The protective effect of the aqueous extract of *Sida acuta* BURM.F on lead nitrate-induced genotoxicity

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**Abstract**

This study investigated the protective effective of *Sida acuta* leaf extracts against the genotoxic effect of lead nitrate, a toxic heavy metal that easily permeate the ecosystem. The genotoxic and anti-genotoxic effects of the aqueous extract of *S. acuta* on onion cells (*Allium cepa* L.) was evaluated using the *Allium cepa* L. assay. Onion bulbs were exposed to 0.25 – 2.5 mg.mL\(^{-1}\) concentrations of the plant extract for analyses of induction of cytogenetic damage. There was observed a concentration-dependent decrease in mitotic index of the *A. cepa* roots cells compared to the negative control. Lead nitrate significantly induced chromosomal aberration in *A. cepa* root cells. This effect, however, was significantly ameliorated by the *S. acuta* leaf extract. This effect was demonstrated by the lower frequency of chromosome aberrations in lead nitrate treated root cells after exposure to the extract. Furthermore, the extract restricted the extent of lead-induced cytological aberrations in *A. cepa*. The findings in this study suggested the mitodepressive, antiproliferative and anti-genotoxic potentials of the extract.

**Introduction**

Plants are source of bioactive compounds with numerous benefits to mankind. Some of them act as antioxidants and anti-mutagens while others act through various mechanisms to prevent or cure diseases and or modulate the harmful effects of xenobiotic effects. This has led to increase in consumption of herbs and herbal supplements worldwide for various reasons. Some people believed it can cure diseases that orthodox medicine are unable to cure (Welz *et al.* 2018) while others believe it is affordable, natural, and thus non-toxic. However there are reports of some medicinal plants being toxic in man or animals (Oyeyemi *et al.* 2015; Ruan *et al.* 2019). Hence the need to establish the safety of medicinal plants. *Sida acuta* is a common wireweed, a species of the flowering plants of the Mallow family (Malvaceae). It is an herbaceous weed widely distributed in the tropical and subtropical countries (Jindal *et al.* 2012). Renowned for its phytoremediation properties, it acts as a phytoextractor and phytostabilizer of heavy metals (Gupta and Sinha 2007; Putshaka *et al.* 2015; Ameh *et al.* 2019) suitable for decontamination of metals in waste contaminated sites. The different parts of the plants are also used traditionally for medicinal purposes. It is used to treat wound (Adetutu *et al.* 2011), liver disorders (Ekpo and Etim 2009), malaria (Iyamah and Idu 2015), nervous and urinary diseases, blood and bile disorders (Sreedevi *et al.* 2009), headaches, infectious diseases, rheumatism (Ememor *et al.* 2013), asthma, renal inflammation, colds, ulcers and worm infections (Caceres *et al.* 1987; Fokou...
et al. 2015). It has been reported to have antioxidant (Adetutu et al. 2011; Nwankpa et al. 2015), antineoplastic and antiproliferative (Jang et al. 2003; Pieme et al. 2010; Mallikarjuna et al. 2013), neuroprotective (Sreedevi et al. 2009), hepatoprotective (Benjumea et al. 2016; Owoeye and Salami 2017; Owoeye et al. 2017), antimicrobial (Oboh et al. 2007; Adetutu et al. 2011; Akinnibosun and Pela 2015), antiplasmodial (Karou et al. 2003; Nguyen-Pouplin et al. 2007), analgesic (Konaté et al. 2012), anti-hyperglycemic and anti-inflammatory activities (Arciniegas et al. 2017). Phytochemicals present in the leaves include alkaloids, saponins, tannins, steroids, glycosides and phenolic compounds (Akinnibosun and Pela 2015). Despite its wide usage and potential health benefit, it has been reported to be neurotoxic (Eluwa et al. 2013; Enemor et al. 2013), although non-toxic to the liver and kidney (Konaté et al. 2012). However, little is known about its genotoxic and anti-genotoxicity effects. Studies of genotoxicity and anti-genotoxicity are valuable to establish the safety and efficacy of herbs or natural products (Bast et al. 2002).

Allium cepa assay is a widely used plant assay for evaluation of genotoxic potential of different compounds/mixtures including medicinal plants (Oyeyemi and Bakare 2013; Sharma et al. 2018). It detects genomic mutation (Bonciu et al. 2018) with results comparable to those obtained in animal cell lines and is therefore an excellent model to determine the potential genotoxicity of compounds or mixtures.

In view of the widespread use of S. acuta in traditional medicine and dearth of information on its anti-genotoxic potentials, this study seeks to investigate the genotoxic and anti-genotoxic effect of the aqueous extract of the leaves of S. acuta. Genotoxicity tests can confirm the efficacy of a phytoremediation agent and also give an insight into the safety of medicinal plants for consumption.

**Experimental**

**Collection of plants**

Leaves of S. acuta were collected within Ondo City and authenticated by a taxonomist at the Department of Forestry, Federal University of Technology, Akure, Nigeria. Voucher specimen was deposited in the Herbarium of the same department (FUTA HERBARIUM: 149). The leaves were air dried and pulverised using electronic blender. The pulvrised leaves were extracted by boiling in water at 100 °C as practised by the local people. The extract was filtered and concentrated using rotatory evaporator at 60 °C. The concentrated extract was stored at 4 °C until use.

**Allium cepa assay**

*Allium cepa* (onion) bulbs of same size were procured from a local market. These were sun dried for two weeks. The dried *A. cepa* bulbs were used for the modified *A. cepa* assay (Fiskesjö 1997; Oyeyemi and Bakare 2013) to evaluate the genotoxic and anti-genotoxic effect of the extract. For the genotoxicity test, onions bulbs were placed on 100 mL beakers filled with tap water for 24 h. After 24 h, three onions per concentration variant were transferred into beakers with four different concentrations (0.25 – 2.5 mg.mL⁻¹) of the aqueous extract of S. acuta (ESA) for 48 h to permit two complete cell cycles. These concentrations were chosen based on the result of our preliminary range finding test where 0.25 – 20 mg.mL⁻¹, were tested. The test sample was changed after the first 24 h. Tap water served as negative control and lead nitrate (10 ppm) served as positive control. After 48 h exposure to ESA, the meristematic cell region of the roots were excised and prepared for microscopic analysis. Onion bulbs were rooted in tap water for 24 h, transferred into lead nitrate (PbNO₃) solution for 24 h and then into the extract for another 24 h. Negative and positive controls are tap water and PbNO₃ respectively. As in the genotoxicity study, the meristematic cell regions of the roots were excised after 24 h exposure to the extract and prepared for microscopic analysis. ESA extracts at the above mentioned concentration range (0.25 – 2.5 mg.mL⁻¹) were assessed for protective effect against lead, under the same Pb conditions applied. Slide preparation for both the genotoxicity and anti-genotoxicity studies was carried out as previously described (Oyeyemi and Bakare 2013). Briefly, the root tips (cut at 48 h) were fixed in...
Table 1. Summary of the cytological effects of aqueous extracts of *Sida acuta* on *Allium cepa* cells.

<table>
<thead>
<tr>
<th>Extract concentration [mg.mL⁻¹]</th>
<th>Nº. Dividing cells</th>
<th>Mitotic index [Mean ± SD]</th>
<th>Sticky Chrom.</th>
<th>Disturbed spindle</th>
<th>F Lag Chr.</th>
<th>A Chr.</th>
<th>Scattered / disoriented Chr.</th>
<th>Total aberration</th>
<th>Frequency of aberrant cells [Mean ± SD]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>221</td>
<td>5.53±2.34</td>
<td>2</td>
<td>7</td>
<td>–</td>
<td>–</td>
<td>6</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>0.25</td>
<td>237</td>
<td>5.93±2.02</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>9</td>
<td>5</td>
<td>30</td>
<td>46</td>
</tr>
<tr>
<td>0.5</td>
<td>164</td>
<td>4.10±0.64</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>16</td>
<td>34</td>
</tr>
<tr>
<td>1.25</td>
<td>110</td>
<td>2.75±0.96*</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4</td>
<td>3</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>2.5</td>
<td>94</td>
<td>2.35±1.44*</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>PbNO₃₃</td>
<td>184</td>
<td>4.60±0.69</td>
<td>14</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>32</td>
<td>57</td>
</tr>
</tbody>
</table>

Extract *c* = 0 mg.mL⁻¹ = Negative control (Tap water), PbNO₃ - *c* = 0.01 mg.mL⁻¹, Chr.= Chromosome, F = Fragment, A = Anaphase bridge, * Significantly different from negative control, ● Significantly different from positive control.

ethanol:glacial acetic acid (3:1 v/v), hydrolyzed in 1 N HCl at 60°C for 5 min and then washed in distilled water. Root tips were squashed on each glass slide and stained with acetocarmine for 10 min. Six slides were prepared per concentration, out of which four were scored.

A total of 1,000 cells per slide and 4,000 cells per concentration were scored for both the genotoxicity and anti-genotoxicity studies. The mitotic index (MI) and frequency of aberrant cells were calculated as follows:

\[
MI [%] = \frac{\text{number of dividing cells/total number of cells}}{100}
\]

\[
\text{Frequency of aberrant cells [%]} = \frac{\text{number of aberrant cells}}{\text{number of cells scored (1,000)}} \times 100
\]

Statistical analysis

Statistical analysis was performed using IBM-SPSS 23.0 software. Data were presented as mean ± standard deviation. Analysis involving comparison of means was carried out using one-way analysis of variance (ANOVA) followed by Duncan posthoc test where necessary. *P*-value of < 0.05 was considered significant.

Results

Genotoxicity study

This study investigated the potential genotoxic effect of the aqueous extract of the leaves of *S. acuta* using the *A. cepa* assay. The ESA extract caused a reduction in MI of onion cells after 48 h exposure compared with the negative control. The reduction in MI was significant (*P* > 0.05) at 1.25 and 2.5 mg.mL⁻¹ (Table 1). The tested doses of *S. acuta* did not significantly induced chromosomal aberrations except at the lowest concentration (0.25 mg.mL⁻¹). The frequency of aberration decreased with increasing concentration (Table 1). The frequency of aberration at 1.25 and 2.5 mg.mL⁻¹ is lesser than the background frequency observed in the control. There were normal chromosomes (Fig. 1) while various types of aberrations which include spindle aberration (Fig. 2a), vagrant chromosome (Fig. 2b-d), C-mitosis (Fig. 2e), disturbed spindle (Fig. 2f), anaphase bridge (Fig. 2f-2g), sticky chromosome (Fig. 2h-2i) polar deviation at telophase (Fig. 2j).

Anti-genotoxicity study

In the anti-genotoxicity study, the ability of the ESA extract to mitigate lead nitrate induced genotoxicity in *A. cepa* cells was investigated. The extract significantly (*P* < 0.05) reduced MI across the tested ESA concentrations compared to both the negative and positive controls. Lead nitrate significantly (*P* < 0.05) increased the frequency of chromosome aberrations compared to that of the negative control. This frequency was restored to level comparable to that of the negative control by the extract (Table 2).

Discussion

Humans are constantly exposed to genotoxins in the environment. Some of these only cause alteration in the somatic cells, which although may not be passed onto the next generation, may have serious health implications. Herbs have been consumed over the ages for the prevention
and treatment of several ailments with the presumption that they are safe (Elgorashi et al. 2002). However, concern has been raised over the safety of these herbs as some herbs with therapeutic effects have also been reported to be toxic (Oyeyemi et al. 2015; Amadi et al. 2018; Ruan et al. 2019). This study investigated the genotoxicity and anti-genotoxicity of the aqueous extract of *Sida acuta*, a widely used medicinal plant.

Our data showed a decline in the MI in both the genotoxicity and anti-genotoxicity studies. This is in line with previous reports on several medicinal plants and/or natural products (Shetty et al. 2017; Sharma et al. 2018). Suppression of MI correlates with cytotoxicity in plant cells (Shetty et al. 2017) and is thus an indication that *S. acuta* extracts at given concentrations are cytotoxic in this study. This suggested that the extract interfered with the cell cycle (Oyeyemi and Bakare 2013), inhibited DNA synthesis and cell cycle progression, which resulted into decreased cellular proliferation (Qin et al. 2015). This could probably be the basis of the antiproliferative effect of *S. acuta* reported in various cancer cell lines (Pieme et al. 2010; Thondawada et al. 2016).

The chromosome aberration induced by the extract in this study was, however, very low and comparable with that observed in the negative control. The extract at the high concentrations reduced the background frequency of chromosome aberration. This implies it is not genotoxic and can even prevent occurrence of spontaneous damage to the genetic material. The chromosome aberrations observed were physiological aberrations due to spindle inhibition (spindle aberration at anaphase (Fig. 2a), vacant chromosomes (Fig. 2b-2d), C-mitosis (Fig. 2e) disturbed spindles (Fig. 2f) and disoriented chromosomes (Fig. 2j) or chromatin dysfunction such as stickiness (Fig. 2h). This shows that the extract probably contains spindle inhibitor(s). Inhibition of mitotic spindle is an important strategy in the development of anticancer drugs, as it inhibits the cell cycle progression (Zhu et al. 2016). The observed genotoxicity could due to the presence of alkaloids and tannins in this plant and treatment of several ailments with the presumption that they are safe (Elgorashi et al. 2002). However, concern has been raised over the safety of these herbs as some herbs with therapeutic effects have also been reported to be toxic (Oyeyemi et al. 2015; Amadi et al. 2018; Ruan et al. 2019). This study investigated the genotoxicity and anti-genotoxicity of the aqueous extract of *Sida acuta*, a widely used medicinal plant.

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### Table 2. Summary of the cytological effects of the aqueous extracts of *Sida acuta* on *Allium cepa* cells pre-treated with lead nitrate.

<table>
<thead>
<tr>
<th>Extract concentration [mg.mL$^{-1}$]</th>
<th>N$^\circ$ Dividing cells</th>
<th>Mitotic index [Mean ± SD]</th>
<th>Sticky Chr.</th>
<th>Disturbed spindle</th>
<th>F Chr.</th>
<th>Lag Chr.</th>
<th>Total aberration</th>
<th>Frequency of aberrant cells [Mean ± SD]</th>
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<tr>
<td>0</td>
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<td>2</td>
<td>7</td>
<td>–</td>
<td>–</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>0.25</td>
<td>65</td>
<td>1.63±1.48*●</td>
<td>1</td>
<td>6</td>
<td>–</td>
<td>–</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>0.5</td>
<td>61</td>
<td>1.53±0.32*●</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>1.25</td>
<td>51</td>
<td>1.28±0.30*●</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>32</td>
<td>1.05±0.13*●</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Pb(NO$_3$)$_2$</td>
<td>195</td>
<td>4.89±0.62*●</td>
<td>36</td>
<td>20</td>
<td>3</td>
<td>8</td>
<td>6</td>
<td>23</td>
</tr>
</tbody>
</table>

Extract $c = 0$ mg.mL$^{-1}$ = Negative control (Tap water), Pb(NO$_3$)$_2$ $c = 0.01$ mg.mL$^{-1}$ Chr.= Chromosome, F = Fragment, A = Anaphase bridge, * Significantly different from negative control, ● Significantly different from positive control.

![Fig. 1](image1.png)

**Fig. 1.** Normal chromosomes observed in *Allium cepa* cells exposed to aqueous extract of *Sida acuta*:
(a) interphase; (b) prophase; (c) metaphase; (d) anaphase; (e) telophase.
Fig. 2. Chromosomal aberrations induced in *Allium cepa* root cells by the aqueous extract of *Sida acuta*: (a) spindle aberration at anaphase; (b, c) vagrant chromosome at anaphase; (d) vagrant chromosome at metaphase; (e) C-mitosis; (f) disturbed spindle with anaphase bridge; (g) anaphase bridge; (h, i) sticky chromosome; (j) polar deviation at telophase.

(Oboh and Onwukaeme 2007). These phytochemicals have been associated with chromosomal damage (Oyeyemi and Bakare 2013). Heavy metals are big menace in the environment. They threaten human health as they easily enter human body through the food chain. Lead (Pb\(^{2+}\)) is one of the most toxic heavy metals (Abdullah et al. 2014). Lead moves into and throughout ecosystems and contaminate the vegetation, air, water and soil (Samal et al. 2017). The toxicity of lead nitrate in several systems has been reported. Its genotoxicity in *A. cepa* has been established (Oyeyemi and Bakare 2013; Atoyebi et al. 2015). ESA significantly mitigated the genotoxicity induced by PbNO\(_3\) in this study as the frequency of chromosome aberration was reduced below that observed in the negative control. This buttressed the phytoremediation ability of *Sida acuta*, as phytoremediation agents are known to deplete the genotoxicity of effluents or any other environmental contaminants (Di Gregorio et al. 2015; Basilico et al. 2017). The observed chromosomal aberration are also structural and not numerical. Structural chromosomal alterations occur as a result of DNA breaks, inhibition of DNA synthesis or replication of altered DNA. Chromosomal aberrations such as chromosome bridges and breaks, are indicators of a clastogenic action while abnormal segregation of chromosomes (lag chromosome, scattered/disoriented chromosome and C-metaphases) occur either spontaneously or by the action of aneugenic agents (Nefic et al. 2013). PbNO\(_3\) induced disoriented chromosomes predominantly, showing that it is an aneugenic agent. However ESA, mitigated this aneugenic effect demonstrating its antigenotoxic effect. One of the mechanisms of lead induced genotoxicity is oxidative stress (Dai et al. 2012; Taie et al. 2019). ESA having antioxidant property (Subramanya et al. 2015) possibly inhibited bioaccumulation of Pb\(^{2+}\) in *A. cepa* cells. *S. acuta* is phytostabilizer of several heavy metals (Ameh et al. 2019) with the ability to inhibit the mobility/bioavailability of Pb\(^{2+}\), thus preventing PbNO\(_3\) induced oxidative stress and ultimately DNA damage and or genotoxicity. Noteworthy, the observed anti-genotoxic effect was accompanied by severe cytotoxicity as indicated by very low MI. In oocytes, when there is DNA damage, the spindle assembly checkpoint induces mitotic index arrest to inhibit the progression of cells harbouring the DNA damage (Marangos
et al. 2015). This pathway is not naturally activated in somatic cells (Collins et al. 2015). S. acuta being a natural spindle inhibitor probably activates this pathway in cells harbouring DNA/chromosomal damage resulting in the arrest of the cell cycle progression in those cells. Prolonged mitotic inhibition leads to inhibition of further cell proliferation or induction of apoptosis (Hain et al. 2016). Hence the complete inhibition of proliferation observed at the high concentration. Alkaloid isolated from S. acuta triggered apoptosis in human gastric adenocarcinoma cells (Ahmed et al. 2011). The low MI observed in the anti-genotoxic study may corroborate the apoptosis inducing effect of the plant.

In this study, the aqueous extract of S. acuta showed antiproliferative, mitodepressive and anti-genotoxic effects. Its ability to modulate the mitotic spindle is probably one of its key mechanisms of action. However, caution should be taken against indiscriminate consumption of the extract as it has the potential to be cytotoxic.

**Conclusion**

This study shows the potential of S. acuta to inhibit lead induced genotoxicity. S. acuta is a spindle inhibitor with the potential to interfere with cell cycle and inhibit mitosis. This buttresses its potential as an anticancer agent. There is a need to further explore the effect of S. acuta on cell cycle and proliferation so as to gain further insight into its molecular mechanism of action.

**Acknowledgement**

I acknowledge Miss Oluwatomiisin Ojo for her assistance in air drying the plant.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

**References**


Oyeyemi IT, Yekeen OM, Oduisu PA, Ologun TM, Olaleye OI, Bakare AA (2015) Genotoxicity and anti-genotoxicity of aqueous and hydro-methanol extracts of Spondias mombin (L), Nymphaea lotus (L) and Luffa cylindrica (L) using animal bioassays. Interdiscipl. Toxicol. 8: 184-192.


