Genotoxic Effect of Herbal Mixture of *Phyllantus amarus*, *Xylopica aethiopica* and *Sida cordifolia*

1Gbenle OA, 2Taiwo IA, 3Odeigah PGC, 3Ofoma ON

1Department of Biomedical Engineering, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria.
2Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, Nigeria.
3Department of Biochemistry, College of Medicine, University of Lagos, Idi-Araba, Lagos.

Corresponding Author

OA Gbenle
Department of Biomedical Engineering, College of Medicine, University of Lagos, Lagos, Nigeria.
Email: gbenlesegz@yahoo.com; Tel.: +2348029085197

ABSTRACT

Background: The herbal mixture of *Phyllantus amarus*, *Xylopica aethiopica* and *Sida cordifolia* is commonly used as a decoction for blood tonic. Genotoxicity has been shown in the individual plants. However, the genotoxic effect of this herbal mixture has rarely been investigated.

Objective: To evaluate the genotoxic effect of herbal mixture of *Phyllantus amarus*, *Xylopica aethiopica* and *Sida cordifolia* using multiple genetic assay systems.

Materials and Methods: Root tips of *Allium cepa* were treated with various concentrations of the herbal mixture (31.5, 63, 94.5 and 126mg/L) and distilled water respectively for 48 h. The root tips of *A. cepa* were then subjected to cytological examination using Jette Rank method.

Results: Root lengths of actively growing *A. cepa* grown in the various concentrations of the herbal mixture decreased with increasing concentration of the herbal mixture over the experimental period. The treated roots showed various morphological abnormalities compared to the control roots grown in distilled water which showed no abnormal morphology. The herbal mixture induced various types of chromosomal and mitotic abnormalities which include binucleated cells, C-anaphase, sticky chromosomes and telophase bridges. Stickiness, vagrants and binucleated cells were the commonest of all observed aberrations as they were found at almost all concentrations of the polyherbal preparation. Frequency of aberration was found to increase with increasing herbal mixture concentration. Mitotic index decreased while mitodepression increased with increase in herbal mixture concentration. Likewise, the control had a mitotic index of 8.60%. The highest mitotic index of the mixture treated root was observed at 31.5mg/L (7.30%) and the lowest was observed at 126mg/L (3.56%).

Conclusion: The result suggests that the herbal mixture of *Phyllantus amarus*, *Xylopica aethiopica* and *Sida cordifolia* may have genotoxic effect.

Keywords: Genotoxic, herbal mixture, chromosomes, binucleus, aberration.

INTRODUCTION

A medicinal plant is any plant with one or more of its organs containing substances that can be used for therapeutic purpose or which can be used as precursors for the synthesis of drugs (1). There are about 250,000 to 500,000 species of plant on earth (2), a relatively small percentage (1–10%) is used as foods by animals including humans, but more are used for medicinal purposes (3). More than 400,000 species of tropical flowering plants have medicinal properties; this has really improved traditional medicine (4).

The use of medicinal plants is an important part of traditional medicine. Some of the most important uses of plants are food and medicine. Various parts of plants have been reported to be used in the treatment and cure of diseases such as pile, malaria, skin diseases, high blood pressure, ulcer, yellow fever and measles (5). Traditionally, the usage of plants in curing diseases has deep roots in history of man (6). Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. Some plant decoctions are of great importance in treating several diseases (7). Medicinal uses of plants for treatment of diseases prevail in Africa, especially among Nigerians. The curative plants vary among the natives in different localities. It has been reported that medicinal plants are effective against microorganisms; as a result, plants are one of the bedrocks for modern medicine to attain new principle (8). As regards this, plants have given western pharmacopoeia about 7,000 different pharmaceutically important compounds and a number of top-selling drugs of modern time (9).

The plants studied in this research are *Phyllantus amarus*, *Xylopica aethiopica* and *Sida cordifolia*. The extracts of the studied plants are combined and used mainly as a decoction for blood tonic in humans (5). This research aimed at investigating the genotoxic effect of the herbal mixture on *Allium cepa*. The objectives are to evaluate the genotoxic effect of the herbal mixture and determine and identify the effects of the herbal mixture at microscopic and macroscopic levels.
**MATERIALS AND METHODS**

**Plant Collection and Authentication**

Plants used in this study were obtained from Ojuwuye market, Mushin, Lagos, Nigeria. *Phyllantus amarus* (LUH 8561), *Xylopica aethiopica* (LUH 8563) and *Sida cordifolia* (LUH 8562) were authenticated at the Department of Botany, Faculty of Science, University of Lagos, by Dr G. I. Nodza, and voucher specimen were deposited.

**Plant Materials and Extraction**

The shoots of the plants were air-dried in the shade for two weeks and pulverized separately into fine powder using mortar and pestle. Equal masses of each plant powder were then mixed; 40g of the mixture was soaked in 1 L of water for 4 h at room temperature and sieved after thorough mixing to get the herbal mixture. The filtrate obtained was stored in a container at 4°C until use. This served as the stock solution. The concentration of the stock solution was determined by evaporation to be 1400 mg/L. From this, other concentrations (1mg/L, 10mg/L, 100mg/L and 1000mg/L) were obtained by dilution using the formula $C_1V_1 = C_2V_2$.

**Root Growth Inhibition Test**

The root growth inhibition test was performed as a 96 h semi-static exposure test using four different concentrations (1mg/L, 10mg/L, 100mg/L and 1000mg/L) of the herbal mixture. Healthy onion bulbs of medium size were selected. The outer scale and bottom of the bulbs were removed to expose the ring of the primordial. The peeled onion bulbs were then exposed to the herbal mixture at different concentrations of 1mg/L, 10mg/L, 100mg/L and 1000mg/L respectively for four days. Distilled water was used as control. The test solution was replaced by fresh solution after every 24 h. At the end of the exposure period, the length of the root bundle was measured (in cm) with a ruler. This was carried out in quadruplicates and the mean value obtained. From the weighted averages for each concentration and the control, the percentage root growth inhibition in relation to the negative control and the EC$_{50}$ (the effective concentration where root growth amounts to 50% of the control) for each extract was determined (10).

**Genotoxicity Assay**

This procedure was carried out as described by Fiskejo (10). Four different sample concentrations were used (the EC$_{50}$ concentration, 50%, 150% and 200% respectively of EC$_{50}$ which were 63mg/L, 31.5mg/L, 94.5mg/L and 126mg/L respectively) with distilled water used as control. Onion bulbs were exposed to these concentrations of the herbal mixture for 48 h. The test solution was changed every 24 h. After growing of the onions root tips, the root tips were cut off, fixed in 1 M HCl and prepared for microscopy. Only one slide from each onion bulb was prepared. Ten root tips at a length of 10mm were cut and placed in Bijou bottle containing 10 ml (1:3 of glacial acetic acid and absolute alcohol) of acid alcohol. Thus, root cells became fixed. The root was placed on a slide and the terminal root tips (2 mm) were cut off for further microscopic preparation. The rest of the materials were discarded from the slide and excess liquid was sucked up by a piece of blotting paper. Two drops of freshly filtered 2% orcein solution were added and mixed with the root tips. Cover slip was placed on the root cells and allowed to absorb stain for 10 min.

**Slide Preparation and Microscopic Examination**

Root tips were squashed on the cover glass and pressed slightly down with the thumb. The cover slip was fixed carefully to the slide with nail hardener. Prepared slides were kept carefully for microscopic examination. All slides were coded and examined blind. The microscopic analysis included the mitotic index (MI) and scoring of chromosomal aberrations in cells. The mitotic index was found by counting all stages of mitosis out of total cells. The slides were thoroughly examined and cells scored for aberrations. Two categories of aberrations were scored, namely bridges and fragments. Other less fragment aberration such as multipolarity, C-mitotic anaphases, and others were also scored. Three slides were prepared for each concentration and they were all examined under a phase-contrast microscope with oil immersion lens (×40). Good slides were selected from several prepared slides and subsequently photographed using a Zeiss photomicroscope.

**RESULTS**

The growth inhibition is shown in Table 1, with the root growth values expressed as a percentage of the control. There was a decrease in the mean values of root length as concentration increased (Table 1).

<table>
<thead>
<tr>
<th>Plant Extract Concentration (mg/L)</th>
<th>Mean Values of Four Root Length (cm)</th>
<th>Root Growth Values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>4.00 ± 0.05</td>
<td>100.00</td>
</tr>
<tr>
<td>1</td>
<td>3.70 ± 0.13</td>
<td>92.50</td>
</tr>
<tr>
<td>10</td>
<td>2.80 ± 0.19</td>
<td>70.00</td>
</tr>
<tr>
<td>100</td>
<td>1.20 ± 0.16</td>
<td>30.00</td>
</tr>
<tr>
<td>1000</td>
<td>0.50 ± 0.08</td>
<td>12.50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant Extract Concentration (mg/L)</th>
<th>Mean Values of Four Root Length (cm)</th>
<th>Root Growth Values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>4.00 ± 0.05</td>
<td>100.00</td>
</tr>
<tr>
<td>1</td>
<td>3.70 ± 0.13</td>
<td>92.50</td>
</tr>
<tr>
<td>10</td>
<td>2.80 ± 0.19</td>
<td>70.00</td>
</tr>
<tr>
<td>100</td>
<td>1.20 ± 0.16</td>
<td>30.00</td>
</tr>
<tr>
<td>1000</td>
<td>0.50 ± 0.08</td>
<td>12.50</td>
</tr>
</tbody>
</table>

Variations in root length of *Allium cepa* with response to varying concentrations of herbal mixture is shown in Plates 1–5. A pronounced reduction in root length was observed as the concentration of the herbal mixture increased.

**Plate 1: Control (distilled water).**
Plates 1–5: Variations in Root Length of *Allium cepa* with response to varying concentrations of Herbal Mixture of *Phyllantus amarus*, *Xylopica aethiopica* and *Sida cordifolia*.

The mitodepressive effects of the herbal mixture on *Allium cepa* increased as the concentration increased (Table 2).

Table 2: Mitodepressive Effect of Herbal Mixture of *Phyllantus amarus*, *Xylopica aethiopica* and *Sida cordifolia* on root cell of *Allium cepa*.

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Total Examined Cells</th>
<th>Dividing Cells</th>
<th>Mitotic Index (%)</th>
<th>Mitodepression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>500</td>
<td>43</td>
<td>8.60</td>
<td>–</td>
</tr>
<tr>
<td>31.5</td>
<td>451</td>
<td>33</td>
<td>7.30</td>
<td>28</td>
</tr>
<tr>
<td>63.0</td>
<td>406</td>
<td>29</td>
<td>7.10</td>
<td>36</td>
</tr>
<tr>
<td>94.5</td>
<td>378</td>
<td>19</td>
<td>5.00</td>
<td>88</td>
</tr>
<tr>
<td>126</td>
<td>365</td>
<td>13</td>
<td>3.56</td>
<td>138</td>
</tr>
</tbody>
</table>

The mitotic aberration effect of the herbal mixture on *Allium cepa* is shown in Plates 6–13. More mitotic aberrations were observed as the concentration increased.

DISCUSSION

In this study, the root growth of *Allium cepa* decreased as concentration of herbal mixture of *Phyllantus amarus*, *Xylopica aethiopica* and *Sida cordifolia* increased. This shows that the herbal mixture may be mutagenic. The study also showed a concentration-dependent reduction in mitotic index observed in roots treated with various concentrations of the herbal mixture (11). It was observed that there were cytological aberrations in *Allium cepa* root tips. This shows that the herbal mixture may be capable of inducing genotoxicity at the chromosomal level. Mixture of *Phyllantus amarus*, *Xylopica aethiopica* and *Sida cordifolia* induced a number of mitotic aberrations such as...
Genotoxic Effect of Herbal Mixture of *P. amarus*, *X. aethiopica* and *S. cordifolia*

Plate 6: Normal Metaphase (0mg/L).

Plate 7: Normal Telophase (0mg/L).

Plate 8: Normal Anaphase (0mg/L).

Plate 9: Bridge and Fragmentation (94.5mg/L).

Plate 10: Bridge Anaphase (126mg/L).

Plate 11: Binucleated Cells (94.5mg/L).

Plate 12: Aberrated Metaphase (126mg/L).
fragments, C-anaphase, C-metaphase and stickiness. Frequency of aberration was very minimal in the control; there was increase in frequency of aberration as herbal mixture concentration increased. These mitotic aberrations may be due primarily to the disturbance of the spindle fibre formation and cell plate. The most important abnormalities observed in this study were stickiness, vagrants and binucleus. In this study, abnormalities such as chromosome stickiness were produced at both metaphase and anaphase stages by the herbal mixture. Bridges were also noticed in herbal mixture treatment of higher concentrations. When it involves one or more chromosome pair, it results in single, double or multiple bridges which could be attributed to general stickiness of chromosome and subsequent failure of anaphase separation (7) or it may be the result of chromosome bridge reunion (12). The degree of the frequency of the genotoxic aberrations is usually at high dosage of plant extracts as herbal medicine (13). Stickiness appears to be the most frequent mitotic abnormality in this study. Binucleus observed may be as a result of dysfunction of the spindle apparatus. Caffeine treatment of telophase stage cell induces formation of binucleus (11).

The results obtained with the Allium cepa test in this study are similar to those obtained with the testing of different chemicals on other organisms, including eukaryotes and prokaryotes (14).

CONCLUSION
The results from this study suggest that combination of extracts of Phyllanthus amarus, Xylopica aethiopica and Sida cordifolia may be mutagenic. The continuous ingestion of this herbal mixture as medicine, which is very rampant especially in the urban-rural settings, could act as mutagen to man. It is therefore very important, as the result of this project suggests, that the intake of this herbal mixture as medicine be monitored and regulated because of its genotoxic effect on living organisms.

Conflict of Interest
None declared by the authors.

REFERENCES
1. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons Ltd, New York. 1984; 244.

Plate 13: Vagrant Chromosome (126mg/L).
