a great number of genotoxic agents, which contributes to its increasing application in environmental monitoring. *A. cepa* is commonly used as a test organism because it is cheap, easily available and has advantages over other short-term tests. This study determined if there are possible genotoxicity effects induced by tubli *(Derris elliptica)* root extract in *A. cepa* root tip cells. *A. cepa* was exposed to *D. elliptica* root extract with different concentrations, 0.2%, 0.5%, and 1% for T_1, T_2, and T_3, respectively, and the control, T_0 treated with distilled water. Both the control and the experimental setups were treated respectively for 6 hours. The highest aberrant cells were observed when *A. cepa* were exposed in T_1 revealing a percentage of 1.13%. The type of chromosomal aberration detected in *A. cepa* was mostly found in the metaphase and anaphase stages. The common abnormalities were breaks, fragments, and bridges while nucleated and multipolar cells were evident in the telophase stage. This study shows a significant difference in the mitotic index in the total percentage of aberrant cells in *Allium cepa* showing possibilities of the genotoxicity and cytotoxicity of high existence of rotenone in *Derris elliptica* root. This indicates the interference of toxic agents like rotenone in the normal growth of cells. Plants with such abnormalities may transfer altered genetic makeup not only to their offspring but also to humans when consumed as food thereby causing further complications.

***Keywords* — *Allium cepa*, chromosomal aberrations, *Derris elliptica*, genotoxicity, rotenone**

INTRODUCTION

While the newest physical and chemical agents facilitate human life, these agents may also cause some health problems due to their mutagenic and cytotoxic effects on living organisms. Mutations are changes in the genetic material that is heritable and essentially permanent (Singh et al., 2021). A chromosome aberration is a type of mutation; it is an unpredictable change that occurs in a chromosome, this can result in changes in the number of chromosomes in a cell or changes in the structure of a chromosome (Holeckova et al., 2021).

The use of plants as complementary and alternative medicine is increasing day by day. Plants have been considered raw materials for alternative medicine and have anti-mutagenic effects against chemicals and environmental factors. In 2015, World Health Organization reported that traditional medicine is used by almost 70% of the world's population, and in some countries incorporated extensively into the public health system. On the other hand, these extracts may also have mutagenic and cytotoxic effects on different organisms.

Popular knowledge of the usage of plants by humans is said to be on thousands of years of experience wherein people have recognized and utilized medicinal plants in their daily activities and source of living for survival (Camacho et al., 2012). Despite preliminary findings about the therapeutic advantages of medicinal plants, some of their constituents may be potentially toxic, mutagenic, carcinogenic, or teratogenic in some ways (Gadano et al., 2016).

Derris plants are evergreen lianas growing in the wild and subtropical areas of Asia. In the Philippines, the *Derris* species locally known as "tubli" usually thrives in thickets along riverbanks and streams or forests at low elevations from Luzon to Mindanao. They were once widely cultivated as important natural resources for insecticidal products (De Jesus, 2012). Throughout history, plants have provided a source of inspiration for novel drug compounds, as plant-derived medicines have truly made a great contribution to human health and well-being (Ekpo and Etim, 2019). *D. elliptica* has been gradually utilized as herbal remedy for the cure of various ailments even though they have not been explored scientifically. It is traditionally used for antisepsis and applied to abscesses and it is used against leprosy and itching (Orwa et al., 2016).

They were also used by native people to treat the infestation of insects and some other pests. These plant extracts are also extensively used in cattle to control ticks and other ectoparasites (Yoon, 2016). For hundreds of years since human beings used *Derris* root powder specifically for fishing. These properties are due to the presence of rotenone (Uy et. al., 2015). Rotenone is not stable in air, light, and alkaline conditions. It is rapidly broken down in soil and water. Therefore, almost all toxicity may be lost after 2–3 days of exposure, so it does not pollute the environment; nor does it accumulate in the food chain like the persistent synthetic insecticides (Starr et al., 2013).

Higher plants have been proposed as test organisms for the detection of genotoxic substances in the environment. Several plant test systems are already in use and are found to be as sensitive and reliable as other short-term tests (Liman et al., 2014). *Allium cepa* is one of these plants, which has been used in different studies to detect chromosome aberrations induced by chemicals (Mbuni et al., 2020). The use of non-animal test methods, including in vitro studies, provides important tools to enhance our understanding of the hazardous effect of chemicals, and for predicting these effects in humans. In vitro systems are used principally for screening purposes, and for generating toxicological profiles (Singh et al., 2018). Numerous chemicals can generate the breakage or interchange

of DNA segments between chromosomal structures (Chowdhury et al., 2014).

The *Allium* test is one of the few direct methods for measuring damage in systems that are exposed to mutagens and carcinogenic potential and enables the evaluation of the effects of these damages through the observation of chromosomal alterations (Bonciu et al., 2018). The root tips of plant species have been used for the study of induced chromosomal aberrations. Root tips of different *Allium* species are used the most frequently as experimental material. The plants served as a bioindicator of genotoxicity and possess some advantages over other organisms in certain circumstances (Bosio et al., 2012). Plants usually have chromosomes and low chromosome numbers. The root meristem contains a high proportion of cells in mitosis (Nefic et al., 2013).

A. cepa is distinctive in regards to its efficiency in detecting genetic damage and was introduced by Levan (1938). The first modification of the *A. cepa* test for environmental monitoring was introduced way back in 1985 by Fiskesjö. The chromosome assays on plants are rapid and inexpensive and do not require elaborate laboratory facilities and a wide range of genetic endpoints is available. The chromosomes of plants and animals are morphologically similar, and appear to respond to treatment with mutagens in a similar way to those of mammals and other eukaryotes.

The study focused on the genotoxicity effects of tubli *(Derris elliptica)* root extract in *Allium cepa* root tip cells. It determined if there is an observed and identified type of chromosomal aberrations during mitotic activity upon the application of excessive use of the substance rotenone from the root of *Derris elliptica* to the *Allium cepa* plant cell. Further, it also emphasizes the analysis of mitotic index, phase index, and percentage of aberrant cells performed in mitotic activity.

MATERIALS AND METHODS

Collection of Plant Material

Root samples of *Derris elliptica* were collected from the botanical garden of the Central Bicol State University of Agriculture, Pili, Camarines Sur. Only roots with a diameter size less than 1.5 cm were used. The fresh roots were cut into small pieces of about 50-60 mm in length and tangled with strings. *Derris* roots were wrapped with a thick cloth and soaked in 5 liters of water overnight to sustain the bioactive compound rotenone. It was stored in a basin and kept in a dark room with a temperature of 28-30°C.

Preparation of Plant Root Extracts

Traditional root extraction method was followed in this study. Fresh root samples (1,000 g) were washed thoroughly with tap water to remove the remaining soil and other unwanted materials. Roots were hammered and macerated in a basin. At first, the pure extract was intended to obtain but since it was so sticky and has a thick texture, 1 liter of distilled water was added to obtain the needed consistency of the extract. The roots were rinsed and filtered carefully in cheesecloth and placed the recovered extract in a separate container. The extract was then sieved through 15 cm Whatman #4 filter paper directly in a well-closed bottle and set aside in a refrigerator for at least 6-8 hours at 4°C.

Dilution of Plant Root Extracts

From the stock solution of root extracts, graded or serial dilution of 1:10 was done to prepare the treatments. It is a series of sequential dilutions used to reduce a more usable concentration.

In the determination of the genotoxicity effect of tubli *(D. elliptica)* root extract on the onion plant, three replicates of onion bulbs

were planted in an identical container with different concentrations of the extract were set. The serial concentration of 1:10 for T_1, T_2, and T_3 were treated respectively and exposed to extract for 6 hours at 28°C-32°C room temperature. *A. cepa* was exposed to *D. elliptica* root extract with different concentrations, 0.2%, 0.5%, and 1% for T_1, T_2, and T_3, respectively. While the control group (T_0) was used, without any exposure to *D. elliptica* root extract.

Allium cepa Genotoxicity Assay

Twelve (12) common onions (*Allium cepa*, 2n = 16) were obtained; it was peeled and the dried roots were removed in the test system. Cleaned identical containers were prepared. For setup, there were three replicates per treatment (T_0, T_1, T_2, and T_3). Each container was filled with distilled water where the red onion bulbs were placed and germinated. Then, the onions were placed in the corresponding 300 mL round container and filled with water up to the brim, at least ¼ part of the onion is submerged in the water. The mouth of the bottle must be of right size enough and adequate to hold the bulb to have in contact with the water surface so as not to let the onion bulb fall into the container (Figure 1). The roots were observed to grow within 2-3 days and the water was regularly replaced daily to prevent contamination of the root growth.

After two days, the onions in T_1, T_2, and T_3 with their three replicates were exposed to 0.2%, 0.5% and 1% *D. elliptica* root extract respectively for 24 hours (Sayono, et al., 2019).

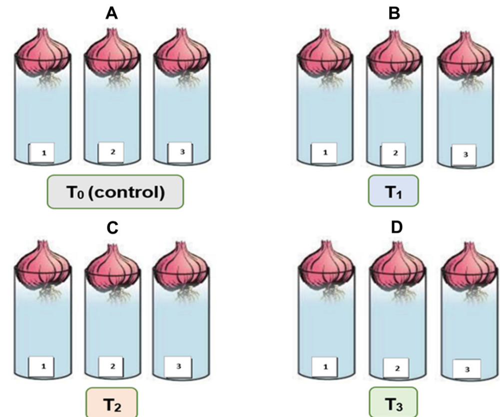

Figure 1. Experimental Set-up for *Allium cepa*. A: To (control group); B: T_1; C: T_2; and D: T_3.

After a day of exposure, approximately 2-3 cm long from the tip were gathered from 11:00 o'clock in the morning to 2:00 o'clock in the afternoon (Eren et al., 2014), the time when usually onion cells are actively dividing and mitotic activity is at its peak (Akarsha et al., 2021).

A modified protocol for squashing the root tips was adapted (Quilang et al., 2012). The root tips were then fixed in a petri dish with a Farmer's solution maintaining the ratio of one (1) part glacial acetic acid and three (3) parts 95% ethanol. From the obtained root tip, about 2 mm from the root cap was cut and treated with distilled water and placed on a clean glass slide. The cells were exposed to 1N Hydrochloric acid (HCl) for at least 3-5 minutes in order to soften the tissues and make them transparent. Root tips were covered with a cover slip and carefully squashed or crushed by another slide using a pencil's eraser to flatten the tips until the cells were nearly in a single layer.

Root tips were removed from 1N HCl and stained with acetocarmine or Giemsa dye with a ratio of 2 mg Giemsa to 16 mL of water for at least 3-5 minutes. Using a piece of tissue paper, the excess stain was removed. The slide was gently heated over the flame of an alcohol lamp. Thereafter, the

slide was sealed with a clear manicure for observation and initial preservation.

Determination of Chromosomal Aberration in *A. cepa* Induced by *D. elliptica* Root Extract

Twelve (12) fully-grown onion roots were obtained. There were three replicates per treatment (T_0, T_1, T_2, and T_3). For the T_0 control group set-up, each onion in the replicate was placed in a corresponding 75 mL container filled with distilled water up to the brim. About ¼ part of the onion is submerged in the water. While the T_1, T_2, and T_3 (experimental set-ups) were exposed to root extracts with 1:10 serial or graded concentration. Both the control and the experimental set-ups were treated respectively for 6 hours. The root tips were closely obtained from 11:00 am to 2:00 pm, the time when the cells are actively dividing and the highest mitotic activity was observed.

Fluorescence stereomicroscope was used to view the normal and aberrant cells. Each replicate in the treatments was sequentially labeled and viewed one at a time under the microscope. A total of 500 cells were evaluated and examined for each serial concentration. Using a digital camera, types of chromosomal aberrations in the different stages of mitosis were identified and determined.

Statistical Analysis

Statistical data were analyzed and performed using Mitotic Index, Phase Index, and Total Percentage of Abnormal Cells. For testing the significance, Analysis of Variance (ANOVA) was used with $p<0.05$ as the minimal level of significance.

In the mitotic index (MI); phase index (PI) and percentage of abnormal cells study, about 500 cells were counted as follows:

Mitotic Index

$$MI = \frac{\text{Total Dividing Cells}}{\text{Total Cells}} \times 100$$

Phase Index

$$PI = \text{Total Cells} \times \frac{100}{\text{Total Dividing Cells}}$$

Total Percentage of Abnormal Cells

$$T_{Abn} = TC_{Abn} \times \frac{100}{\text{Total Dividing Cells}}$$

RESULTS AND DISCUSSIONS

The usefulness of *A. cepa* root tips as a method for monitoring the genotoxic effects of test substances was first performed by Fiskesjö (1985) as cited by Adegbite et al. (2014). This form of genotoxicity assay is very appropriate for testing mutation aside from their role in the economy and human consumption (Nefic et al., 2013). *A. cepa* also served as an excellent bioindicator that exhibits sensitivity to harmful contaminants that may indirectly affect other eukaryotes and humans as well based on their reactions (Ozkara et al., 2015).

The chromosome aberration can be an abnormality, disorder, anomaly, or mutation characterized by a missing, extra, or irregular portion of chromosomal DNA. It can be from an unusual number of chromosomes or a structural abnormality in one or more (Daniel et al., 2022).

Aberrations in chromosomes were observed during cell division. There were different stages of mitosis in *A. cepa* upon exposure to the root extract of *D. elliptica*. As shown in Figure 2A, the prophase stage reveals where chromosomes are starting to form; Figure 2A1 has prominent binuclei at prophase; while 2A2 micronucleus was present at the lower part. This type of aberration has a high amount of chromatin in the other cell nuclei. In metaphase, several aberrations were observed like sticky metaphase wherein the chromosomes are clumped and have high condensation; C-metaphase, wherein

there are chromosomal breakages, chromosomes are scattered in the equatorial plate; chromosomes that stay out of place during cell division, this is due to chromosomal breaks and fragments as shown in Figure 2: B1 and B2 respectively. While in Figure 2B, normal metaphase was evident. The chromosomes align at the equator of the cell. the chromosome fragments. In anaphase, the abnormalities observed were chromosome bridges formed between the separating groups of anaphase chromosomes that are being drawn opposite; apparently, chromosome breaks and multipolar are observed, respectively. (Figure 2C1-2C2); while normal anaphase was detected in Figure 2C. Lastly, the abnormalities observed in telophase were vacuolated cells or ball telophase (Figure 2D1); a form of mitosis with characteristically clumped chromosomes and binucleate cells is evident as shown in Figure 2D2, while normal telophase was observed where spindle fibers are still visible between the two nuclei and the cytoplasm has been divided (Figure 2D).

Results revealed that most of the aberrations observed were at the metaphase and anaphase stages. Few aberrations were recorded in the interphase, prophase, and telophase. Chromosome aberrations such as sticky chromosomes, breaks, and fragmented structures were commonly encountered. The binucleate cells and chromosome bridges occurred most frequently, while other aberrations were rarely found (Figure 2). Observed lagging chromosomes are usually noticed due to the inhibition of spindle fiber that makes some tend to separate during cell division (Potapova, 2017).

The reduction in mitosis as observed in this study implies that rotenone contains substances that have mitotic depressive properties. This may occur by inhibiting DNA synthesis and the formation of microtubules. Possibly, it could be an arrest of the 24h-cycle at G_1 and G_2 phases or disruption of nucleoprotein synthesis and a low level of ATP to supply the energy required for spindle elongation, chromosomal movement, or

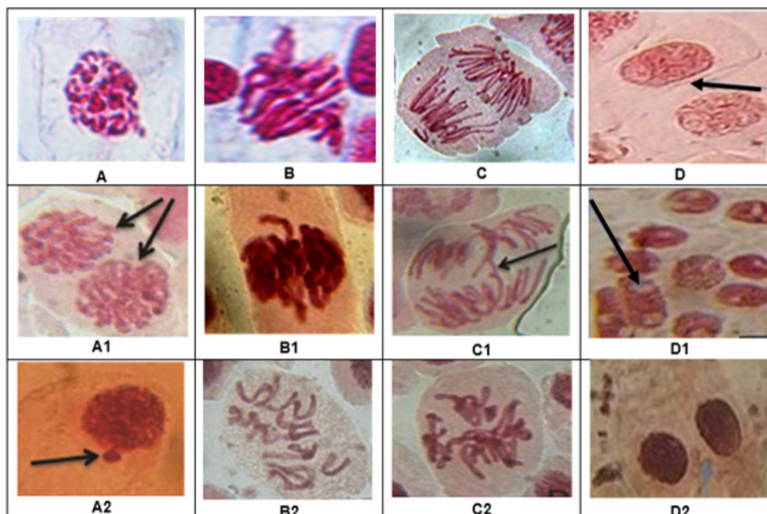

Figure 2. Micrographs of chromosomal aberrations observed after exposure of *A. cepa* root tip meristematic cells to *D. elliptica* root extract. (A) normal prophase; (A1) binuclei at prophase; (A2) micronucleus at prophase; (B) normal metaphase (B1) sticky metaphase; (B2) C-metaphase; (C) normal anaphase; (C1) anaphase with chromosome bridge and chromosome loss; (C2) chromosome breaks and multipolar; (D) normal telophase; (D1) vacuolated cells; and (D2) binucleate cells (Magnification ×400).

microtubule formation (Majewska et al., 2003). However, these hypotheses require further investigation.

Mitotic index (MI) is the ratio between the number of cells in a population undergoing mitosis versus the number of cells in a population not undergoing mitosis. It is essential to measure the proliferation status of a cell population (Proudlock, 2016).

Results showed that the aberrant cells from T_2 to T_3 decreases as the concentrations of extract decreases. The cells that were exposed to the extract in T_1 reveals the highest result in the percentage of aberrant cells with a result of 1.13% as shown in Table 1. Similar to the report gathered from Bakare et al. (2012), mitotic depression in *A. cepa* root cells was also recorded in the treatment but not observed in the control (Raji et al., 2018) with an average of 101.7 phase indexes as the mitotic index in T_0 has the highest average of mitotic average of 98.3 and the lowest was in T_1 with an average of 82.6 mitotic indexes. The phase index in T_1 was the highest average of 121.1 and the lowest was in T_0 (Table 1).

This result only showed that there is a relationship between MI and the treatment as the concentration increases the mitotic activity decreases and this is due to the blocking of the G_1 stage suppressing DNA synthesis (Bertoli et al., 2013).

A growing literature suggests that rotenone blocks mitosis and inhibits cell proliferation by perturbing microtubule assembly dynamics (Srivastava et al., 2007).

Table 1. Chromosomal aberration in *A. cepa* exposed with *D. elliptica* root extract.

Tr	Rep	P	M	A	T	TDC	TC	MI	PI	TAC	%AC
	1	468	6	10	8	492	500	98.4	101.6	0	0
T_0	2	470	7	8	6	491	500	98.2	101.8	0	0
	3	468	7	8	8	491	500	98.2	101.8	0	0
	Total	**468.6**	**6.6**	**8.6**	**7.3**	**491.3**	**500**	**98.3**	**101.7**	**0**	**0**
	1	408	2	2	0	412	500	82.4	121.4	6	1.2
T_1	2	412	1	2	1	416	500	83.2	120.2	6	1.2
	3	404	2	3	2	411	500	82.2	121.7	5	1.0
	Total	**408.0**	**1.6**	**2.3**	**1.0**	**413.0**	**500**	**82.6**	**121.1**	**5.6**	**1.13**
	1	440	3	5	1	450	500	90.0	111.1	5	1
T_2	2	430	2	3	2	435	500	87.0	114.9	3	0.6
	3	421	3	3	0	428	500	85.6	116.8	3	0.6
	Total	**430.3**	**2.6**	**3.6**	**1.0**	**437.6**	**500**	**87.5**	**114.3**	**3.6**	**0.73**
	1	458	2	6	2	468	500	93.6	106.8	2	0.4
T_3	2	460	3	7	1	471	500	94.2	106.1	3	0.6
	3	466	2	5	1	474	500	94.8	105.5	3	0.6
	Total	**461.3**	**2.3**	**6.0**	**1.3**	**471.0**	**500**	**94.2**	**106.1**	**2.6**	**0.53**

Legend: **Tr**=Treatment; **P**=Prophase; **M**=Metaphase; **A**=Anaphase; **T**=Telophase; **TD**=Total Diviving Cells; **TC**=Total Cell Counted; **MI**=Mitotic Index; **PI**=Phase Index; **TAC**=Total Aberrant Cells; **%AC**=Total Percentage of aberrant cells.

At its effective inhibitory concentration range, rotenone-depolymerized spindle microtubules of both cell types that suppressed the reassembly of microtubules in living cells, suggesting that it can suppress microtubule growth rates (Panda et al., 2007).

The genetic results show that the efficiency of the extracted solvent in rotenone may affect the abnormalities in the cells. This supports the report of Alin et al. (2016) that there were factors to be taken into account to have an optimum and reliable outcome for the cytotoxic effect on the cells. Rotenone caused a time- and dose-dependent decrease in cell viability (Samrat et al., 2021). Rotenone also induced alterations in mitochondrial morphology (Heinz, 2017), which in contrast to its effect on peroxisomes was dependent on the generation of its microtubule-active properties (Passmore et al., 2017).

CONCLUSION

The current study showed effects of plant extracts exposed to rotenone concentrations on chromosomal aberrations in onion root tips. This revealed that the variable nature of chromosomal abnormalities due to exposure of *A. cepa* to *D. elliptica* which was observed at different phases of mitosis. Most of which are noted in metaphase, like sticky metaphase, breaks, fragments, and C-metaphase. In anaphase, bridges, and are multipolar cells, while ball telophase and binucleated cells are revealed. The presence of such abnormalities indicates the interference of toxic agents in the normal growth of cells. Plants with such abnormalities may transfer altered genetic makeup not only to their offspring but also to humans when consumed as food thereby causing further complications. Because of these mutagens, the plants are affected not just on the chromosome but also in the expression of their genes, and phenotype.

The effect of rotenone on root-mitosis simulates in the type of abnormal meta- and anaphases (with X-shaped chromosomes) and the induction of tetraploid cells. Rotenone, on the other hand, study shows a significant difference in the mitotic index and in the total percentage of abnormal cells in *A. cepa*. Many diseases show a direct correlation with the parameters used to measure the division behavior of cells. Thus, a detailed understanding of the cellular and molecular mechanisms of cell division by cell proliferation and cytotoxicity tests is critical in distinguishing between normal and healthy cells. However, the fact that the details of the intracellular milieu are not completely understood yet. It is logical to anticipate in vitro cytotoxicity tests to measure cell proliferation and cytotoxicity will continue to be the first choice for investigation.

Data Availability

The data for our cell counts were used to support the findings of this study and are available from the corresponding author upon request.

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Conflicts of Interest

The author declares no conflicts of interest.

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