Analyses of Cytotoxic and Genotoxic Potentials of *Loranthus micranthus* using the *Allium cepa* Test

1B.A. Iwalokun, 2A.O. Oyenuga, 3G.M. Saibu and 4J. Ayorinde

1Department of Biochemistry and Nutrition, Nigerian Institute of Medical Research (NIMR), Yaba Lagos, Nigeria

2Department of Microbiology, Lagos State Polytechnic, Nigeria

3Department of Biotechnology, University of Western Cape, Bellville, South-Africa

4Department of Biochemistry, University of Lagos, Nigeria

Abstract: *Loranthus micranthus* (LM) is one of the Nigerian folk medicinal plants used chronically for the management of immuno-depressive illnesses such as diabetes mellitus, cancer and hypertension. There has not been report on the cytotoxic and genotoxic effects of the plant. This study was conducted to investigate the cytotoxic, mitodepressive and genotoxic effects of LM against *Allium cepa* root cells. The roots of *Allium cepa* (onion bulb) were exposed to different concentrations (2.5-40 mg/mL) of *L. micranthus* aqueous leaf extract (LMAE) using NaN₃ (100 ug/mL) and distilled water as positive and negative controls and examined macroscopically and microscopically for toxic effects. Phytochemical screening of the plant was also carried out using conventional methods. LMAE was found to significantly (p<0.05) inhibit root growth of *Allium cepa* with an EC₅₀ of 28.2 mg/mL and elicit a strong negative association (r = -0.97; p<0.05) in concentration with the bulb root length, suggesting a dose-dependent root growth inhibition. In comparison with the negative control, sodium azide used at 100 μg/mL as a positive control decreased mitotic index by 50%, while LMAE dose-dependently decreased MI by 2.4-27.4% at 5-40 mg/mL concentration range except at 2.5 mg/mL in which 11.9% increase in MI was observed. The observed dose-dependent alterations in cell division by LMAE was also not significant (p>0.05) at 5 mg/mL compared to the control and did not display significant variation in activity between 20 and 40 mg/mL concentrations respectively (p>0.05). Furthermore, LMAE at 10 mg/mL was found to produce the highest number of aberrant cells but failed to elicit c-mitosis found only at 40 mg/mL. LMAE at 5 mg/mL produced the least number of aberrant cells and also failed to induce micronucleus and binucleated cell formation found mostly at 40 mg/mL. Chromosomal aberrations including stickiness, multipolar anaphase and lagging chromosomes, breaks and bridges were induced by all the extract concentrations tested but not dose-dependently. Tannins, alkaloids, saponins and flavonoids were also present in the extract. Our findings indicate that LM is cytotoxic, mitodepressive and genotoxic to *Allium cepa* especially at doses beyond pharmacological range *in vitro* and suggest for safety reasons, the continuous use of this plant at lower concentrations for human phytomedicine coupled with a need to conduct further *in vivo* genotoxic tests.

Key words: *Allium cepa* test, cytotoxicity, genotoxicity, *Loranthus micranthus*

INTRODUCTION

The plant *Loranthus micranthus*, is a semi-parasitic plant and one of the few species of plants belonging to the *Loranthaceae* family that thrive favorably on a wide spectrum of host trees in Nigeria (Osadebe and Ukweze, 2004). In Nigeria and other developing countries, other reported *Loranthaceae* plants include *L. bewengi* (Obatomi et al., 1994), *L. globosus* (Islam et al., 2007), *L. parasiticus* (Okuda et al., 1987), *L. ferruginea* (Ameer et al., 2009), *L. yadoriki* (Wang et al., 2000) and *L. tanakae* (Kim et al., 2004). These semi-parasitic plants also called African mistletoes, generally use their modified root system to obtain nutrients from host trees such as *Persia Americana, Azadirachta indica, Kola acuminata, Parkia globosus, Baphia nitida, Citrus* sp. and *Pentaclethra macrophylla* (Osadebe and Ukweze, 2004; Osadebe et al., 2004; Obatomi et al., 1994; Islam et al., 2007). In traditional medicines, *L. micranthus like other Loranthaceae is used* either as a decoction or a component of polyherbal formulations in the treatment of diabetes mellitus and hypertension (Osadebe and Ukweze,
diabetes and hypertension (Osadebe and Ukweze, 2004; Osadebe et al., 2004; Obatomi et al., 1994; Obatomi et al., 1996). The leaf extract of *L. micranthus* has also been scientifically validated to elicit anti-diabetic activity in streptozotocin and alloxan -induced diabetic rats (Uzochukwu and Osadebe, 2007). In a recent study by Edem and Usloh (2009), a dose-related decrease in blood glucose was observed in rat administered various concentrations of *L. micranthus* aqueous leaf extract. Osadebe and Omeje (2009) also demonstrated that *L. micranthus* possessed immunomodulatory activity using in vitro assay, suggesting that the plant holds promise as a potential source of efficacious and cost-effective bioactive substances against immunosuppression diseases. Studies have also associated the use of *L. micranthus* and other plants in the Loranthaceae family such as *L. ferruginea* and *L. yadoriki* with improved cardiovascular performance and correction of cardiovascular abnormalities such as hypertension, dyslipidaemia and vasculopathy (Ameer et al., 2009; Wang et al., 2000). The plant as a Loranthaceae has also been credited for weight reduction property via inhibition of fatty acid synthase activity in mice by Tian et al. (2004). We have also observed *L. micranthus* methanolic leaf extract to be bacteriocidal against gram negative bacteria (Iwalokun et al., unpublished) thereby corroborating the findings of Osadebe and Akabogu (2006) that the methanolic and petroleum ether leaf extracts of *L. micranthus* harvested from Kolanut tree displayed antibacterial activity against *Escherichia coli* and *Bacillus subtilis* and antifungal activity against **Candida** and **Aspergillus** isolates in vitro. Ethylacetate bark fraction of *L. globosus*, a Loranthaceae on *Mangifera indica* tree used extensively in Bangladesh has also been reported to elicit broad spectrum antibacterial activity against gram positive and gram negative bacteria with an LC$_{50}$ of 10.83 ug/mL in vitro (Islam et al., 2004).

Generally, the utilization of medicinal plants including Loranthaceae plants in the treatment of a spectrum of human diseases has increased tremendously in the last 5 years and concerns have been raised about the potential of biodiversity loss (Akerele et al., 1991) and the need to promote sustainability as medicinal plants are used by over 70% of the world population for livelihood, primary health care, self medication and national health services (Akerele et al., 1991; Heinrich et al., 2010). This development has been attributed to their better accessibility, affordability, acceptability and the belief that medicinal plants work (Heinrich, 2010). *L. micranthus* has been found to be tolerable and safe when administered orally to laboratory animals with an LD$_{50}$ > 5000 mg/kg (Edem and Usloh, 2009). However, there is paucity of data on safety and tolerability of the plant when used as a phytomedicine in humans. Since, *L. micranthus* is used in the traditional management of

**MATERIALS AND METHODS**

**The plant material:** A fresh plant specimen of *Loranthus micranthus* was harvested from a Kolanut tree (*Kola acuminata*) from Sagamu, Ogun State during the beginning of rainy season in March, 2009. The plant was taken to the Department of Botany, University of Lagos, Nigeria for authentication. After authentication, a voucher sample of the plant was kept in the herbarium of the Department of Botany, University of Lagos, Nigeria.
Aqueous extract preparation: The leaves of *L. micranthus* plant were rinsed in sterile water to remove contaminants followed by drying in a ventilated oven at 40°C for 3 days. On day 4, the leaves were ground into fine powder (passage through a 30-mesh sieve) using a kitchen blender, boiled in sterile distilled water in ratio 1:10 (30 g in 300 mL of sterile water) for 20 min and subsequently cooled to 25°C before filtration through a Whatmann No. 1 filter paper (Whatmann, UK). The resulting crude extract was evaporated to dryness in vacuo using a Rotary evaporator (Memmer, Germany) at 40°C. The sterile distilled water to make 40 mg/mL stock solution. The sticky extract was taken and reconstituted in 10 mL of distilled water in tubes at 25°C for 2 days in the dark. The distilled water was changed every 24 h for 96 h. Bulbs in groups I - V were treated with graded concentrations of *L. micranthus* leaf extracts (40, 20, 10, 5 and 2.5 mg/mL) prepared from the stock solution by 2-fold serial dilutions using distilled water as the diluent. Group VI consisted of rooted onion bulbs placed in distilled water and served as negative control, while the bulbs in group VII were treated with 100 ug/mL of sodium azide (Sigma, USA) to serve as positive control. After 48 h, three roots per bulb were extracted, fixed in ethanol: glacial acetic acid (3:1, v/v) at 4°C for 24 h, hydrolyzed for 5 min with 1N HCl at 70°C, stained with Feulgen’s reagent at 37°C for 20 min and squashed on grease-free slide for cytological examination of the root tip cells using a binocular microscope (Olympus, USA) at x 40 and x 100 magnification. Two-hundred cells per each of the five slides prepared per treatment group were counted using a manual counter. The number of cells at different stages of cell division was scored and Mitotic Index (MI) was expressed as the frequency of aberrant cells (F), defined as the ratio of number of aberrant cells to total number of cells examined. After 96 h, macroscopic change in the root lengths of extract-exposed and control bulbs (an average of 4 roots per bulb) were measured in centimeter using a ruler. Root growth inhibition was measured as $E_{50}$, defined as the effective extract concentration required for root length reduction to 50% of the control (i.e. distilled water). For each treatment group, extract or controlsolution wa changed every 24 h for 96 h.

Phytochemical screening: The leaf extract of *Loranthus micranthus* was assayed qualitatively for the presence of secondary metabolites such as saponins, tannins, alkaloids, anthraquinones, cyanogenic glycosides, cardiac glycosides, terpenoids and flavonoids using the conventional methods described by Sofowora (1982) and other conventional methods.

Statistical analysis: Data were expressed as mean±standard deviation (SD) for the bulb root lengths and percentages for mitotic index and frequency of aberrant cells. $EC_{50}$ and regression equation was obtained from a plot of mean root lengths as a percentage of control and different concentrations of *L. micranthus* leaf extract. Disparity between mean values was evaluated statistically using Student’s Newman Keul’s (SNK) test. Relationships between mitotic index, mean root length and frequency of aberrant cells were also investigated using Pearson correlation analysis. Analyses were done at 95% confidence level using SPSS 11.0 computer program.

RESULTS

The results of the *Allium cepa* root growth response to different concentrations of *Loranthus micranthus* aqueous leaf extract (LMAE) are presented in Table 1 and Fig. 1. In comparison with the distilled water control, the leaf extract of *Loranthus micranthus* was assayed qualitatively for the presence of *L. micranthus* leaf extract on root length of *Allium cepa* after 96 h of exposure.

Table 1: Effect of *Loranthus micranthus* leaf extract on root length of *Allium cepa* after 96 h of exposure

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Root Length (cm), mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Distilled water</td>
<td>2.3±0.18</td>
</tr>
<tr>
<td>50 mg/mL (NaN₃)</td>
<td>2.4±0.20</td>
</tr>
<tr>
<td>40 mg/mL</td>
<td>2.4±0.17</td>
</tr>
<tr>
<td>20 mg/mL</td>
<td>2.4±0.21</td>
</tr>
<tr>
<td>10 mg/mL</td>
<td>2.4±0.20</td>
</tr>
<tr>
<td>5 mg/mL</td>
<td>2.3±0.14</td>
</tr>
<tr>
<td>2.5 mg/mL</td>
<td>2.4±0.17</td>
</tr>
</tbody>
</table>

Data values are mean±SD of root lengths of at least six determinations per group of *Allium cepa* exposed to different concentrations of *Loranthus micranthus* leaf extracts. Figures having different supercriters are statistically significant (p<0.05) (Student’s Newman Keul’s Test)
Fig. 1: Effect of Loranthus micranthus leaf extract at different concentration on Allium cepa root growth after 96 h of exposure. Each data point was calculated from mean of at least 6 determination. $r = $ Correlation coefficient

Table 2: Antiproliferative activity and chromosomal aberrations due to Loranthus micranthus at different extract concentrations on Allium cepa meristematic cells after 48 h of exposure

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of cells examined</th>
<th>M. No. dividing cells</th>
<th>Mean MI $\pm$ SD</th>
<th>$\Delta$ MI (%)</th>
<th>Chromosomal aberration</th>
<th>No. of aberrant cells</th>
<th>Frequency of aberrant cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>1000</td>
<td>64</td>
<td>8.4±0.8$^a$</td>
<td>0</td>
<td>c-mitosis, VC, Stickiness, MNC, BNC, MPA, Lagging, Breaks, Bridges</td>
<td>0</td>
<td>0$^a$</td>
</tr>
<tr>
<td>NaN$\textsubscript{3}$</td>
<td>1000</td>
<td>44</td>
<td>4.4±2.0$^b$</td>
<td>-50.0</td>
<td>-27.4</td>
<td>0</td>
<td>0$^b$</td>
</tr>
<tr>
<td>40 mg/mL</td>
<td>1000</td>
<td>61</td>
<td>6.1±0.7$^c$</td>
<td>-19.0</td>
<td>-2.4</td>
<td>0</td>
<td>0$^c$</td>
</tr>
<tr>
<td>20 mg/mL</td>
<td>1000</td>
<td>68</td>
<td>6.8±1.2$^d$</td>
<td>-16.7</td>
<td>-2.3</td>
<td>0</td>
<td>0$^d$</td>
</tr>
<tr>
<td>10 mg/mL</td>
<td>1000</td>
<td>70</td>
<td>7.0±0.9$^e$</td>
<td>-16.7</td>
<td>-2.3</td>
<td>0</td>
<td>0$^e$</td>
</tr>
<tr>
<td>5 mg/mL</td>
<td>1000</td>
<td>82</td>
<td>8.2±0.7$^f$</td>
<td>-2.4</td>
<td>-2.3</td>
<td>0</td>
<td>0$^f$</td>
</tr>
<tr>
<td>2.5 mg/mL</td>
<td>1000</td>
<td>94</td>
<td>9.4±0.4$^g$</td>
<td>11.9</td>
<td>-2.3</td>
<td>0</td>
<td>0$^g$</td>
</tr>
</tbody>
</table>

$^a$Frequency of aberrant cells = (No. of aberrant cell/No. of cells examined) × 100; Figures with different superscripts are statistically significant (p<0.05) (Student’s Newman Keul’s Test); DW = Distilled water; MI = Mitotic index; VC = Vagrant chromosome; MNC = Micronuclei cells; BNC = Binucleate cells. $\Delta$MI = Percentage increase (+) or decrease (-) in mitotic index compared to the DW control = 100 (1 – MI$\text{test}$/MI$\text{control}$).

Dose-dependent ($r = -0.97$; p<0.05) reduction in Allium cepa mean root length that was significant (p<0.05) at 5-40 mg/mL ($3.6 – 8.6±0.08-0.42$ vs. $9.7±0.36$ cm) but not significant (p>0.05) at 2.5 mg/mL ($9.2±0.17$ vs. $9.7±0.36$ cm). Treated concentrations of LMAE for 96 h was observed with an EC$\text{50}$ of 28.2 mg/mL, suggesting cytotoxicity (Fig. 1). Significant (p<0.05) reduction in Mitotic index (MI) of the Allium cepa meristematic cells with increasing concentration of LMAE after 96 h of exposure was also observed after 48 h of exposure when compared to the control ($6.1- 8.2 + 0.7-1.2$ vs. $8.4±0.8$) except at 2.5 mg/mL (MI = $9.4±0.4$) in which the MI disparity with the control was not significant (p>0.05).

Unlike NaN$\text{3}$, which elicited chromosomal aberrations 29 meristematic cells of Allium cepa, no chromosomal aberrations were seen in Allium cepa placed in the distilled water control. However chromosomal aberrations, suggestive of clastogenicity and impaired tubulin biogenesis were observed in 22-31 of the 1000 Allium cepa cells examined at each of the different concentrations of LMAE (Table 1 and Fig. 2). Highest (n = 31) and lowest (n = 22) number of aberrant cells were
Fig. 2: Mitotic and chromosomal aberrations after exposure of *Allium cepa* root tip meristematic cells to *Loranthus micranthus* aqueous leaf extract. (a). Micronucleus, (b). Binucleated cell (c). Chromosomal bridges

Table 3: Bivariate correlations between mean root length, mitotic index and frequency of aberrant meristematic root cells of *Allium cepa* exposed to different concentrations of *Loranthus micranthus* aqueous leaf extracts for 48-96 h

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean root length vs. MI</td>
<td>0.86</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean root length vs. Aberrant cells frequency</td>
<td>0.28</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MI vs. Aberrant cell frequency</td>
<td>-0.054</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MI versus Extract concentration</td>
<td>-0.83</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Aberrant cell frequency vs. Extract Concentration</td>
<td>-0.28</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

*r* = Correlation coefficient; *p*≤0.05 was considered to be significant

observed at 10mg/mL and 20 mg/mL respectively (Table 2). C-mitosis was observed only at 40 mg/mL of LMAE, the concentration at which highest number cells with chromosomal bridges (*n* =5) and micronuclei (*n* = 3) but lowest number of vagrant chromosomes (*n* = 5) were also seen (Table 2). Micronuclei formation and binucleated cells were not observed in *Allium cepa* treated with 2.5 and 5.0 mg/mL LMAE, while the highest number of cells with vagrant chromosomes (*n* = 7 vs. 2-6) was found at 20 mg/mL of LMEA.

Bivariate correlation analysis further revealed non-significant associations between aberration frequency, extract concentration and mitotic index (*r* = (-0.054) - (-0.28); *p*>0.05) but significant (*p*<0.05) association that was direct (*r* = 0.86) between Mitotic Index (MI) and mean root length but indirect (*r* = -0.83) between extract concentration and MI of *Allium cepa* meristematic cells (Table 3).

Phytochemical screening of LMEA revealed the presence of saponins, tannins, flavonoids, phenolics and cardiac glycosides, while anthraquinones and cyanogenic glycosides were absent (Table 4).

**DISCUSSION**

Research on medicinal plants with proven pharmacological activities and therapeutic benefits especially in the management of chronic diseases for which their use is prolonged, now focuses on the investigation of their roles in human mutagenesis/carcinogenesis. The results of this study showed that *Loranthus micranthus* aqueous extract (LMAE) elicits inhibition of root growth, anti-mitotic activity and clastogenic events in *Allium cepa*. The inhibition of *Allium cepa* root growth by LMAE was elicited at an EC<sub>50</sub> of 28.2 mg/mL. This is an indication for cytotoxicity on onion root cells, which has also been found for a few other Nigerian folk medicines. They include *Ocimum gratissimum*, *Morinda lucida*, *Cybopogon citratus*, *Carica papaya*, *Azadirachta indica*, *Mangifera indica*, *Terapluera tetraptera*, *Plumbago zeylanica*, *Xylopia aethiopica* *Newbouldia laevis*, *Alstonia boonei*, *Enantia chlorantha* and *Rauvolfia vomitoria* (Akintonwa et al., 2009; Oyedare et al., 2009; Akinboro and Bakare, 2007). Oyedare et al. (2009) reported cytotoxic effect of *A. indica*, *C. papaya*, *C. citratus* and *M. indica* against *A. cepa* root with an EC<sub>50</sub> of 0.6, 0.8, 3.0 and 1.4 g%, respectively. Although these plants also displayed dose-dependent inhibition of *A. cepa*
root growth, their disparate EC₅₀ values connote differences in their mechanisms of action.

Root growth in *Allium cepa* like other plants is due to expansion of cells in the elongation zone of the root tip where cellular differentiation occurs (Cordoba-Pedrosa *et al.*, 2004). The biological processes involved in cellular expansion include water uptake, nitrogen mobilization, increased sugar synthesis and plasma and tonoplast membrane flexibility (Gonzalez-Reyes *et al.*, 1999; Buddentsev *et al.*, 2010). Metabolites such as ascorbate and enzymes such as asparagine synthase and membrane ATPases have been described as promoters and mediators of these biological processes (Buddentsev *et al.*, 2010). Alterations in these biological processes including disrupted lipid biosynthesis by plants and toxins have been linked to reduced cell wall expansibility, loss of vacular homeostatic regulation, cellular cytotoxicity, cell necrosis and root growth inhibition (Gonzalez-Reyes *et al.*, 1999). Oxidative stress characterized by depletion of the root reduced glutathione (GSH) has been demonstrated to mediate these pathologic pathways (Cordoba-Pedrosa *et al.*, 2004; Al-meshal, 1987). Therefore, the observed inhibition of root growth by LMAE in this study suggests that the extract contain substances that impair one or more other biological processes which mediate cell expansion and differentiation at the elongation region of *Allium cepa* root tip. Plants and toxins such as *Calotropis procera* and podophytoxins have been described to impact negatively on these processes to cause root growth inhibition/arrest (Sehgal *et al.*, 2006).

With exception at 2.5 mg/mL, we observed LMAE at other concentrations to cause reduction in mitotic index by 2.4-27.4% that was significant at 10-40 mg/mL concentration range. Direct correlation was also found between mean root length and mitotic index. Our findings thus provide an indication for mitodepressive activity of LMAE on *Allium cepa* at these concentrations. They also suggest that cytotoxicity is linked to mitodepression of *Allium cepa* root meristem due to the extract, meaning that LMEA affects both meristematic and differentiated cells in the root tip of *Allium cepa*. The mitodepressive activity of folkmedicines in Nigeria has also been reported for *O. gratissimum, Azadirachta indica, Mangifera indica, Morinda lucida* and *Cymbopogon citratus* (Akintonwa *et al.*, 2009; Oyedare *et al.*, 2009; Akinboro and Bakare, 2007; Williams and Omoh, 1996), while extracts from for example, *Terminalia chebula lanceolata* (Jafferey and Rathore, 2007) have been found to elicit anti-genotoxic activity, protecting *Allium cepa* from heavy metal/chemical - induced genotoxicity and cell death. C-mitosis was only observed in *Allium cepa* root exposed to 40 mg/mL of LMEA. This indicates a relatively higher level of clastogenic constituent at this concentration and further corroborates the highest mitotic index reduction observed at this concentration. Meanwhile, reduction in mitotic index by <20% has been associated with sub-lethal effects on chromosomes of *Allium cepa* (Badr and Ibrahim, 1987). In this study, reduction in mitotic index by >20% of the distilled water control was only observed at 40 mg/mL concentration of the extract.

In aetiology terms, c-metaphase has been explained to occur due to inhibition of microtubule formation during mitosis and this may lead to aneuploidy and cell death, while stickiness is due to interchromosomal linkages of sub-chromatid strands coupled with excessive formation of nucleoproteins and inappropriate protein-protein interaction (Odeigah *et al.*, 1997; Turkoglu, 2007; Chattopadhyay *et al.*, 2004; Badr and Ibrahim, 1987). The latter is also believed to have resulted from altered physico-chemical properties of DNA due to interactions with other chemicals viz-aviz: mutagens, carcinogens and clastogenic agents (Badr and Ibrahim, 1987). The presence of dicentric chromosomes and unequally exchanged chromatids undergoing translocation has been
reported to be responsible for chromosomal bridges at anaphase. In the event of chemical interactions with DNA, breakages may occur and subsequent inhibition of repair mechanisms may lead to base mismatch, mutation and chromosomal aberrations such as fragrant chromosomes and DNA breaks (Fiskesjo, 1997; Turkoglu, 2007; Badr and Ibrahim, 1987; Majewska et al., 2003). Consequently, at anaphase, acentric chromosomes or chromosomes with multipolarity may develop (Majewska et al., 2003). In this study, chromosomal breaks and multipolar anaphase chromosomes were observed at all substrate concentrations but relatively at concentrations less than 20 mg/mL suggesting that these aberrations are sub-lethal when occurred temporarily. Aberrations observed at interphase stage of cell cycle include binucleated cells and micronucleus formation. Inhibition of cytokinesis following telophase is responsible for binucleated cell formation (Majewska et al., 2003). In Allium cepa, such inhibition arrest cell plate formation and this has been attributed to phlamogram inhibition at the early stage of telophase (Fiskesjo, 1997; Rank et al., 2002; Badr and Ibrahim, 1987; Majewska et al., 2003). Micronuclei are formed due to the failure to compartmentalize polarized chromosomes at telophase, micronuclei disrupt microtubule and may cause acentric chromosome formation leading to aneuploidy or polyploidy in the daughter cells after mitosis. Generally, these aberrations were observed to be mostly caused by LMAE at 40 and 20 mg/mL concentrations, suggesting their greater genotoxic and tubergeric effects on Allium cepa when compared with lower concentrations of the extract. Furthermore, some of the chromosomal aberrations elicited by LMEA at 40 mg/mL were also comparable to effects due to NaN₃, a known mutagenic agent. Medicinal plants such as O. gratissimum, Morinda lucida and Cymbopogon citratus have also been found to cause lethal chromosomal aberrations in Allium cepa at higher concentrations. These plants also function as prophase poisons at higher concentrations to bring mitosis to a halt (Akintonwa et al., 2009; Oyedare et al., 2009; Akinboro and Bakare, 2007; Williams and Omoh, 1996). However, complete arrest of mitosis by these plants was not observed at lower concentrations in the range tested for L. micranthus in this study.

Phytochemical screening of LMAE revealed the presence of terpenoids, flavonoids, tannins and phenolics. These secondary metabolites have previously been reported by Osadebe et al., (2009) as factors responsible for antibacterial, antidiabetic and antihypertensive activity of L. micranthus. In our recent study, we observed the methanolic extract of L. micranthus to elicit growth inhibition of gram negative bacteria such as Pseudomonas aeruginosa, Salmonella typhimurium, S. enteritidis and S. typhi and gram positive bacteria including Staphylococcus aureus, S. epidermidis and Bacillus licheniformis in vitro at ≤5 mg/mL (Iwalokun et al., unpublished). In a recent study, we also observed crude and bioguided fractionated extracts of L. micranthus to inhibit dose-dependently turpentine - induced tumor necrosis production by Hep2 cells between 50-200 ug/mL concentration (Iwalokun et al., unpublished). Our findings not only corroborate the earlier findings of Edem and Usoh (2009) and Osadebe and Akabogu (2006) in which in vivo acute toxicity investigation of L. micranthus was not observed at 5000 mg/kg, but also indicate that effective in vitro pharmacological activity of L. micranthus can be achieved at micrograms range, which are non-cytotoxic and sub-lethal concentrations. Ironically, flavonoids such as quercetin, rutin and kaempferol, tannins and other phenolics such as hydroxynamic acid were considered to play a role in mitodepression and chromosomal aberrations in Allium cepa following exposure to extracts from plants credited for their anti-inflammatory and antioxidant activity. They include Inula visciosa, M. lucida, Carica papaya and Ocimum gratissimum (Akinboro and Bakare, 2007; Williams and Omoh, 1996). Both L. ferruginea and L. globosus have been reported to contain flavonoids and terpenoids as factors for their antiviral, cytotoxic, hypotensive and vascular relaxation properties (Islam et al., 2007; Ameer et al., 2009). The ability of tannins and flavonoids to induce genotoxic effect has been attributed to their chemical composition and structure since some species of tannins and flavonoids lack these genotoxic effects. Concentrations of these metabolites in biological systems are also vital in the elicitation of clastogenicity. However, studies have shown that by complexing with cellular proteins and DNA, both tannins and flavonoids evoke free radical generation, which at the expense of a depleted antioxidant system could cause lipid peroxidation, DNA damage and cell death. Studies have also shown that tannins interact with alkaloids in biological systems to annul the genotoxic effects of alkaloid-induced genotoxicity in Allium cepa thereby suggesting a protective effect for tannins on Allium cepa. On the whole, antioxidant defense reservoir is vital in quenching free radicals emanating from tannin or flavonoid interactions with cell macromolecules and promoting prevention of alkaloid-induced genotoxicity in Allium cepa. Therefore, future studies on the antioxidant defense mechanism in cultivars of Allium cepa tested are essential since variations in antioxidant enzymes among the various species of Allium have been envisaged in several studies (Cordoba-Pedrosa et al., 2004; Gonzalez-Reyes et al., 1999; Budentsev et al., 2010).

In conclusion, the results of this study indicate in vitro cytotoxic, mitodepressive clastogenic and tubergeric activity of Loranthus micranthus against Allium cepa especially at doses beyond its pharmacological range in
vitro, suggesting a need for safe dose administration of the plant in human phytomedicine and further in vivo safety studies.

REFERENCES


