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Genotoxicity Screening of the Crude Ethanolic Extract of *Artocarpus heterophyllus* (langka) Unripe Fruit Using *Allium cepa* (Onion) Assay

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Abstract: ***The crude ethanolic extract of Artocarpus heterophyllus (langka) unripe fruit was found out to lower blood sugar in alloxan-induced hyperglycemic white mice (Mus musculus).This study was conducted to determine the genotoxicity (mitotic index (MI) and percent chromosomal aberration (%CA) of the crude ethanolic extract of A. heterophyllus unripe fruit using A. cepa (onion) assay. Onions with 1.5-2.0cm length of the roots were grown in different concentrations of A. heterophyllus extract. The 3 roots with a length of 2mm from the tips were harvested and fixed. Squash preparations were made for each of the concentration, positive and negative control in 3 replicates and 3 trials. Results showed that 15mg (MI=0.782±0.022; %CA=0.06±0.003) and 20mg (MI= 0.754±0.017; %CA=0.04±0.019) of the crude ethanolic extract per ml of ml of distilled water did not show genotoxicity based on the mitotic index (MI) and %chromosomal aberration (CA). They were comparable to the negative control used (dist. water) (MI=0.756±0.028; CA=0.08±0.022) (P<0.05) and not comparable to the positive control (Hydroxylamine HCl) (MI=0.179±0.023; %CA=1.36±0.134). The 25mg (MI=0.682±0.035; CA=0.05±0.018) of the extract per ml of ml of distilled water showed genotoxicity based on the mitotic index but did not show genotoxicity in terms of %chromosomal aberration. The mitotic index of 25mg/ml is significantly lower than the negative control but not comparable to the positive control. The study established the non genotoxicity of 15mg and 20mg of the crude ethanolic extract of the crude ethanolic extract of A. heterophyllus unripe fruit extract per ml of distilled water. The 25mg exhibited genotoxicity in terms of mitotic index but not in chromosomal aberration. This study could serve as basis of future studies of bioactivities of A. heterophyllus unripe fruit extract.***

Keywords: genotoxicity, *Artocarpus heterophyllus*, *Allium cepa* assay, mitotic index

1. Introduction

Background of the Study

Artocarpus heterophyllus (langka) belongs under Moraceae family. It is an evergreen fruit tree cultivated in many tropical regions. Its phenol compound from its wood exhibited anti-proliferative effect on cancer cell line [1].While the butanol fractions of the root bark and fruits have antibacterial effect [2]. Its leaf extracts has antioxidant activity, anti-hyperglycemic and anti-hyperlipidemic effects [3]. The flavonoid fraction from its leaf exhibited hypoglycemic effect [4]. The polyphenolic compound from it stegmen showed antitumor activity [5]. Its leaves have wound healing activity [6] (Gupta *et al*, 2009). In one study, the crude ethanolic extract of the unripe fruit of *A. heterophyllus* at 15mg/20 gram body weight of mice exhibited anti-hyperglycemic activity by 36% inhibition of fasting blood sugar (FBS) in alloxan induced-diabetes in white mice (*Mus musculus*).

A. heterophyllus plant has many medicinal uses. Therefore there is a need to determine its genotoxicity effect especially its fruit due to its anti-hyperglycemic effect. This can be determined using the *Allium cepa* test. In this test, the roots are grown in direct contact with the compound of interest to be tested like the plant extract. *A. cepa* test is a very good *in vivo* model that can predict of the possible damage to the DNA of eukaryotes. This test uses a model that is adequately sensitive to detect numerous substances that cause chromosomal alterations. The *A. cepa* test is one of the few direct methods for measuring damage in systems that are exposed to mutagens or potential carcinogens. It enables also the evaluation of the effects of these damages through the observation of chromosomal alterations. Therefore, the data can be extrapolated for all animal and plant biodiversity. The analysis of chromosomal alterations can be equal to the test of mutagenicity mainly for the detection of structural alterations. It is also possible to observe numerical chromosomal alterations (Ping *et al*. 2012).

Rationale of the Study

It was shown that the crude ethanolic of *A. heterophyllus* unripe fruit has anti-hyperglycemic activity. Therefore there is a need to determine its possible genotoxicity effect.

Objectives of the Study

This study was conducted to determine the genotoxicity of the crude ethanolic extract of *A. heterophyllus* unripe fruit using *A.cepa* (onion) assay. Specifically, the study aimed to determine the concentration of *A. heterophyllus* that exhibit genotoxicity activity through mitotic indices and percent aberrant cells.

Significance of the Study

The result of this study could serve as basis of safe use of the of *A. heterophyllus* unripe fruit as anti-hyperglycemic agent in the future subject for further study.

2. Materials and Methods

Plant Collection and Identification

The onion was bought from the Iloilo Super Market, Iloilo City, Philippines. Before use, the outer scales of the bulbs and the dry bottom plates were removed away without destroying the root primordial [7]. *A. heterophyllus* (Fig. 1.) was properly identified based on the book of Quisumbing

(1978) [8] and Orwa *et al.*, (2009) [9]. The fruit was collected from Barangay Piapi, Hamtic, Antique, Philippines.

Figure 1: Artocarpus heterophyllus (langka) fruit

A. heterophyllus is locally called langka with English name jackfruit. It is an evergreen tree reaching the height of 8 to 25m, canopy dense with rarely buttress trunk. It has greyish-brown and scaly trunk. It has glossy leaves with dark green color on the top and light green underside and alternately arranged. It has a racemoid inflorescence. It has a multiple, spiky, yellow fruit and has waxy, oval to oblong shaped seeds [9].

Extraction of Plant Components

The *A. heterophyllus* unripe fruit was washed with tap water and rinsed with distilled water. After the removal of the peelings, it was chopped and ground using a blender. The ground unripe fruit was homogenized in 90% ethanol (2g plant material/5ml ethanol) for 5 days with shaking every 12 hours. Homogenized ground unripe fruit was filtered. The filtrate was dried. The crude ethanol extract was used for the genotoxicity screening.

Onion Genotoxicity Assay for Chromosome Abnormality Screening

The plant used as test material was the onion bulb (*Allium cepa*) L. (2n= 16). Three clean and healthy bulbs of onion were chosen for each treatment (15, 20, 25 mg of extract/ml of distilled water). The dry scales of bulbs were removed. The onions were grown in distilled water, at room temperature for 2-3 days. When the roots reached 1.5-2 cm in length, they were transferred to the treatments. The treatments were changed daily to ensure that they are freshly prepared.

The negative control was prepared by exposing the bulbs to distilled water only and for the positive control, onion bulbs were placed in Hydroxylamine hydrochloride (500µg/mL). The solution was changed daily. On the 3rd, 3 root tips from each bulb was harvested, fixed in Carnoy's fixative (1:3 acetic: alcohol) for 24hours. The roots were subjected to slides preparation [10].

Slides Preparation

After pre-treatment, the root tips were washed 4 times with distilled water. They were hydrolyzed with 1 N HCl at 60-70◦C for 5 min. After hydrolysis, the roots were washed with tap water. One to two millimeter of the root tips were cut and placed on the slide. A drop of aceto-orcein was added on the root tip and left for 2min. After proper fixation and staining, appropriate squash preparations was made for each of the treatments and control. A cover slip was lowered carefully on the slide to avoid bubbles.

Observation of Specimens

Effects of the different concentrations of crude ethanolic extract of *A. heterophyllus* together with the negative and positive control on different chromosome plates were observed under light microscope at 1000x magnification. The number of cells in mitosis was noted and the mitotic index was computed using the formula:

$$\text{Mitotic index} = \frac{\text{number of cells in mitosis}}{\text{total number of cells}} \times 100$$

The chromosomal abnormalities that were recorded were: c-metaphases, stick chromosome, chromosome breaks and losses, bridged anaphases, multipolar anaphases, and micronucleated and binucleated cells. Other abnormality includes aberrant interphases (vacuolated nuclei and binucleated cells), were recorded [11, 12].

Percent of aberrant cells were determined by examining and counting minimum of 100 cells per slide (9 slides were observed for each treatment). The experiment was replicated 3 times in 3 trials. The percent of aberrant cells was computed using:

$$\text{percent of aberrant} = \frac{\text{number of aberrant cells}}{\text{total number of cells}} \times 100$$

Statistical Data Analysis

Data obtained such as the mitotic indices and percent aberrant cells were analyzed using one way Analysis of Variance Technique (ANOVA) at significant level of $p<0.05$ using Statistical Tool for Agricultural Research (STAR).The post hoc test that used was the least significant difference (LSD).

Waste Disposal

Allium cepa bulbs and roots were sanitized with Lysol spray and disposed to biological hazard container. Waste chemicals were discarded on the assigned containers.

3. Results

Result showed that 15mg and 20mg of the crude ethanolic extract per ml of distilled water did not show genotoxic activity based on the mitotic index and % chromosomal aberration. Their mitotic indices and % chromosomal aberrations were comparable to the negative control used (dist. water) ($P<0.05$). Their results were not comparable to the positive control (Hydroxylamine hydrochloride). However, the 25mg of the crude ethanolic extract per ml of ml of distilled water showed genotoxic activity based on the mitotic index but did not show genotoxic activity in terms of % chromosomal aberration. The mitotic index of 25mg/ml is significantly lower than the negative control but not comparable to the positive control.

Table 1: Result of genotoxicity of the crude ethanolic extract of *A. heterophyllus* in onion (*A. cepa*)

Treatments extract (mg/ml)	*%chromosomal aberration (mean±SEM)*	*Mitotic index (mean±SEM)*
15	0.782±0.022[a]	0.06±0.003[b]
20	0.754±0.017[a]	0.04+0.019[b]
25	0.682±0.035[b]	0.05±0.018[b]
negative control	0.756±0.028[a]	0.08±0.022[b]
positive control	0.179±0.023[c]	1.36±0.134[a]

Note: a>b>c; the same letter means comparable

4. Discussion

The study showed that 15mg and 20mg of the crude ethanolic extract of unripe fruit *A. heterophyllus* per ml of distilled water did not show genotoxic activity based on the mitotic index and % chromosomal aberration in onion bulb. However, the 25mg/ml slowed down the cell division but did not cause chromosomal aberrations. The 15 and 20mg of the extract did not hinder the cell division and did not induce the formation of chromosomal aberrations such as c-mitosis, laggard, bridges, stickiness, vagrant cells and micronuclei based on the *A. cepa* assay. *A. cepa* assay for genotoxicity test is an economic cheap, reliable and valuable test used by several researchers. The reduction of the mitotic index might be explained as being due to the obstruction of the onset of prophase, the arrest of one or more mitotic phases, or the slowing of the rate of cell progression through mitosis [13].

According to Ping *et al*., (2012) [10], the induction of micronuclei in root meristem of *A. cepa* or in any cell of any other organism is a sign of chromosome damage. It is a sign of interruption of the process of mitosis. The formation of micronucleus is due to the formation of a new membrane around the chromatin matter. This leads to the failure of the chromosome to move to the poles during the anaphase of mitosis. The chromatin matter may come from anomalous disjunction of chromosomes due to spindle abnormalities. It may arise also from the breakage of chromosomes resulting in formation of acentric fragments, dicentric chromosomes and chromatin bridges. A compound that induces of micronuclei is a spindle inhibitor or a clastogen. *A. heterophyllus* fruit contains lignans, isoflavone and saponins. It contains also phenollic compound [14]. These compounds may have cause the induction of micronuclei, although they have medicinal importance.

The 15 and 20mg/ml of the crude ethanolic extract of unripe *A. heterophyllus* showed non genotoxic in onion bulb unlike the *Loranthus micranthus* [15], toasted cassava granules popularly known as *garri* [16], and *Euphorbia hirta* which exerted significant genotoxic and mitodepressive effects at 1,000 µg/mL [10].

These findings on the genotoxicity were true only when the 15, 20 and 25mg/ml of the crude ethanolic extract of unripe *A. heterophyllus* were tested in onion roots.

5. Conclusion

The study established the non genotoxic activity of the crude ethanolic extract of the crude ethanolic extract of *A. heterophyllus* unripe fruit extract using onion at 15, 20 mg/ml of distilled water in terms of mitotic index and % chromosomal aberration. The 25mg/ml of the extract did not exhibit genotoxic activity in terms of % chromosomal aberration but lowered mitotic index.

This study could serve as basis of future studies on the use of the crude ethanolic extract of *A. heterophyllus* unripe fruit specifically on the genotoxicity.

6. Future Scope

For further studies, use concentrations lower than 25mg/ml of the crude ethanolic extract of *A. heterophyllus* unripe fruit in other bioactivities to be conducted. Since this study was limited only to the genotoxicity of the crude ethanolic extract of *A. heterophyllus*, other extractants can be used like distilled water because it has been used as vegetable and since no study has been conducted. The use of other positive control is suggested also as basis of comparison of genotoxic activity.

7. Acknowledgement

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Skills Acquired and Trainings/Seminars Attended

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- Postcoital antifertility testing
- Abortifacient testing
- Teratotogenicity testing
- Mutagenicity Testing
- Analgesic Testing

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Communicating researches to stakeholders by DOST
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cytotoxicity testing using brine shrimp
antimicrobial Testing
antihyperglycemic Testing
anti- arthritis Testing
anti-diarrheal Testing
anti-inflammatory
anti- depressant Testing
genotoxicity
larvicidal assay
anti-pediculocidal assay
molluscicidal Testing
phytochemical Analysis