Chromosomal Aberrations Induced in Root Tips of *Allium cepa* by Squeezed *Garri* Extracts

Daniel I. Olorunfemi and Emmanuel O. Ehwre

Abstract: The potential genotoxic effects of squeezed extracts from toasted cassava granules popularly known as *garri*, a popular Nigerian cassava meal, obtained from different fermentation days were investigated using the *Allium cepa* assay. The squeezed extracts were prepared with potable water as practiced conventionally by soaking 1-day fermented, 2-day fermented, 3-day fermented and 4-day fermented *garri* and squeezing out the liquid. A series of 5 onion bulbs were exposed to 1, 2.5, 5 and 10% (effluents, v/v) concentrations of each of the extracts for macroscopic and microscopic analyses. There was fermentation and concentration-dependent and statistically significant (*P* < 0.05) inhibition of root growth by the extracts when compared with the control. The EC$_{50}$ obtained for 1 day, 2 day, 3 day and 4 day fermented toasted cassava granules were 2.5, 2.8, 3.1 and 4.0 respectively. All the tested extracts were observed to have mitodepressive effects on cell division in the increasing order 1-day > 2-day > 3-day > 4-day fermented *garri* extracts. The results further go to confirm findings from other studies that proper fermentation aids in the reduction of toxic cyanogenic components present in poorly processed cassava products including *garri*.


Key words: *garri*, genotoxicity; mitotic index; cyanide; *Allium cepa*

1. Introduction Among the numerous processed forms of cassava roots (*Manihot esculenta*, Crantz), roasted granular product popularly known as *garri* is the most widely consumed in the West Africa sub region (Ofuaya and Akpoti, 1988). In Nigeria, it is a staple for many of the 374 ethnic groups (Akoroda, 1992). It is popularly referred to as the common man’s bread (Meludu *et al*., 2001). Its relative low cost, ease of handling and preparation before serving as a meal and its improved shelf life makes it amenable to the increasing trend in commercialization and urbanization (Irtwange and Achimba, 2009). The most widespread method of *garri* consumption in Nigeria is by pouring it into measured quantity of boiling water to produce a thick paste popularly called *eba* which is eaten by dipping small balls of it into soup or stew and swallowed. *Garri* is also consumed as snack when dispersed in cold water (soaked *garri*) and eaten directly with or without sweeteners (like sugar and salt), coconut, banana, peanut and smoked fish (Ogiehor and Ikenebomeh, 2006). The quality of *garri* available in the local market varies from batch to batch among the traders. Variations are observed in the colour, fibre content, moisture, particle size, starch content and toxic residual which are due to the cyanogenic glucosides, linamarin and lotaustralin present in all parts of the plant with the possible exception of the seeds (Coursey, 1973). The variations in commercially prepared *garri* are caused by cassava variety, age at the time of harvest, processing method and equipment and duration of fermentation (Irtwange and Achimba, 2009).

Fermentation is a method by which cyanogenic glucosides in *garri* can be reduced. The time allowed for fermentation is critical, if too short, the detoxification process will be incomplete, resulting in a potentially toxic product, if too long the product will have a strong sour taste and the texture will be poor (Azam-Ali *et al*., 2003). Collard and Levi (1959) reported that the acceptability of *garri* is influenced by its sourness and is directly related to the degree of fermentation. Poorly processed cassava products are undoubtedly associated with increased cyanogenic potential or ability to generate hydrogen cyanide, a well known poison with potential acute and chronic metabolic effect in humans.

The cyanogenic glucoside content is an important quality parameter of *garri*, due to its toxicity, if consumed in amount greater than 30ppm (Olarewaju and Boszoromanyi, 1975). Studies have shown that increased total cyanogens content of *garri* diet deplete as fermentation period increased (Ihedioha and Chineme, 1999). There is likelihood that cyanide has been linked to pancreatic islet damage and diabetes (Akanji, 1994). Various test systems, using human and animal models (especially with animals e.g. rats) has been used to provide data, as a scientific basis for improving the processing of cassava product; however literature is scanty on the use of plant models. *Allium cepa* assay
is undoubtedly a rapid and sensitive first tier assay for the cytological screening of toxic effluents and chemicals (Fiskesjö, 1993). This study was undertaken to access the toxicity or otherwise of squeezed garri extracts processed with varied fermentation periods.

2. Materials and Methods

One-day fermented garri was collected from Ugbejobo market located in Ovia-South-East Local government Area while two-day fermented garri was collected from Abudu market in Orhionwon Local Government Area. 3-day fermented garri was purchased from Auchi in Estako West Local Government Area while and four-day fermented garri was got from Ibilbo in Akoko-Edo Local Government Area.

Ugbejobo market is located on the ever-busy Benin-Sagamau expressway while Abudu market is on the Benin-Asaba dual carriage way. Auchi market is located on the Benin-Abuja highway while Ibilbo market is on the Ibadan-Ife-Abuja highway. Traffic on these roads is extremely high.

2.2 Preparation of Extracts

400g of garri was measured and dispersed into a plastic bowl containing 120cl of tap water at room temperature to soak the garri. This was left for 5 minutes to soak properly, after which it was stirred and the extract squeezed out and sieved with the aid of a cheese cloth. The residual garri particles were discarded.

2.3 Allium cepa Assay

Onion bulbs (Allium cepa L., 2n=16) bulbs of the purple variety of average size (15-22 mm diameter) were purchased locally in Benin City, Edo State in Nigeria. They were sun-dried for six weeks and the dried roots present at the base of the onion bulbs were carefully shaved off, with a sharp razor blade to expose the fresh meristematic tissues. The bulbs were then placed in freshly prepared distilled water to protect the primodial cells from drying up. To account for a number of bulbs in the population that would be naturally slow or poor growing, seven replicate bulbs were used for each test sample and control (tap water) and the best five bulbs were chosen at the approximate time for examination (Rank and Nielsen, 1993). The bulbs were removed from the distilled water and placed on a blotting paper to remove excess water.

For root growth inhibition evaluation, freshly prepared stock extracts were diluted into five concentrations of 20, 10, 5, 2.5, and 1%. Seven onion bulbs were utilized for each concentration of each extract and the control (tap water). The base of each of the bulbs was suspended on the extracts inside 100mL beakers in the dark for 72 h. Test extracts were changed daily. At the end of the exposure period, the roots of five onion bulbs with the best growth at each concentration were removed with a forceps and their lengths measured (in cm) with a metre rule. 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 controls) for each extract was determined (Fiskesjö, 1985). The effect of each sample on the morphology of growing roots was also examined.

Four types of garri samples based on the number of days of fermentation they were subjected to were purchased from four local markets in Edo State of Nigeria and used for this study. The markets are well known for stocking specific types of garri, a reflection of the processing methods employed by the respective farmers in these local communities.
between the control and the different concentrations of the extract were analyzed by means of the Student’s unpaired \( t \)-test. \( P \) values of < 0.05 were considered to be statistically significant. All statistical analyses were carried out using SPSS®14.0 statistical package.

3. Results

Table 1 shows the results of the effects of the squeezed extracts of garri on root growth of Allium cepa. Maximum root growth was achieved in the control and without any morphological deformities. The roots were whitish in colour, straight and unbroken. At tested concentrations, root growth was highest at the 1% concentration of the 4-day fermented extract. On the other hand, mean root length was least at 10% 1-day fermented garri extract. Inhibition of root growth was concentration-dependent and statistically significant \( (P < 0.05) \) at tested concentrations. Morphological deformities observed in tested concentrations were short, bent, spiral and crochet-like roots. There was no root growth at a concentration of 20% of all extracts. The \( EC_{50} \) for the 1-day fermented, 2-day fermented, 3-day fermented and 4-day fermented extracts were 2.5, 2.8, 3.1 and 4.0 respectively.

Results of the microscopic analysis are presented in Table 2. Compared to the mitotic index (MI) value of 92.8% in the control, there was concentration-dependent decrease in the mitotic indexes in all concentrations and fermentation periods. In particular, the lowest MI value of 83.6 was recorded for 10% 1-day fermented garri extract. There were no dividing cells at the 20% concentration of the extracts. All the tested extracts induced chromosomal aberrations in all the concentrations of the extracts (Fig. 1) and they were statistically significant \( (p < 0.05) \).

### Table 1 - Root length of A. cepa grown in squeezed garri extracts

<table>
<thead>
<tr>
<th>Conc (%)</th>
<th>Mean Root length±S.E.</th>
<th>RG(%) of control</th>
<th>95% CL</th>
<th>Mean Root length±S.E.</th>
<th>RG(%) of control</th>
<th>95% CL</th>
<th>Mean Root length±S.E.</th>
<th>RG(%) of control</th>
<th>95% CL</th>
<th>Mean Root length±S.E.</th>
<th>RG(%) of control</th>
<th>95% CL</th>
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<tbody>
<tr>
<td>0</td>
<td>3.75±0.21</td>
<td>0.34</td>
<td>3.75±0.21</td>
<td>0.34</td>
<td>3.75±0.21</td>
<td>0.34</td>
<td>3.75±0.21</td>
<td>0.34</td>
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<tr>
<td>1</td>
<td>2.13±0.17</td>
<td>0.98</td>
<td>2.31±0.16</td>
<td>0.50</td>
<td>3.23±0.17</td>
<td>0.70</td>
<td>3.31±0.25</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2.5</td>
<td>2.04±0.14</td>
<td>1.10</td>
<td>2.08±0.18</td>
<td>0.50</td>
<td>2.83±0.11</td>
<td>0.56</td>
<td>3.08±0.22</td>
<td>0.52</td>
<td></td>
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<td></td>
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<tr>
<td>5</td>
<td>1.56±0.08</td>
<td>0.44</td>
<td>1.28±0.05</td>
<td>0.68</td>
<td>2.10±0.18</td>
<td>0.60</td>
<td>2.65±0.04</td>
<td>0.70</td>
<td></td>
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<td>10</td>
<td>0.33±0.08</td>
<td>0.46</td>
<td>0.44±0.07</td>
<td>0.40</td>
<td>1.17±0.07</td>
<td>0.40</td>
<td>1.53±0.09</td>
<td>0.40</td>
<td></td>
<td></td>
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<tr>
<td>EC (_{50} )</td>
<td>2.5%</td>
<td>No root growth</td>
<td>No root growth</td>
<td>2.8%</td>
<td>3.1%</td>
<td>No root growth</td>
<td>4.0%</td>
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</table>

RG (%) of control expressed as % root growth of the control.
95%CL: 95% Confidence limit.
* \( P < 0.05 \), level of significance of root growth inhibition compared with the control. Values are Mean ± SEM

### Table 2 - Cytological effects of effluents of garri, akpu and lafun on cells of Allium cepa

<table>
<thead>
<tr>
<th>Conc (%)</th>
<th>No. of dividing cells</th>
<th>Mitotic index</th>
<th>% of aberrant cells</th>
<th>No. of dividing cells</th>
<th>Mitotic index</th>
<th>% of aberrant cells</th>
<th>No. of dividing cells</th>
<th>Mitotic index</th>
<th>% of aberrant cells</th>
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<td>0</td>
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<td>92.8</td>
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<td>-</td>
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<td>-</td>
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<tr>
<td>1</td>
<td>450</td>
<td>90</td>
<td>18</td>
<td>454</td>
<td>90.8</td>
<td>14</td>
<td>455</td>
<td>90.8</td>
<td>10</td>
<td>458</td>
<td>91.6</td>
<td>03</td>
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<tr>
<td>2.5</td>
<td>430</td>
<td>87</td>
<td>19</td>
<td>449</td>
<td>89</td>
<td>16</td>
<td>450</td>
<td>90</td>
<td>11</td>
<td>452</td>
<td>90</td>
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<td>425</td>
<td>85</td>
<td>21</td>
<td>440</td>
<td>88</td>
<td>18</td>
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<td>89</td>
<td>15</td>
<td>446</td>
<td>89.2</td>
<td>08</td>
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<tr>
<td>10</td>
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<td>83.6</td>
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<td>430</td>
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<td>25</td>
<td>434</td>
<td>86.8</td>
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<td>436</td>
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<td>20</td>
<td>-</td>
<td>-</td>
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</table>

*5000 cells (5 slides) per concentration of each effluent and the control
Figure 1. Stages of mitotic division in cells of *Allium cepa* treated with water extracts of *garri*. (a–e) Normal stages of mitotic division. (a) interphase (b) prophase (c) metaphase (d) anaphase (e) telophase (f) chromosome bridges (g) sticky chromosomes (h) spindle disturbance at prophase. Magnification 1000×.

Discussion

"Garri" is one of the most popular forms in which cassava is consumed in West Africa (Ofuya and Akpoti, 1988). Production methods vary from one locality to another resulting in products of non-uniform quality and the quality of *garri* available in the local market varies from batch to batch among the traders (Ogiehor and Ikenebomeh, 2006). There is likelihood that cyanide has been linked to pancreatic islet damage and diabetes (Akanji, 1994) and fermentation has been reported to be a veritable tool for reducing the total cyanide content of *garri* to appreciable safe levels (Ihedioha and Chineme, 1999; Asegbeloyin and Onyimonyi, 2005). Soaked *garri* is a ready-to-eat meal as it does not involve preparation with boiling water and accompaniment of soup or stew. This mode of *garri* consumption in West Africa and Nigeria in particular is as popular as boiling water to produce a paste popularly called *eba* which is eaten by dipping small balls of it into soup or stew and swallowed in morsels. *Garri* soaking is an easier and more convenient mode of *garri* consumption as a snack than *eba* (Ogiehor, 2002).

In this study, the potential cytotoxic and genotoxic effects of squeezed extracts of *garri* on *Allium cepa* were evaluated. There was a linear relationship between macroscopic and microscopic parameters for all the extracts. There was concentration-dependent decrease in root growth and the order of induction of root growth inhibition based on EC50 values was 1-day > 2-day > 3-day > 4-day fermented *garri* extracts. These values indicate that the samples were toxic; comparatively, the 1-day fermented squeezed *garri* extract has more inhibitory and mitodepressive effects than all the other extracts. This might be due partly to the cynogenic component in the *garri* as in all poorly fermented cassava products (Coursey, 1973).

Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. Our results showed among other aberrations, induction of sticky chromosomes, bridges and disturbance of spindle fibres at different stages of mitotic division in the onion root cells. In *A. cepa*, whenever chromosome aberrations occurred, there were almost always certain growth restrictions (Fiskesjö, 1997). Most of these aberrations are lethal which can cause genetic effects, either somatic or inherited (Swierenga et al., 1991). The total root-growth inhibition in the onion bulbs and complete arrest of cell division by the squeezed extract of *garri* at the 20% concentration may suggest turbagenic activity of these extracts.

In spite of repeated warnings from research findings (Sanni, 1994; Asegbeloyin and Onyimonyi, 2007) on the danger of consumption of inadequately
fermented *garri*, there is presently no legislation on sale of cassava processed into *garri* the same day in Nigeria by appropriate regulatory bodies. Furthermore, post process handling practices such as spreading on the floor, display in open bowls in the market and sales points and the use of various packaging materials to haul finished products from rural to urban areas where large buyers live have been identified as possible additional ways exacerbating contamination (Ogiehor and Ikenebomeh, 2006). Results from this study further go to confirm suggestions from other studies that proper fermentation and good product handling aid in the reduction of toxic components of substances present in poorly processed cassava products especially *garri*.

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