Asian Journal of Biochemistry, Genetics and Molecular Biology

Volume 15, Issue 3, Page 56-64, 2023; Article no.AJBGMB.107539
ISSN: 2582-3698

Genotoxic and Cytotoxicity Activities of Leaf Extract of *Setaria megaphylla*

Okokon J. E. [a], Ochigbo E. B. [a], Johnny I. I. [b], Anagboso M. O. [c] and Ebong N. O. [d*]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Akwa-ibom State, Nigeria.
[b] Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
[c] Department of Microbiology, Madonna University, Elele, Rivers State, Nigeria.
[d] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Madonna University, Elele, Rivers State, Nigeria. Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors OJE, OEB, JII, AMO and ENO initiated and designed this study. Authors OEB, JII, AMO and OJE carried out the experiments and drafted the manuscript. Authors ENO, OJE and AMO performed the statistical analysis, edited and reviewed the manuscript. Authors OJE, OEB, JII, and ENO read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2023/v15i3339

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/107539

Original Research Article

Received: 04/08/2023
Accepted: 07/10/2023
Published: 20/10/2023

ABSTRACT

Setaria megaphylla a medicinal plant, used in ethnomedicine for the treatment of malaria, diabetes and inflammatory diseases was investigated for cytotoxic and genotoxic effects on cells of the root meristem of *Allium cepa*. Bulbs of Onion were exposed to 2.5 mg/ml, 5mg/ml, and 10 mg/ml concentrations of the leaf extract for macroscopic and microscopic study. Tap water was used as a negative control and Methotrexate (0.1 mg/ml) as a positive control. Depending on concentration by the extract, there was statistically significant ($P < .05$) inhibition of root growth when compared with the negative control group. All the tested concentrations of the extract were observed to have

**Corresponding author: E-mail: nwakaebong@gmail.com;*

cytotoxic effects on cell division in *A. cepa.* The extract- induced micronuclei (MNC) formations and chromosomal aberrations in *A. cepa* root tip cells were significant (P < .05) when compared with control group. The extract treatment further induced, ghost cells, cells membrane damage, cell death, and bi nucleated cells. These results implies that *Setaria megaphylla* leaf extract possess cytotoxic and genotoxic effects on *A. cepa* which is as a results of its phytochemical constituents.

Keywords: Setaria megaphylla; genotoxicity; cytotoxicity; Alium cepa test.

1. INTRODUCTION

Setaria megaphylla (Steud) Dur & Schinz (Poaceae), a perennial grass grown in tropical and subtropical areas of the World [1] is traditionally used for the treatment of malaria and diabetes among others [2]. Preliminary reports have demonstrated that the leaf extract has antiplasmodial [2,3] antidiabetic, hypoglycaemic [4,5], anti-inflammatory, analgesic [6], cytotoxic, immunomodulatory, antileishmanial [7], antidepressant [8], inhibitory effect on α-amylase and α-glucosidase [9] activities. Phytochemical analysis of the leaf extract shows that it contains compounds such as 1-triacontanal, 1-triacontanol, 1-dotriacontanol, 1-triacontyl cerotate, stigmasterol [5], flavonoid, carbohydrate, terpenes, saponins, tannins, anthraquinones, cardiac glycosides (Z,Z,Z)-8,11,14-eicosatrienoic acid, phthalic acid, diisooctyl ester, vitamin E, ᵞ-elemene, urs-12-ene, bicyclogermacrene, α-muurolene, germacrene-A, and guaiol have been reported [6,7]. In this study, genotoxic and cytotoxic potentials of the leaves extract of *S. megaphylla* on *Allium cepa* test are reported.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The leaf of *Setaria megaphylla* was collected from farms in Uruan area of Akwa Ibom State, Nigeria. A taxonomist in the department of Botany and Ecological Studies, University of Uyo, identified the plant and deposited a voucher specimen (UUPH. 221 d) in the Department of Pharmacognosy and Natural Medicine herbarium at the University of Uyo, Uyo, Nigeria.

2.2 Extraction

The plant parts (leaves) were cleaned and air-dried on laboratory table for 2 weeks. The dried materials were crushed using a pestle and mortar. The pulverized material was macerated in 50% ethanol for 72 hours. A rotary evaporator 40ºC was used to evaporate to dryness the filtered liquid ethanol extract. The extract was stored in a refrigerator at 4˚C until use for experiment in this study.

2.3 *Allium cepa* Test

Small bulbs of onion, *A. cepa*, were procured from Jos, Northern region of Nigeria. The outer scales of the bulbs and the dry bottom plate were separated without destroying the root primordia using a small sharp knife and collected in a jar of water before initiating the test. The plant extract (20 g) was dissolved in 200 mL of distilled water. Concentrations of 2.5 mg/mL, 5 mg/mL and 10 mg/mL respectively were prepared differently from the stock solution. Test concentration of the plants' extract at 2.5 mg/mL, 5 mg/mL, and 10 mg/mL concentrations made in 15 mL beakers were arranged in a series of 5 per test concentration and filled up for each concentration. One *A. Cepa* bulb was placed on top of each beaker, with the root primordia downward toward the liquid. Tap water was used as negative control and Methotrexate (0.1 mg/mL) was used as positive control. After 24 hours, the test samples were changed in the controls and all test concentrations and photographs of the growing *A. cep*a roots were captured. This continued for 72 hours, after which the roots were counted per beaker in all the tested concentrations and mean root number was calculated. Similarly, the roots' lengths were measured using a meter rule and the mean root length was calculated. These were also done for the control. Several root tips were cut at a length of 10 mm from the bulbs at 8:30 am, and respectively fixed in 3:1 (v/v) ethanol: glacial acetic acid and 1N HCL before putting them in sample bottles and storing in a refrigerator just before usage.

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 forceps and were 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 slight with a thumb. “The cover slip was sealed with a clear finger nail polish and each slide was examined using a Light Microscope at a magnification of x40. Microphotographs were taken to show chromosomal aberrations. The number of aberrant cells per total cells scored at each concentration of each sample were used to determine the mitotic index and frequency of chromosomal aberration” [10]. The mitotic inhibition was determined using the following formula:

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

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

%root growth of control = Overall mean root length of test solution / Overall mean root length of control x 100

To measure the cytotoxicity and genotoxicity, the following parameters were used: (i) the mitotic index (MI) was evaluated as the ratio between the number of mitotic cells and the total number of cells scored and expressed as percentage and (ii) chromatin aberrations (stickiness, bridges, breaks and polar deviation) were used as endpoints for determination of cytogenetic effects and micronuclei (MNC) were scored in interphase cells per 500 cells.

2.5 Statistical Analysis

Data obtained from this study were analyzed 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 i.e. $P \leq .05$.

3. RESULTS

3.1 Physiochemical Analysis

The effect of *Setaria megaphylla* leaf extract on different physicochemical parameters (root number and root length) of *Allium cepa* root tips are presented in Table 1. The results from all the tested concentrations of *Setaria megaphylla* leaf extracts show that it resulted in significant inhibition in the growth of roots when compared to negative control and positive control. The inhibition of root number and root length was greater as concentrations of the extract increased. The average root length in negative and positive control (methotrexate) groups were 4.66±1.26 and 0.25±0.22 cm respectively. However, significant decrease in average root lengths in 10 mg/mL treatment group when compared the negative control; (0.34±0.01 cm) for *S. megaphylla* (Table 1). Average root lengths in treatment groups were decreased depending on concentration, significantly (P < .05) when compared to negative control. The morphology of the root was almost normal in the negative control group, but at 2.5 mg/mL of *S. megaphylla* leaf extract treated group, the roots appeared a little brownish and at 5 and 10 mg/mL of *S. megaphylla* leaf extract (Table 1).

3.2 Cytogenetic Analysis

Table 2 shows the effect of *Setaria megaphylla* leaf extracts on cytogenetic parameters of *Allium cepa* roots. Cytogenetic analysis performed showed that the extract caused concentration-dependent and significant (P < .05) decreases in the mitotic index when in comparison to that of negative control. The leaf extract of *S. megaphylla* at 10 mg/mL had mitotic index of 24.20± 3.51 as compared to 57.60±12.34 recorded in the negative control group (Table 2).

Cytogenetic alterations caused by the leaf extract are shown in Table 3. Chromosome and cytological alterations were observed in negative control, methotrexate, *Setaria megaphylla* leaf extract-treated groups as depicted in Table 3. An examination of chromosome aberrations observed showed that in the different treatments, the fragments were of chromosome type especially in the highest concentration of the extract (Table 3) (Fig. 1(a). The chromosome breaks observed revealed the clastogenic effect of extract. This was significant (P < .05) when in comparison to negative control group. Polar deviations and sticky metaphase (chromosomes moving in wrong directions) were also observed (Figs. 1(b)) in the extract-treated groups but were more frequent in the group treated with the highest concentration of extract (10 mg/mL). Sticky metaphase were also observed in the extract- treated groups. These abnormalities were observed to increase with increasing concentrations of the extract. A concentration-dependent and statistically significant (P < .05) increase in total aberrant cells (aberrant cells consist of chromosome breaks, stickiness and polar deviation) in comparison with the negative control (Table 3) was observed with the leaf extract with the highest concentrations exerting the highest effect and higher frequencies of aberrations. Genotoxic activity of the extract were further demonstrated using *A. cepa* by inducing micronuclei in the root tip meristem cells. Formation of micronucleus in 500 cells for each slide (‰MNC value) was not concentration-dependent as the groups treated with methotrexate and 10 mg/mL of *S. megaphylla* had highest numbers of cells with micronuclei in the test compared to negative control, which were statistically significant (P < .05) (Fig. (c)) . The increase also developed in the positive control. In *Allium* test, an extreme toxic effect of extract was observed due to great occurrence of sticky metaphases, leading to cellular death (reduction in mitotic index). Also, various frequencies of cells with membrane damage (Fig. 1(d)),binucleated cells (Fig. 1(c)), and nucleus damage (Figs. 1(d)) were found. Furthermore, apoptotic cells (Fig. 1(d)) were identified in the extract-treated groups.

4. DISCUSSION

In this study, toxic effect of *Setaria megaphylla* leaf extract was assessed by evaluating root growth and root morphology of *Allium cepa*. Varying concentrations of the extract were observed to suppress the growth of roots and these were statistically significant when compared to control group. Also, the extract caused colouration of the root tips of *Allium cepa* depending on the concentration. This colouration ranged from light brown to dark brown coloration of the roots. Cytological parameters such as the mitotic index and number of chromosome abnormalities, as well as chromosome breaks, polar deviations and stickiness were used to assess geno- and cytotoxicity. The mitotic index (MI) of meristematic cells of *A. cepa* treated with methotrexate (3.00%) was significantly decreased when compared to control. It was observed that onion roots treated with *S. megaphylla* were significantly (40.40%, 32.20% and 24.20%) inhibited in comparison to the negative control were observed (Table 2). The inhibition of root growth was found to be dependent on decrease of mitotic index. The drop in mitotic index below 22% in comparison to negative control can have fatal impact on the organism [11], while a decrease below 50% usually has less fatal effects [12] referred to as cytotoxic limit value [13]. “Mitotic index measures the portion of cells in the M-phase of the cell cycle and its inhibition could be understood as cellular death or a delay in the cell proliferation kinetics” [13]. “This decrease in mitotic activity could be as a result of inhibition of DNA synthesis or a blocking in the G2 phase of the cell cycle thus, prohibiting the cell from entering mitosis” [14]. “Earlier reports have described mitodepressive effects of some herbal extracts as well as the ability to block the synthesis of DNA and nucleus proteins” [15,16]. Inhibition of mitosis have been reported with a lot of herbal extracts. [17,18,19]. “The reduced mitotic index in roots of *A. cepa* treated with *S. megaphylla* leaf extract maybe due to either disruptions in the cell cycle or chromatin dysfunction induced by extracts-DNA interactions. The outcome of this study implies that the tested extract concentrations have inhibitory, mito-depressive effects on root growth and cell division of *A. cepa*, hence, it can impede DNA synthesis and reduce the number of dividing cells in roots caused by the cytotoxic effects of compounds found in the extract. The detection of sticky metaphase reinforces the theory of the toxic effect of the extract. The normal appearance of metaphases with sticky chromosome usually gets lost, and are seen with a sticky “surface,” initiating chromosome agglomeration” [20]. “It has been found that stickiness maybe due to the effect of pollutants and chemical compounds interacting with the physicochemical properties of DNA, protein or both, on the formation of complexes with phosphate groups in DNA, on DNA abridgment or on production of inter- and intra-chromatid cross links” [21]. “Changes in chromosome structure due to a break or exchange of chromosomal material are referred to as Chromosomal aberrations (CA). Almost all CA observed in cells are fatal, but there are many related aberrations that are viable and that can affect genes, either somatic or inherited” [22]. “Chromosome fragments been present in this study, indicates chromosome breaks, and can be an aftermath of anaphase/telophase bridges” [23]. “The extract understudy was found to interfere with the cell cycle as well as affect chromatin organization or DNA replication, leading to chromosome breaks. Recurrence of total CA increased significantly following exposure to the extracts which suggests clastogenic activity (Table 3). The extract significantly induced the production of micronuclei (MNC) in root cells of *A. cepa* at concentrations of 2.5–10 mg/mL. However, MNC frequency decreased in *A. cepa* roots treated at the highest concentration of the extracts (10 mg/mL), because of high cytotoxicity. Frequent cells with micronuclei is a good sign of the cytogenetic effects of tested chemicals. Micronuclei (MN) often arise from the acentric fragments or lagging chromosomes that fail to fuse into the daughter nuclei during telophase of the mitotic cells and can result in cellular death due to the deletion of primary genes" [24,25]. Previous research have suggested MNC-induced effect of numerous plant extracts such as *Lavandula stoechas* and *Ecballium elaterium* [17,26], *Azadirachta indica* [27], *Psychotria* species [18].

"In this study, it was observed that membrane damaged cells occurred in groups treated with 5 mg/mL and 10 mg/mL of the extract. The results shows that cytotoxicity and membrane damage may occur with the extract over certain concentrations may cause cytotoxicity and membrane damage. These results further support the cytotoxicity activities reported on *Setaria megaphylla* leaf extracts" [7]. "Multinucleated and bi nucleated cells have been detected in extracts treated groups. This is due to the prevention of cytokinesis or formation of cell plate. Microtubules have been involved in formation of cell plate and the extracts the process, resulting in inhibition of cytokinesis. Ghost cell is a dead cell in which the outline is usually visible, but its nucleus and cytoplasmic structures cannot be stained" [26]. Some ghost cells were observed in different frequencies following this study (Fig. 1). This could have been as a result of the activities of the phytochemical constituents of the extract causing nucleus impairment and prevention of cytoplasmic structures, thus resulting in ghost cells. Again, the extract also caused DNA damage and cell death and/or apoptosis in different frequencies in this study. Furthermore, high concentrations (5 mg/ml and 10 mg/ml) of the extract were found to cause the induction of cell death and/or apoptosis. Cell death is an essential biological process of living organism which is induced by high concentrations of such as toxin, stress, heavy metals, chemicals and others. In this study, the results shows that the extract of *S. megaphylla* can cause cytogenetic changes (cytoplasmic shrinkage, DNA fragmentation, nuclear condensation, membrane blebbing, cytoskeleton alterations and appearance of apoptotic bodies) and cell death in *A. cepa* root tips (Figs. 1(a), 1(b), 1(c), and 1(d)), suggesting cytotoxic and genotoxic activities of the extract.

Therefore, proper use of this plant in ethnomedicine is recommended and high doses should be avoided as it can cause cytotoxic and/or genotoxic effects.

Table 1. Cytotoxicity of *Setaria megaphylla* 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	34.10±4.62	4.66±1.26
Methotrexate	0.1	8.16±3.28[a]	0.25±0.22 [a]
Setaria megaphylla	2.5	13.60±1.86[a]	3.24±0.30 [a]
	5.0	24.40±4.50	1.42±0.12 [a]
	10.0	13.20±1.80[a]	0.34±0.01 [a]

Values are expressed as mean ±SEM (n=5). Significant at P < .05 when compared to negative control

Table 2. Total and dividing cells counted following microscopic examination and mitotic values in control and treatment concentrations

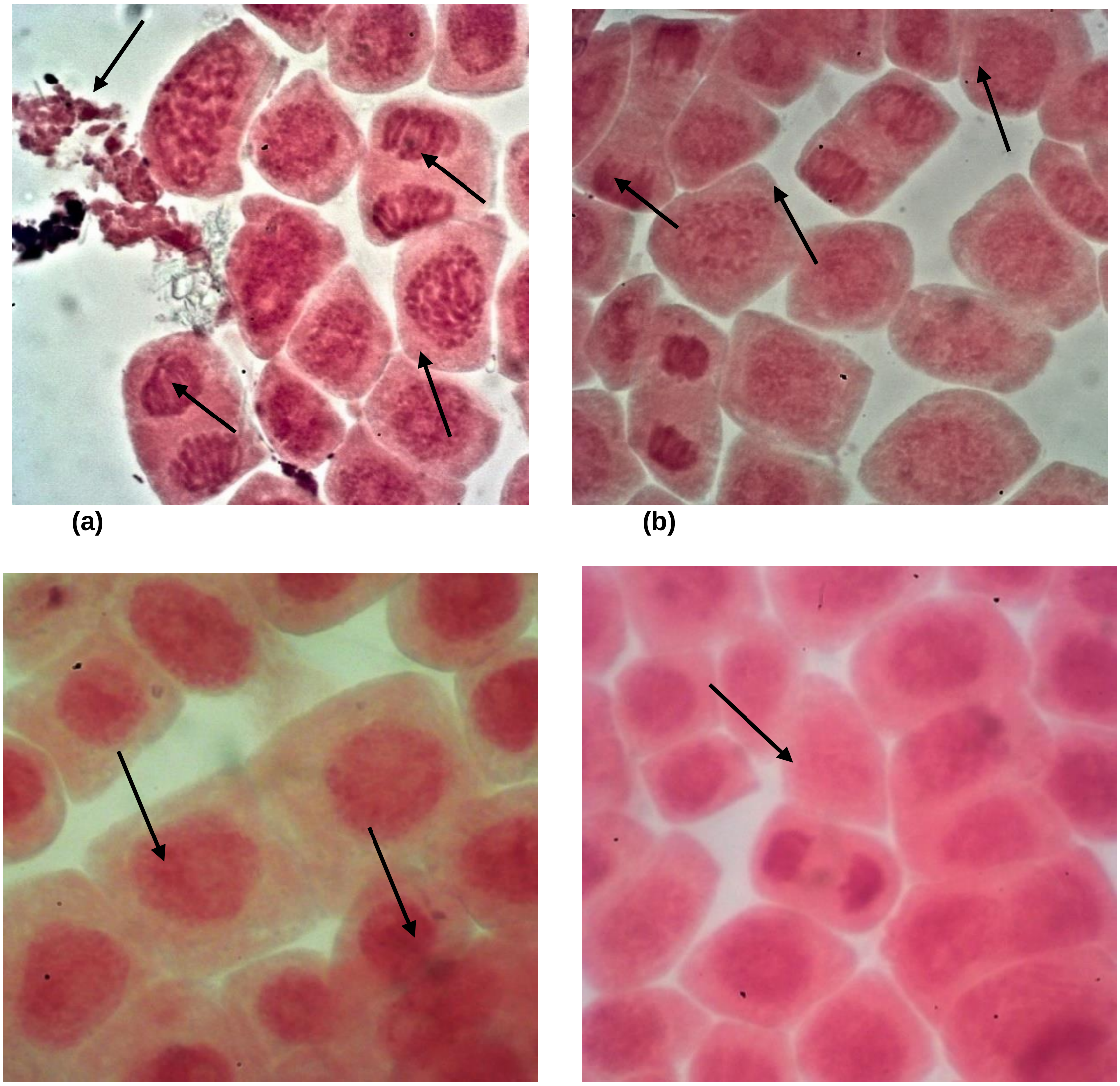
Treatment group	Concentration of extract (mg/mL)	Total Number of cells	Dividing cells	M.I (%)± S.E
Negative control	Tap water	500	288	57.60±12.34
Methotrexate	0.1	500	15	3.00±0.68[a]
Setaria megaphylla	2.5	500	202	40.40±4.56
	5.0	500	151	32.20±4.95[a]
	10.0	500	121	24.2±3.51[a]

Values are expressed as mean ±SEM (n=5). Significant at P < .05 when compared to negative control

Table 3. Chromosomal and mitotic aberrations in the root meristematic cells of *Allium cepa* after treatment of extract of *Setaria megaphylla*

Treatment group	Concentration of extract (mg/mL)	Chromosome breaks (%)±S.E	Stickiness (%) ±S.E	Polar deviation (%)±S.E	Aberrant cells (%)±S.E	MNC (%)± S.E
Negative control	Tap water	-	0.11±0.08	0.31±0.04	1.05±0.56	-
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]
Setaria megaphylla	2.5	3.45±0.22[a]	18.15±1.04 [a]	1.88±2.68[a]	30.56±4.57[a]	1.34±0.16[a]
	5.0	2.85±0.56[a]	25.18±5.57[a]	3.44±0.24[a]	36.41±5.96 [a]	1.02±0.38[a]
	10.0	3.26±1.06[a]	52.46±6.78 [a]	2.53±0.28[a]	62.14±6.94	2.66±1.04[a]

Values are expressed as mean ±SEM (n=5). Significant at P < .05 when compared to negative control



(a) (b)

(c) (d)

Fig. 1. Photomicrography showing the mitotic and chromosomal aberrations after the *Setaria megaphylla* extract treatments in *Allium cepa* root tip meristem cells viewed with light microscopy at magnification X40. (a) visible fragments and polar deviation (b) polar deviations and stickyness at metaphase (c) micronucleus (d) ghost cells

5. CONCLUSION

The leaf extract of *Setaria megaphylla* in this analysis revealed that it possesses cytotoxic and genotoxic effects, as seen in the effects elicited by all test concentrations of the plant extract 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), the inhibition of cellular mitotic processes, and the general abnormalities observed in all root bulbs treated with the extract further indicate cytotoxic potentials of *S. megaphylla* leaf not only in experimental plant tissues, but also quite possibly in animal systems as well.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Van Oudtshoorn FP. Guide to grasses of South Africa. Briza Publications, Cape Town; 1999.

2. Okokon JE, Ubulom PM, Udokpoh AE. Antiplasmodial activity of Setaria megaphylla. Phytother Res. 2007;21:366-368.
3. Okokon JE, Okokon PJ, Sahal D. *In vitro* antiplasmodial activity of some medicinal plants from Nigeria. Int J Herbal Med. 2017;5(5):102-109.
4. Okokon JE, Antia BS.. Hypoglycaemic and antidiabetic effect of Setaria megaphylla on normal and alloxan induced diabetic Rats. J Nat Remedy. 2007;1:134-138.
5. Okokon JE, Davies K, Lekara J, Iwara K, Thomas P, Li W-W. Antidiabetic and hypolipidemic activities of solvents fractions of Setaria megaphylla and its phytochemical characterisation. Phytomedicine Plus. 2022;2(2022): 100182.
6. Okokon JE, Antia BS, Ita BN. (Antiinflammatory and antinociceptive effects of ethanolic extract of Setaria megaphylla leaves in rodents. Afr J Biomed Res. 2006;9:229-233.
7. Okokon JE, Dar A., Choudhary MI. Immunomodulatory, cytotoxic and antileishmanial activities of Setaria megaphylla. Int J Phytomed. 2013;4:155-160.
8. Okokon JE, Otuikor JE, Davies K. Psychopharmacological study on ethanol leaf extract of Setaria megaphylla. Afr. J Pharmacol Ther. 2016;6(4);166-172.
9. Okokon JE, Davies K, Lekara J, Iwara K, Kumar H. Effect of solvents leaf fractions of Setaria megaphylla on alpha amylase and alpha glucosidase activities in rats. Adv Pharm J. 2021;6(4):111-115.
10. Bakare AA, Mosuro AA, Osibanjo O. Effect of simulated leachate on chromosomes and mitosis in roots of *Allium cepa* L. J. Environ. Biol. 2000;21:263–271.
11. Antonsie-wiez D. Analysis of the cell cycle in the root of Allium cepa under the influence of Leda krin," Folia Histochemica et Cytobiologica. 1990;26:79–96.
12. Panda BB, Sahu UK. Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of Allium cepa by the organophosphorus insecticide fensulfothion. Cytobios. 1985;42(167-168):147–155.
13. Sharma CB. Plant meristems as monitors of genetic toxicity of environmental chemicals. Current Science. 1983;52:1000–1002.
14. Sudhakar R, Ninge Gowda KN, Venu G. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of Allium cepa. Cytologia. 2001;66(3):235–239.
15. Mercykutty VC, Stephen J. Adriamycin induced genetic toxicity as demonstrated by *Allium cepa* test," Cytologia. 1980;45(4):769–777.
16. Schulze E, Kirschner M. Microtubule dynamics in interphase cells. Journal of Cell Biology. 1986;102(3):1020–1031.
17. As T, kınelik C, Aslanturk OS. Cytotoxic and genotoxic effects of Lavandula stoechas aqueous extracts. Biologia. 2007;62(3):292–296.
18. As T, kin C, elik, Aslantu¨rk OS. Antimitotic and antigenotoxic effects of *Plantago lanceolata* aqueous extract on Allium cepa root tip meristem cells. Biologia. 2006;61(6):693–697.
19. Akinboro A, Bakare AA. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants. Journal of Ethnopharmacology. 2007;112(3):470–475.
20. Babich H, Segall MA. Fox KD. The Allium test— a simple, eukaryote genotoxicity assay. American Biology Teacher. 1997;59(9):580–583.
21. G¨om¨urgen AN. Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tips of Allium cepa L. Cytologia. 2005;70(2):119–128.
22. Swierenga SH, Heddle JA, Sigal EA. Recommended protocols based on a survey of current practice in genotoxicity testing laboratories, IV. Chromosome aberration and sister-chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocyte cultures," Mutation Research. 1991;246(2):301–322.
23. Sharma A, Sen S. Chromosome botany, Science, Enfield, NH, USA; 2002.
24. Albertini RJ, Anderson D, Douglas GR. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutation Research. 2000;463(2):111–172.
25. Krishna G, Hayashi M. *In vivo* rodent micronucleus assay: protocol, conduct and data interpretation. Mutation Research. 2000;455(1-2):155–166.

26. As TA, kin C, elik, Aslantu¨rk OS. Investigation of cytotoxic and genotoxic effects of Ecballium elaterium juice based on Allium test. Methods and Findings in Experimental and Clinical Pharmacology. 2009;31(9):591–596.

27. Soliman MI. Genotoxicity testing of neem plant (*Azadirachta indica* A. Juss.) using the *Allium cepa* chromosome aberration assay. Journal of Biological Sciences. 2001;1(11):1021–1027.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/107539