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Evaluation of genotoxic and cytotoxic activities of *Solenostemon monostachyus* (P. Beauv.) Brig. (Lamiaceae) using *Allium cepa* test

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ABSTRACT

Background: Solenostemon monostachyus (P. Beauv.) Brig. (Lamiaceae), a medicinal plant used traditionally in the treatment of diseases, was investigated for genotoxic and cytotoxic effects using the *Allium cepa* test.

Methods: The effect of the leaf extract on the root meristem cells of *Allium cepa* was investigated using onion bulbs exposed to 2.5 mg/mL, 5 mg/mL, and 10 mg/mL concentrations of the extract for macroscopic and microscopic analysis. Tap water was used as a negative control and Methotrexate (0.1 mg/mL) was used as a positive control.

Results: There was statistically significant ($p< 0.05$) inhibition of root growth depending on concentration by the extract when compared with the negative control group. All the tested concentrations of the extract were observed to exert cytotoxic effects on cell division in *A. cepa.* The mitotic indices were 8.20±3.56%, 4.80±0.86&% and 3.60±0.56% for 2.5mg/mL, 5.0 mg/mL and 10.0 mg/mL of the extract. The extract-induced chromosomal aberrations and micronuclei (MNC) formations (2.33±0.65% for the 2.5 mg/mL of the extract) in *A. cepa* root tip cells were significant ($p<0.05$) when compared with control group. The leaf extract treatment further induced cell death, ghost cells, cell membrane damages, and binucleated cells. .

Conclusion: These results suggest that the leaf extract of *S. monostachyus* possesses cytotoxic and genotoxic effects on *A. cepa*.

Keywords: *Allium cepa*, cytotoxic, genotoxic, *Solenostemon monostachyus*

1. INTRODUCTION

Solenostemon monostachyus P. Beauv (Lamiaceae), a medicinal plant, is well distributed in West and Central Africa. It is an annual succulent weed, which can grows up to 100 cm tall [1] . The aerial parts of the plant are used ethnomedicinally by the Ibibios of the Niger Delta of Nigeria to treat stomach ulcer, fever/malaria [2], hemorrhoid, and other inflammatory diseases. Phytoconstituents such as diterpenoids [3], flavonoids, coumarin, and polyphenol [4] have been isolated from the leaves. Compounds such as b-pinene, oct-1-en-3-ol, b caryophyllene, octan-3-ol, and (*E*,*E*)-a-farnesene have been identified in the essential oils from the leaves [1]. Biological activities such as antioxidant [4-6], antihypertensive [7], antimicrobial [8], antiulcer [9],antidiabetic and hypolipidemic [10], antipyretic and antimalarial [11], antiinflammatory and antinociceptive [12], hepatoprotective and nephroprotective [13], antidepressant [14] and inhibitory action on alpha amylase and alpha glucosidase activities [15] have been reported on the leaves. Genotoxic and cytotoxic potentials of leaf extract of *Solenostemon monostachyus* were evaluated using *Allium cepa* test in this study.

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MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials:
Onion bulbs

2.1.2 Chemical and Reagents
Ethanol, glacial acetic acid, orcein (Sigma-Aldrich, USA),

2.1.3 Equipment and Apparatus
Grinding machine, weighing balance (Ohaus, USA), desiccator, measuring cylinder, Microscope (Olympus, Japan)

2.2 Methods

2.2.1 Plants collection
The plant material *Solenostemon monostachyus* (leaves) were collected in compounds in Uruan area, Akwa Ibom State, Nigeria in April 2024. The plant was identified and authenticated by Prof Margaret Bassey, a taxonomist in Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria (FPUU 573).

2.2.2 Extraction
The leaves were washed and shade-dried for two weeks. The dried plants' materials were further chopped into small pieces and reduced to powder using an electric grinder. The powdered leaves material (1.5 kg) was macerated for 72 h in 50% ethanol. This was thereafter filtered and the liquid filtrate was concentrated and evaporated to dryness in *vacuo* 40°C using a rotary evaporator (BuchiLab, Switzerland). The extract was stored in a refrigerator at -4°C, until used for the proposed experiments.

2.2.3 Allium cepa test.
Allium cepa (onion) bulbs (small in size), were bought from Itam market, Itu LGA, Akwa Ibom State, Nigeria. The bulbs were foremostly identified and authenticated by a taxonomist in the Department of Botany and Ecological Study, University of Uyo. The bulbs were thereafter prepared for the study by cutting off dried old roots using a small knife at the bottom base of the bulbs without destroying the root primordia [16, 17]. The leaf extract (20 g) was dissolved in distilled water (200 mL) and diluted to make varying concentrations of the extract (2.5, 5.0 and 10.0 mg/mL respectively) from the stock solution. The different extract test concentrations (2.5 mg/mL, 5.0 mg/mL, and 10 mg/mL) were filled in 50 mL beakers (5 per test concentration) and arranged serially. One onion bulb was placed with the root primordia touching the surface of the liquid on top of each beaker in the different tested concentrations,. Tap water and methotrexate (0.1 mg/mL) were respectively used as negative and positive controls. After 24 hours, the test samples were changed in the controls and all test concentrations. The experiment was for a period of 72 hours. Thereafter, the root numbers were counted in all the beakers used in the respective tested concentrations and the mean root number per concentration was calculated. The mean root length per concentration was also calculated after measuring the roots' length with a metre rule. The root tips (10 mm) of some roots per bulb were cut off and respectively fixed in 3:1 (v/v) ethanol: glacial acetic acid and 1N HCl in respective sample bottles and stored in a refrigerator [16, 17].

2.2.4 Microscopy
The root tips were each placed in a test tube with 1N HCl and heated at 50ºC for 6 minutes in order to fix and macerated them. Thereafter, the root tips were placed on microscopic slides on a blank background with a forcep and cut off at terminal tips. Two drops of 2% (w/v) orcein stain was added and mixed with the rootlets properly by knocking and stirring with a stirring spatula. Then a cover slip was placed at 45º to avoid air bubbles. After that, the cells were squashed by placing a filter paper on the cover slip and pressed slightly with a thumb. The cover slip was sealed with a clear fingernail polish and each slide was examined using a Light Microscope at a magnification of x40. Microphotographs were taken to show chromosomal aberrations. The mitotic index and frequency of chromosomal aberration were calculated based on the number of aberrant cells per total cells scored at each concentration of each sample [18,19]. The mitotic inhibition was determined using the following formula:

$$\text{Mitotic index} = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cells} \times 100$$

$$\%\text{Aberrant cells} = \frac{Number\ of\ Aberrant\ cells}{Total\ number\ of\ cells} \times 100$$

$$\%\text{root growth of control} = \frac{Overall\ mean\ root\ length\ of\ test\ solution}{Overall\ mean\ root\ length\ of\ control} \times 100$$

The parameters used in evaluating cytotoxicity and genotoxicity are (i) the mitotic index (MI) (ii) chromatin aberrations (stickiness, bridges, breaks and polar deviation) and micronuclei (MNC) were scored per 500 cells [18, 19].

2.3 *Statistical Analysis.*
Data obtained from this work were analysed statistically using one –way ANOVA followed by Tukey-Kramer multiple comparison test using Instat Graphpad software, (San Diego, USA). Differences between means were considered significant at 5% level of significance ie $p \leq 0.05$.

RESULTS

3.1 Physicochemical Characterization.

The effect of *Solenostemum monostachyus* leaf extract on the physicochemical parameters (root number and root length) is presented in Table 1. This result shows that all tested concentrations of *Solenostemum monostachyus* leaf extract caused significant inhibition in the growth of roots in comparison to negative and positive control groups. The inhibition of root number and root length was greater with increasing concentrations of the leaf extract with the highest concentration giving almost complete inhibition of root growth. The average root length in negative and positive control (methotrexate) groups were 4.56±0.19 and 0.10±0.01 cm respectively. However,the mean root length in the 10 mg/mL treatment group was decreased significantly compared to that of the negative control; 0.10±0.00 cm for *S. monostachyus* (Table 1). Mean root lengths in treatment groups were decreased depending on concentration, significantly ($p<0.05$) when compared to negative control. The root morphology was almost normal in the negative control treatment group, but at 2.5 mg/mL of *Solenostemum monostachyus* leaf extract, the roots turned slightly yellow and at 5 and 10 mg/mL of *Solenostemum monostachyus* leaf extract, the roots appeared brownish at the tips. (Table 1).

3.2 Cytogenetic Analysis.

Table 2 shows the effects of *Solenostemum monostachyus* leaf extract on the cytogenetic parameters of *Allium cepa* roots. Cytogenetic analysis performed showed that the leaf extract caused concentration-dependent and significant ($p<0.05$) decreases in the mitotic index when compared to that of the negative control. The leaf extract of *S. monostachyus* at 10 mg/mL had mitotic index of 3.60±0.56 as compared to 69.90±5.64 recorded in the negative control group (Table 2). Cytogenetic alterations caused by the extract are shown in Table 3. Chromosome and cytological alterations were observed in negative control, methotrexate, *Solenostemum monostachyus* leaf extract-treated groups as depicted in Table 3. Analysis of chromosome aberrations observed showed that there were bridges of chromosomes, polar deviation and nuclear damage detected in the different concentration treatments especially in the highest concentration (Table 3) (Figure 1(A,B,C, D,E,F). This was significant ($p<0.05$) when compared to the negative control group. Fragments or clastogenic breaks of chromosomes were observed at 2.5 and 5.0 mg/mL concentrations of the leaf extract (Table 3; Figure 1 (B,C)). Sticky metaphase was also observed (Figures 1(C) in the extract-treated groups but was more frequent in the group treated with the highest concentration of the extract (10 mg/mL). It was however observed that these abnormalities increased as the concentration of the extract increased. A concentration-dependent and statistically significant ($p<0.05$) increase in total aberrant cells (aberrant cells include chromosome breaks, stickiness and polar deviation) as compared with the negative control (Table 3) was observed . However, the highest number of aberrant cells was recorded in the methotrexate-treated group (positive control)(Table 3). Genotoxic activities of the leaf extract were further exhibited by the induction of micronuclei in the root tip meristem cells of *A. cepa.* This was not concentration-dependent as the groups treated with methotrexate and the lowest concentration, 2.5 mg/mL of

Solenostemum monostachyus leaf extract had superior numbers of cells with micronuclei in the test compared to the negative control, which were statistically significant ($p < .05$) (Figure 1(B)). In addition, cells with damaged membrane (Figure 1(E and F)), binucleated cells (Figure 1(B and C)), and damaged nucleus (Figures 1E and F) were found in different frequencies in the extract exposed groups. Also, apoptotic cells (Figure 1A) were detected in the group treated with the leaf extract.

Table 1: Cytotoxicity of *Solenostemum monostachyus* leaf extract on growing roots of Onion (*Allium cepa*)

Treatment group	Concentration of extract (mg/mL)	Average root Number ± S.D	Average root length (cm)± S.D
Negative control	Tap water	21.25±1.54	4.56±0.19
Methotrexate	0.1	2.10±0.02[a]	0.10±0.01[a]
Solenostemum monostachyus	2.5	5.66±1.66[a]	1.91±0.20[a]
	5.0	3.66±0.66[a]	0.45±0.11[a]
	10.0	2.33±0.33[a]	0.10±0.00[a]

Values are expressed as mean ±SEM (n=5). Significant at p<0.05 when compared to negative control

Table 2: Dividing and total cells counted under microscopic observations and mitotic values in control and treatment concentrations

Treatment group	Concentration of extract (mg/mL)	Total Number of cells	Dividing cells	M.I (%)± SE
Negative control	Tap water	500	348	69.60±5.64
Methotrexate	0.1	500	15	3.00±0.68[a]
Solenostemum monostachyus	2.5	500	41	8.20±3.56[a]
	5.0	500	24	4.80±0.86[a]
	10.0	500	18	3.60±0.56[a]

Values are expressed as mean ±SEM (n=5). Significant at p<0.05 when compared to negative control.

Table 3: Chromosomal and mitotic aberrations in the root meristematic cells of *Allium cepa* after treatment with *Solenostemum monostachyus* leaf extract

Treatment group	Concentration of extract (mg/mL)	Chromosome breaks (%)±SE	Stickiness (%)±SE	Polar deviation (%)±SE	Aberrant cells (%)±SE	MNC (%)± SE
Negative control	Tap water	-	0.05±0.06	-	2.00±0.28	-
Methotrexate	0.10	2.34±1.23 [a]	21.34±5.38 [a]	10.55±2.28 [a]	45.13±4.22 [a]	2.28±0.86 [a]
Solenostemum monostachyus	2.5	1.46±0.23[a]	5.19±0.38[a]	0.12±0.01 [a]	32.65±4.82[a]	2.33±0.65[a]
	5.0	2.39±0.78 [a]	9.83±3.35[a]	-	45.34±5.78[a]	-
	10.0	-	16.27±5.98 [a]	-	55.85±6.39[a]	-

Values are expressed as mean ±SEM (n=5). Significant at p<0.05 when compared to negative control.

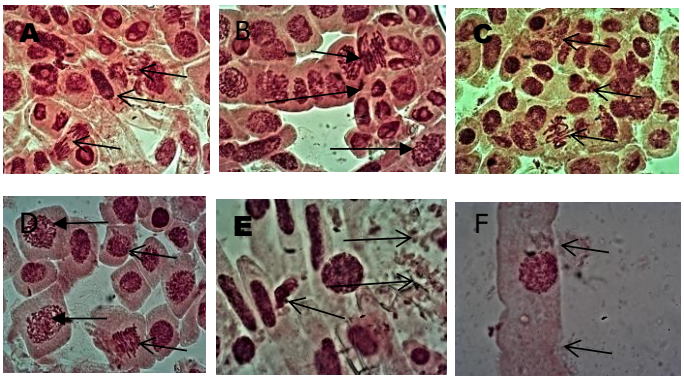

Figure 1: Photomicrograph showing the mitotic and chromosomal aberrations of *Allium cepa* root meristem cells after *Solenostemum monostachyus* leaf extract treatments under light microscope X40 magnification. Arrows indicate (A) apoptotic bodies, cell wall and nuclear damage (B) metaphase, sticky chromosomes (C) sticky

chromosome, bridge, laggard, cell wall and nuclear damage (D) sticky metaphase (E) membrane and nuclear damage (F) cell wall and nuclear damage

DISCUSSION

In this study, the toxic effects of *Solenostemum monostachyus* leaf extract were evaluated by analyzing root growth and root morphology. The various concentrations of the extract used in the study were observed to cause root growth inhibition and these were statistically significant when compared to the control group. In addition, the extract induced changes in the coloration of the roots. Cyto- and genotoxicity were evaluated by determining cytological parameters such as the mitotic index and number of chromosome abnormalities; including chromosome breaks, stickiness, and polar deviations. The mitotic index (MI) of *A. cepa* meristematic cells treated with methotrexate (3.60%) was significantly decreased when compared to the control. Significant inhibition in the onion roots treated with the *Solenostemum monostachyus* leaf extract (8.20%, 4.80% and 3.60% compared to the negative control) was observed (Table 2). The inhibition of root growth was found to be dependent on the decrease in the mitotic index. The decline of mitotic index below 22% in comparison to negative control can have lethal impact on the organism [20], while a decrease below 50% usually has sublethal effects [21] and is called cytotoxic limit value [22]. The mitotic index measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be interpreted as cellular death or a delay in the cell proliferation kinetics [23]. Reduction in the mitotic activity could be due to inhibition of DNA synthesis or a blocking in the G2 phase of the cell cycle, preventing the cell from entering mitosis [24]. Mitodepressive effects of some herbal extracts, including the ability to block the synthesis of DNA and nucleus proteins, were reported earlier [25,26]. Several other herbal extracts have been reported to inhibit mitosis [27-29]. The decreased mitotic index in *A. cepa* roots treated with *S. monostachyus* leaf extract is probably due to either disturbances in the cell cycle or chromatin dysfunction induced by extract-DNA interactions. The results of this study suggest that the tested extract concentrations have inhibitory or mito-depressive effects on root growth and cell division of *A. cepa* and can prevent DNA synthesis. The reduction in the number of dividing cells in roots may have been produced by the cytotoxic effects of the compounds found in the extract. The induction of sticky metaphase indicates the toxic potentials of the extract. Metaphases with sticky chromosome, loses their normal appearance, and they are seen with a sticky "surface," causing chromosome agglomeration [30]. Stickiness has been attributed to the effect of pollutants and chemical compounds on the physico-chemical properties of DNA, protein or both, on the formation of complexes with phosphate groups in DNA, on DNA condensation or on formation of inter- and intra chromatid cross links [31,32]. Chromosomal aberrations (CA) are changes in chromosome structure resulting from a break or exchange of chromosomal material. Most of the CA observed in cells are lethal, but there are many related aberrations that are viable and that can cause genetic effects, either somatic or inherited [33]. The presence of chromosome fragments is an indication of chromosome breaks, and can be a consequence of anaphase/telophase bridges [34]. Fragments were observed in this study in all the extract concentrations- treated groups. The extract was found to not only interfere with the cell cycle, but also affect chromatin organization or DNA replication, causing chromosome breaks. Frequencies of total chromosome aberrations increased significantly following exposure to the extract which indicates clastogenic activity (Table 3). The extract significantly induced the formation of MNC in *A. cepa* root cells at 2.5–10 mg/mL concentrations.The frequency of MNC was found to increased in the groups treated with 2.5 mg/mL of the leaf extract. However, MNC frequency decreased in *A. cepa* roots treated at the highest concentration of the extract (10 mg/mL), due to high cytotoxicity. The frequency of cells with micronuclei is a good indicator of the cytogenetic effects of tested chemicals. Micronuclei (MN) often results from the acentric fragments or lagging chromosomes that fail to incorporate into the daughter nuclei during telophase of the mitotic cells and can cause cellular death due to the deletion of primary genes [35, 36]. Previous studies have suggested MNC-induced effect of various plant extracts such as *Heinsia crinata*, *Lasianthera africana* and *Justicia insularis* [19], *Hippocratea africana* [37], *Setaria megaphylla* [38], *Solanum anomalum* fruit [39], *Croton zambesicus* [40].

In this study, membrane damaged cells were observed in all the treated groups. These results indicated the potential of the extract to exert cytotoxic effect over certain concentrations such as causing membrane damage. Multinucleated and binucleated cells were observed in extract treated groups. This is due to the prevention of cytokinesis or cell plate formation. Microtubules have been implicated in cell plate formation and the extract inhibited the process, resulting in inhibition of cytokinesis. A ghost cell is a dead cell in which the outline remains visible, but whose nucleus and cytoplasmic structures are not stainable [40]. Some ghost cells were observed in various frequencies in this study especially in 10 mg/mL treated groups (Figure 2). This could have resulted from the activities of the phytochemical constituents of the extract leading to nucleus damage and prevention of cytoplasmic structures, thus resulting in ghost cells. In addition, the extract also induced DNA damage and cell death and/or apoptosis in various frequencies in this study. Cell death is a basic biological process of living organism. The cell death is induced by high concentrations of substances such as toxin, stress, heavy metals,

chemicals and others. Researches have revealed the isolation of phytochemical constituents of the extract; such as monoterpenes, diterpenes, flavonoids, polyphenols among others (1,3, 4] in the leaf extract of *S. monostachyus*. Their presence in the leaf extract among other constituents may have contributed significantly to the effects observed in this investigation.

CONCLUSION

The results of this study revealed that *Solenostemum monostachyus* leaf extract exhibited cytotoxic and genotoxic effects in all tested concentrations on the root number, root length and root morphology of the *Allium cepa* meristems after exposure. The degree of chromosomal aberrations (based on increasing extract concentration), inhibition of cellular mitotic processes, and general abnormalities observed in all root bulbs treated with test samples further demonstrate cytotoxic potentials of the leaf extract of *S. monostachyus.*

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Conflict of interest

The authors have not declared any conflict of interests.

Contribution of the Authors

JEO, JG - Research concept and design; JEO, IIJ, UUF,IJU CCO Data analysis and interpretation; JEO,UUF, CCO, Writing the article; JEO,CCO, IJU,IIJ, JG and UUF read and approved the final manuscript.

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