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Research Article

Cytotoxic and Genotoxic Potentials of the Extracts and Fraction of the Leaf of *Datura metel* (Lnn.)

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ABSTRACT

This study evaluated the cytotoxic and genotoxic effects of the leaf extracts of *Datura metel* with a view to understanding the mode of action of the extracts using plant model. The study involved collection, identification, authentication and preparation of methanol (ME) and aqueous extracts (AQE) of the plant. The methanol extract (ME) was partitioned with n-hexane and ethylacetate to yield ethylacetate fraction (EAF). Genotoxic and cytotoxic activities of the extracts and fraction as well as its effect on biochemical parameters were evaluated using *Allium cepa* assay technique. The extracts and fraction caused root growth inhibition, induced chromosomal abnormalities which include formation of sticky chromosomes, chromosome bridges, c-mitosis and other metaphase and anaphase disorders. Decrease in mitotic index, increase in chromosomal aberration and changes in biochemical parameters were observed. In conclusion, the ME, AQE and EAF of the leaves of *D. metel* exhibited growth inhibition, genotoxic and cytotoxic effects through the disruption of the structure of nucleic acid thereby blocking cell division and elongation which in turn could affect the growth of the plant.

Keywords: *Allium cepa, Cytotoxic, Datura metel, Genotoxic, Chromosome.*

INTRODUCTION

Medicinal plants are known to play important roles and contribute immensely to the development and growth of nations (Adachukwu and Yusuf, 2014). Despite the profound therapeutic advantages possessed by medicinal plants, some constituents of medicinal plants have been found to be sources of mutagenic, genotoxic, clastogenic, cytotoxic, carcinogenic and teratogenic compounds (Chandra *et al.*, 2012).

Datura metel is a weed that grows along roadsides, in cornfields, pastures and dumbing sites and has been used for treatment of cough, chest pain, asthma, fever, diarrhea, and acts as an antipyretic (Sayyed and Sha, 2014). Studies have shown that *D. metel* which aggressively colonised its environment is toxic and does not allow neighbouring plants to thrive well (Ekanem *et al*., 2015). It has also been used to kill parasites and microorganisms (Ekanem *et al*., 2015).

The *Allium cepa* test has been used by researchers as a bioindicator of environmental pollution (Ilbas *et al.*, 2012), and has been used to evaluate the genotoxic and cytotoxic potentials of medicinal plants (Dragoeva *et al.*, 2012). This technique is sensitive enough to detect several substances that cause chromosomal alterations. It is an excellent model in which the roots of *A. cepa* grow in direct contact with the substance of interest enabling possible damage to the DNA of eukaryotes to be predicted (Dragoeva *et al*., 2012). The *A. cepa* test is one of the few direct methods for measuring damage in systems that are exposed to mutagens or potential

carcinogens, and enables the evaluation of the effects of these damages through the observation of chromosomal alterations (Anjana and John, 2013). It is an excellent genetic model for evaluating environmental pollutants, detecting mutagens in different environments and evaluating many genetic endpoints (point mutations to chromosomal alterations) (Anjana and John, 2013). The study investigated the genotoxic and cytotoxic activities of the extracts and fraction of *D. metel* with a view to understanding the mechanisms by which the plant affects the growth of other nearby plants.

MATERIALS AND METHODS

Collection and Identification of Plant Material

D. metel (fresh leaves) was collected at Ajebamidele Area, Ile-Ife (7˚49 N, 4˚07 E) and Iperindo (7˚29 N, 4˚30 E), Atakunmosa East Local Government Area, Osun State, Nigeria. The identification and authentication of the plant was done at Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria where the specimen copy was deposited and voucher number (FHI 111862) collected. Bulbs of *A. cepa* were purchased at Akinola Market, Ipetumodu, Osun State, Nigeria.

Experimental Design

Preparation of Aqueous and Methanol Extracts of the Leaf of *D. metel*

Fresh leaves of *D. metel* were air-dried at room temperature for four weeks and then milled into fine powder. Powdered leaf was exhaustively extracted with methanol (80% v/v) and distilled water (aqueous) separately for 72 h. The extracts were collected; the residue was washed and re-extracted with the same solvent. The methanol extract (ME) and aqueous extract (AQE) were filtered through Whatman paper No. 1, followed by centrifugation at 3000 rpm for 15 min on Bench Centrifuge (Model 90-2(Microfield Instrument, Essex, England) and was concentrated to dryness *in vacuo* at 40ºC.

Fractionation of the Methanol Extract (ME)

The methanol extract (15 g) was dissolved in hot distilled water (200 ml) and filtered in a separating funnel, followed by partitioning with n-hexane to remove fat. The aqueous portion was further partitioned with ethylacetate to obtain ethylacetate fraction (EAF). The fractions were pooled together and subjected to evaporation to dryness under reduced pressure.

Root Growth Inhibition Assay

The *A. cepa* root growth inhibition assay was performed as a 96 hr semi-static exposure test as described by Rank (2003) with slight modification. Dried *A. cepa* bulbs were scrapped, the outer scales were removed and remnants of old root were removed. *A. cepa* was exposed for 96 h to different concentrations (0, 100, 200, 300, 400 and 500 µg/ml) of extracts and fraction in 5 replicates. The best developed 10 roots of each onion in each group were measured with ruler and mean root length was calculated.

The percentage growth inhibition was calculated using the expression:

$$\text{Percentage Growth Inhibition} = \frac{\text{Average length of roots in test solution}}{\text{Average length of roots of the control}} \text{ X } 100$$

Evaluation of Cytotoxic and Genotoxic of the Extracts and the Fraction using *A. cepa*

The dried scales of *A. cepa* bulbs were scrapped and the old roots removed to promote the emergence of new roots. The onion bulbs were set-up allowing rootlets to grow. All bulbs were placed separately in a small 100 ml plastic cup, containing distilled water for 24 hours to initiate root growth. The bulbs were transferred into cups containing varying concentrations of the extracts and fraction (0, 100, 200, 300, 400 and 500 µg/ml) for 48 hours in five replicates (Plate 1). The test solutions were changed every 24 h. After 48 hours, the roots were collected and tips were cut, washed with distilled water and fixed in a mixture of ethanol: glacial acetic acid (3:1 v/v) for 24 h according to Fasaanu *et al.* (2013). The remaining roots were harvested and kept for the evaluation of biochemical parameters.

Preparation of Microscope Slides

The root tips were washed with distilled water, hydrolyzed with 1 M HCl for 5 min at room temperature, washed again with distilled water and then placed on the glass slide. The root tip terminal was cut followed by addition of two drops of 2% (w/v) orcein solution, left to stand for proper staining (2 min), and then squashed with a metal rod, additional two drops of 2% (w/v) orcein were added. Then cover-slip was carefully placed and allowed to stand for another 5 min. The excess stain was removed with tissue paper by pressing slightly down with the thumb. Five slides were prepared for each concentration according to Fasaanu *et al.* (2013).

Counting and Scoring of Cell

The slides were view under the light microscope (CHA/CHB & CHU, OLYMPUS OPTICAL co., LTD, Tokyo, Japan) using 40X/0.65 objective lens. The cells were counted and scored for the different cell division stages (interphase, prophase, metaphase, anaphase, and telophase). The number of dividing cells, number of non-dividing cells and number of cells with chromosomal aberration in the microscope fields were counted and recorded.

Mitotic index was calculated as a ratio between the number of dividing cells and the number of cells counted;

Mitotic index = (Number of dividing cells/ number of cells counted) x 100

Percentage chromosome aberration was estimated as the ratio between the number of aberrant cells and the dividing cells;

Percentage chromosomal aberration = (Number of aberrant cells/Number of dividing cells counted) x 100.

Plate 1. Experimental Set-Up for the Study of Cytotoxic and Genotoxic Potentials

The photomicrograph of dividing cells was taken at X 400 magnification using a microscope attached with a camera (DCE-PW130 OLYMPUS OPTICAL co., LTD, Tokyo, Japan) as described by Rank (2003).

Preparation and Estimation of Biochemical Parameters

Preparation and Estimation of Soluble Proteins

Soluble proteins of the roots were prepared using normal saline and 0.1 M NaOH. Typically, the roots (0.5 g) were homogenized first with normal saline (5 ml), centrifuged for 10 min. at room temperature and supernatant (4 ml) collected. The residue was extracted again with 2.5 ml 0.1 M NaOH, centrifuged and supernatant (2 ml) collected. The two supernatants were combined, filtered and used for the estimation of protein concentration. The soluble protein concentrations of homogenates were determined spectrophotometrically according to Lowry *et al.* (1951) method as modified by Schacterle and Pollack (1973) using bovine albumin as standard.

Extraction and Quantification of Total Nucleic Acids

The extraction and quantification of total nucleic acids were carried out according Oyedapo and Olalere, (1994) and Pathak *et al*. (2013) with slight modification.

Quantification of total nucleic acid isolated was determined by taking the absorbance at 260 nm. The extracted nucleic acid was dissolved in 5 ml normal saline (0.85% w/v), varying dilutions of the nucleic acid was prepared while the absorbance readings were taken at 260 nm and 280 nm respectively. The concentration was estimated using the expression

[1 O.D = 50 µg/ml of double-stranded DNA and 1 O.D = 40 µg/ml of RNA (Pathak *et al*., 2013)] and 280 nm to determine the quality of the isolated nucleic acid.

Statistical Analysis

Graph-Pad 5 (prism) was used for the analyses. The data were expressed as Mean ± SEM, n = 5 and One-way Analysis of Variance was used to determine the differences in the data. The level of confidence was set at $p < 0.05$.

RESULTS AND DISCUSSION

Results

The inhibitory potentials of different concentrations of the leaf extracts and ethyl acetate fraction of *D. metel* on root growth of *A. cepa* are shown in Table 1. The extract significantly ($P<0.05$) inhibited the root growth of *A. cepa* exposed to difference concentrations of the extracts. The percentage growth length of *A. cepa* exposed to difference concentrations of ME (100, 200, 300, 400 and 500 µg/ml) were 90.7±5.2, 76.0±4.2, 48.7±4.5, 37.0±7.2, 31.0±3.6 respectively, EAF (100, 200, 300, 400 and 500 µg/ml) were 65.5±0.6, 54.7±0.4, 54.0±0.6, 34.1±0.6 and 20.9±0.6 compared to the control respectively and AQE (100, 200, 300, 400 and 500 µg/ml) were 96.39±0.12, 94.48±0.08, 62.86±0.20, 72.79±0.11 and 69.11±0.17 compared to the control respectively.

Table 1. Root Growth Inhibition of *A. cepa* Exposed to Different Concentrations of Extracts and Fractions of the Leaf of *D. metel*.

Concentrations (µg/ml)	% growth length compared to the control		
	ME	EAF	AQE
0	100 ± 0.00	100 ± 0.00	100.00±0.00
100	90.7 ± 5.20	65.5 ± 0.60	96.39±0.12
200	76.0 ± 4.20	54.7 ± 0.40	94.48±0.08
300	48.7 ± 4.50	54.0 ± 0.60	62.86±0.20
400	37.0 ± 7.20	34.1 ± 0.60	72.79±0.11
500	31.0 ± 3.60	20.9 ± 0.60	69.11±0.17

Data represent mean values ± S.E.M; n=10. Value of $p < 0.05$ was considered statistically significant when compared with the control (0 µg/ml).

ME = Methanol extract, EAF = Ethyl acetate fraction, AQE = Aqueous extract

Findings of this study revealed the cytotoxic and genotoxic effects of methanol extract, ethyl acetate fraction and aqueous extract of the leaf of *D. metel* as shown in Tables 2-4.

Table 2 present the reduction in the mitotic index caused by different concentrations (100, 200, 300, 400, and 500 µg/ml) of ME to be 3.1± 0.8, 2.4±0.4, 1.5 ± 0.2, 1.5 ± 0, and 0.4 ± 0.1 respectively compared with the control (9.2 ± 2.5) and increase in chromosomal aberration, 0.10 ± 0.10, 14.8 ± 2.40, 41.013 ± 4.30, 90.80 ± 1.70, and 86.30 ± 5.20 respectively when compared with the control (0.00 ± 0.00). The mitotic index of *A. cepa* exposed to different concentrations (100, 200, 300, 400, and 500 µg/ml) of EAF were 2.4±0.3, 1.5 ± 0.5, .6±0, and 0.01±0.01 respectively compared to the control (9.2 ± 2.5) and increase in chromosomal aberration, 0.30 ± 0.30, 6.08 ± 4.10, 38.013 ± 3.30, 40.20 ± 2.70, and 67.30 ± 5.20 respectively when compared with the control (0.00 ± 0.00) as shown in Table 3

Table 2. Effect of Varying Concentrations of ME of the Leaf of *D. metel* on the Mitotic Index and the Percentage Chromosomal Aberrations of *A. cepa* Root

Conc (µg/ml)	Interphase	Prophase	M	A	T	AP	AM	AA	AT	SC	VC	B	C-Mitosis	Total Dividing Cells	Total Cells Counted	Total Aberration	Mitotic Index	%Chromosomal Aberration
0	2821±391	23±10	29±5	15±3	4±3	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	71±21	2891±411	0±0	9.2±2.5	0±0
100	2189±162	145±50	47±1	17±2	9±1	0±0	0**±0**	0±0	0 ± 0	1±1	0±0	0**±0**	0±0	218±48	2407±114	1**±1**	3.1±0.8	0.1±0.1
200	4143±707	42± 5	37±7	23±1	6±2	1±1	5±1	3±3	2±1	2±2	0±0	2±2	5±2	124±11	4267±697	19±5	2.4±0.4	14.8±2.4
300	3758±305	17± 4	9±1	10±2	3±1	5±0	13±2	5±0	2±0	1±1	1±1	0±0	1±1	57±4	3806±309	23±1	1.5±0.2	41±4.3
400	4588±492	5±1	2±1	0±0	0±0	4±3	35±5	4±3	0±0	0±0	3±3	0±0	2±2	70±9	4658±501	63±7	1.5±0	90.8±1
500	5168±497	2±1	1±1	0±0	0±0	1±1	11±3	1±1	0±0	3±2	0±0	1±0	0±0	20±4	5187±500	17±4	0.4±0.1	86.3±5

Each value represented Mean ± SEM, n=3 readings. Value of $p < 0.05$ was considered statistically significant when compared with the control (0 µg/ml). There were various forms of chromosomal aberrations observed at each mitotic phase. There was reduction in mitotic index and this was concentration dependent.

Key: M: Metaphase, **A:** Anaphase, **T:** Telophase, **AP:** Abnormal prophase, **AM:** Abnormal Metaphase, **AA**: Abnormal Anaphase, **AT:** Abnormal Telophase, **SC:** Sticky Chromosomes, **VC:** Vagrant Chromosomes, **B:** Bridges

The mitotic index of *A. cepa* exposed to different concentrations (100, 200, 300, 400, and 500 µg/ml) of EAF were 2.4±0.3, 1.5 ± 0.5, .6±0, and 0.01±0.01 respectively compared to the control (9.2 ± 2.5) and increase in chromosomal aberration, 0.30 ± 0.30, 6.08 ± 4.10, 38.013 ± 3.30, 40.20 ± 2.70, and 67.30 ± 5.20 respectively when compared with the control (0.00 ± 0.00) as shown in Table 3. Table 4 revealed the reduction in the mitotic index of different concentrations (100, 200, 300, 400, and 500 µg/ml) of AQE to be 5.01 ± 2.01, 3.08 ± 1.10, 2.05 ± 1.40, 1.16 ± 0.20, and 1.50 ± 0.20 respectively compared with the control (6.56 ± 1.20) and increase in chromosomal aberration, 0.00 ± 0.01, 2.4±0.4, 10.53 ± 1.20, 10.80 ± 1.70, and 22.88 ± 6.70 respectively when compared with the control (0.00 ± 0.00).

The effect of the leaf extracts and fraction of *D. metel* on the nucleic acid concentration of the root of *A. cepa* was shown in Figure 1. There was reduction in the levels of total nucleic acid, DNA and RNA concentrations of *A. cepa* root treated with the extracts and fraction when compared with the control.

Table 3. Effect of Varying Concentrations of EAE of the leaf of *D. metel* on the Mitotic Index and the Percentage Chromosomal Aberrations of *A. cepa* Root

Conc (µg/ml)	Interphase	Prophase	M	A	T	AP	AM	AA	AT	SC	VC	B	CM	Total Dividing Cells	Total Cells Counted	Total Aberration	Mitotic Index	%CA
0	2189±162	145 ±50	47±1	17±2	9±1	0±0	0±0	0±0	0±0	1±1	0±0	0±0	0±0	218±48	2407±114	1±1	9.2±2.5	0.1±0.1
100	2880±815	24±4	25±15	15±0	13±7	0±0	0±0	1±1	0±0	0±0	0±0	0±0	0±0	77±25	2957±840	1±1	2.6±0.1	0.3±0.3
200	2307±160	24 ±2.6	17±1	5±0	1±0	1±1	1±1	2±0	0±0	1±1	0±0	1±1	0±0	50±6	2358±274	3±2.3	2.0 ±0.5	6±4
300	3140±189	20±1.6	4±0	2±0.8	2±0.4	0±0	6±1.6	5±1.2	1±0.4	1±0.4	1±0.4	0±0	1±0.4	42±6.3	3183±95.5	15±3.7	1.0±0.2	38±3
400	3253±379	10±0.9	1±0	1±0.6	1±0.6	1±0.6	0±0	0±0	0±0	0±0	0±0	0±0	0±0	21±1.76	3607±72.5	8±0.9	1.0±0.3	40±2.8
500	6018±358	0±0	0±0	0±0	0±0	0±0	1±0.3	0±0	0±0	0±0	0±0	0±0	0±0	1±0.3	6019±358	1±0.3	0.01±0.006	67±33

Each value represented Mean ± SEM, n=3 readings. Value of $p < 0.05$ was considered statistically significant when compared with the control (0 µg/ml). There were various forms of chromosomal aberrations observed at each mitotic phase. There was reduction in mitotic index and this was concentration dependent.
Key: M: Metaphase, **A:** Anaphase, **T:** Telophase, **AP:** Abnormal prophase, **AM:** Abnormal Metaphase, **AA**: Abnormal Anaphase, **AT:** Abnormal Telophase, **SC:** Sticky Chromosomes, **VC:** Vagrant Chromosomes, **B:** Bridges, CM: C-Mitosis: %CA: %Chromosomal Aberration.

Table 4. Effect of Varying Concentrations of AQE of the Leaf of *D. metel* on the Mitotic Index and the Percentage Chromosomal Aberrations of *A. cepa* Root

Conc. (µg/ml)	Interphase	Prophase	Metaphase	Anaphase	Telophase	AP	AM	AA	AT	SC	VC	B	CM	CA	TDC	TCC	Mitotic Index	%Chromosomal Aberation
0	1756.5± 220.5	76.5± 34.5	28.1± 3.0	21.01± 1.02	8.0± 1.0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.0± 0.0	133.5± 37.5	1890.0± 58.0	6.6± 1.2	0.0± 0.0
100	2063.0± 370.0	57.1± 10.1	24.5± 5.5	12.50± 1.50	45.1± 2.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.0± 0.0	98.5± 21.5	2161.5± 348.5	5.0± 2.0	0.0± 0.0
200	2483.5± 613.5	36.1± 3.6	15.1± 0.5	13.02± 4.50	45.1± 1.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.0± 0.0	69.5± 9.5	2553.0± 604.0	3.1± 1.1	0.0± 0.0
300	2115.1± 766.1	15.0± 3.0	6.0±1.3	5.0± 0.2	4.2± 0.6	0±0	1±0	0±0	0±0	1±0	0±0	0±0	1±0	3.0± 1.0	33.5± 4.0	2148.0± 752.0	2.1± 1.4	10.5± 1.2
400	2794.0± 261.0	18.2 ± 3.0	11.5±2.5	7.5± 1.5	3.5± 1.0	0±0	0±0	2±1	2±0	0±0	1±0	0±0	0±0	4.5± 0.5	40.5± 9.0	2839.0± 270.0	1.2± 0.2	10.8± 1.7
500	2393.5± 59.50	16.5± 0.5	9.2 ±2.0	1.50± 1.50	2.1± 0.5	0±0	2±0	1±1	0±0	3±0	0±0	0±0	2±0	6.5± 1.5	35.5± 0.5	2429.0± 259.0	1.5± 0.2	22.8± 6.7

Each value represented Mean ± SEM, n=3 readings. There were various forms of chromosomal aberrations observed at each mitotic phase. There was reduction in mitotic index, increase in chromosomal aberration and this was concentration dependent.
Key: AP: Abnormal prophase, AM: Abnormal Metaphase , AA: Abnormal Anaphase, AT: Abnormal Telophase, SC: Sticky Chromosomes, VC: Vagrant Chromosomes, B: Bridges, CA: Chromosomal Aberation, TDC: Total Dividing Cells CM: C-Mitosis, TCC: Total Cell counted

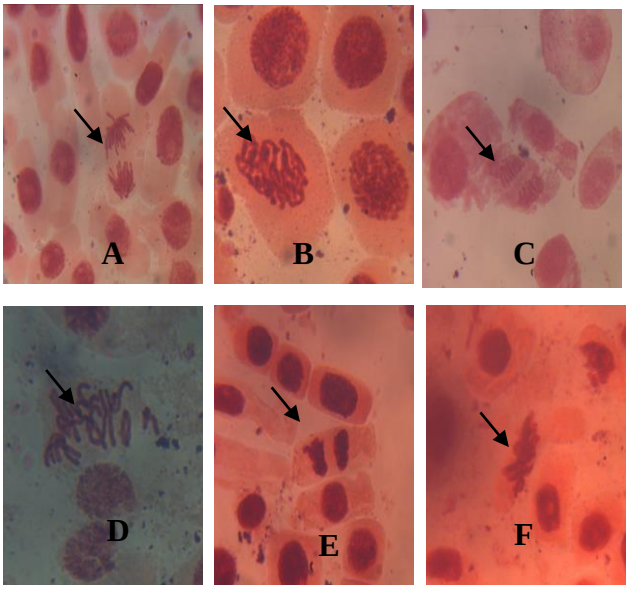

Plate 2. Photomicrograph Showing Chromosome Aberrations Observed in Cells of the Root of *A. cepa* Treated with Different Concentrations of ME for 48 h.

A (control) normal anaphase, **B** (100 µg/ml) normal prophase, **C** (200 µg/ml) sticky telophase, **D** (300 µg/ml) C-mitosis, **E** (400 µg/ml) clumped anaphase, **F** (500 µg/ml) Sticky chromosomes. Magnification: X 400

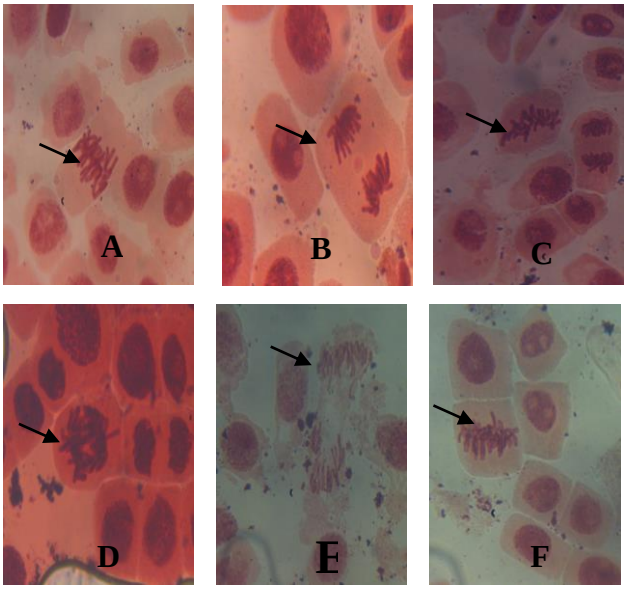

Plate 3. Photomicrograph Showing Chromosome Aberrations Observed in cells of the Root of *A. cepa* Treated with Different Concentrations of EAF for 48 h

A (control) normal metaphase, B (100 µg/ml) normal anaphase, C (200 µg/ml) sticky metaphase (left) and clumped anaphase (right), D (300 µg/ml) disturbed anaphase with chromosome bridge, E (400 µg/ml) multipolar anaphase, F (500 µg/ml) Sticky chromosomes. Magnification: X 400.

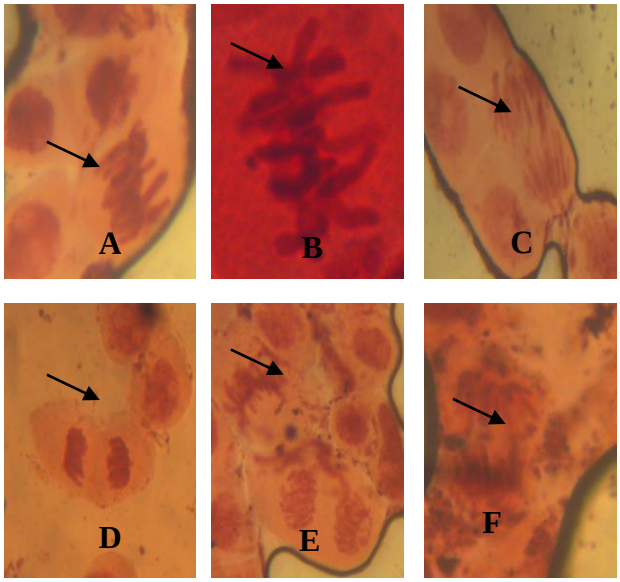

Plate 4. Photomicrograph Showing Chromosome Aberrations Observed in Cells of the Root of *A. cepa* Treated with Different Concentrations of AQE for 48 h

A (control) normal metaphase, B (100 µg/ml) normal metaphase, C (200 µg/ml) normal anaphase, D (300 µg/ml) clumped anaphase, E (400 µg/ml) Sticky metaphase, F (500 µg/ml) abnormal anaphase. Magnification: X 400

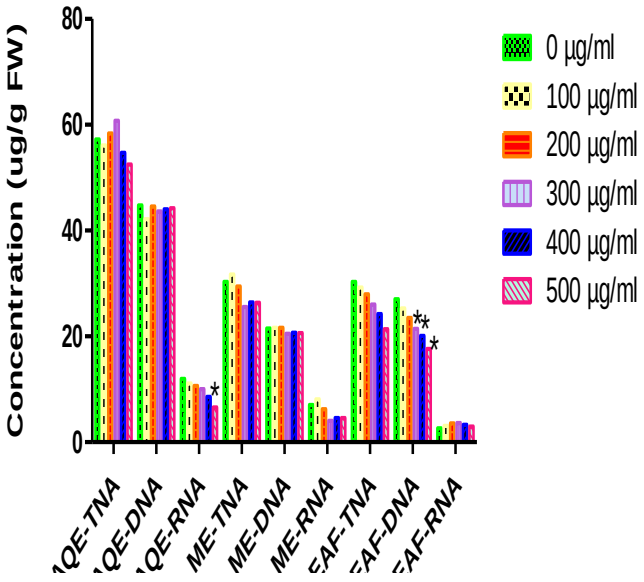

Figure 1. Levels of Nucleic Acid Concentrations of the Root of *A. cepa* exposed to Varying Concentrations of AQE, ME and EAF

Each value represents Mean ± SEM, n=3 readings. Value of $p < 0.05$ was considered statistically significant. The values with * were significant when compared with the control (0 µg/ml). TNA (total nucleic acid), DNA (deoxyribonucleic acid), RNA (ribonucleic acid), AQE = aqueous extract, ME = methanol extract, EAF ethyl acetate fraction, FW = Fresh Weight

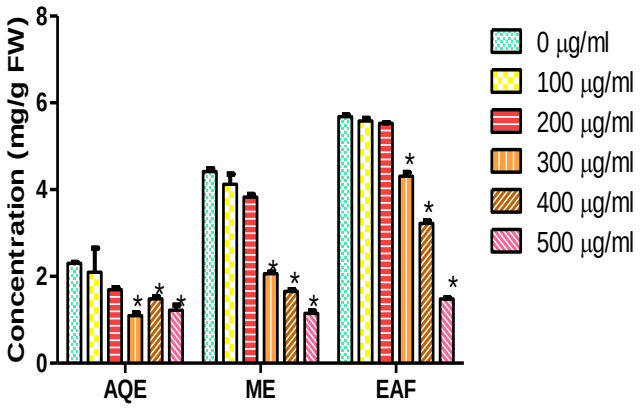

Figure 2. Concentrations of Soluble Protein of the Root of *A. cepa* exposed to Varying Concentrations of AQE, ME and EAF

Each value represents Mean ± SEM, n=3 readings. Value of $p < 0.05$ was considered statistically significant. The values with * were significant when compared with the control (0 µg/ml). AQE = aqueous extract, ME = methanol extract, EAF ethyl acetate fraction, FW = Fresh Weight

Discussion

The root growth inhibition assay is an established method for the detection and quantification of the cytotoxic and genotoxic potentials of various compounds including phytoconstituents of medicinal plants (Dragoeva *et al*., 2015). The root growth inhibition observed in this study may be due to the presence of growth inhibitory allelochemicals in the extract and might also be due to inhibition of cell division which is active at the tip of the root of *A. cepa* exposed to different concentration of the extract (Salam and Kato-Noguchi, 2010). This observation was in agreement with the observations of Iwalokun *et al.* (2011) on growth inhibitory effect of extracts of *Loranthus micranthus* on *A. cepa* root.

A. cepa assay enables the assessment of different genetic endpoints, which are mitotic index and chromosome aberration. Mitotic index is used as an indicator of cell proliferation biomarkers which measures the proportion of cells in the mitotic phase of the cell cycle (Jyrwa *et al.*, 2014). Hence, the decrease in the mitotic index of *A. cepa* meristematic cells could be interpreted as cellular death. The cells of *A. cepa* root tips after treatment with the extracts and fraction showed decreased mitotic index with increasing concentration.

The mitodepressive effect suggests that the extracts and fraction exhibited deleterious effects on cell division. This observation is in agreement with earlier studies (Firbas and Amon, 2013; Anjana and John, 2013; Jyrwa *et al*., 2014; Olorunfemi *et al*., 2015). The results also revealed increase in chromosomal aberration in the root tips of *A. cepa*.

The increase in chromosomal aberration might be due to the obstruction in the phases of cell division and the disruption of proper arrangement of chromosomes at each stage or phase of cell division. The abnormal conditions of the chromosomes of the cells of *A.* cepa after exposure to different concentrations of the extracts and fraction could be due to the presence of genotoxic agents in the plant extract. The abnormalities of chromosomes could be due to the blockage of DNA synthesis or inhibition of spindle formation or the obstruction of their biosynthesis (Fadoju *et al*., 2020). These chromosome aberrations might be due to the obstruction of the onset of prophase, the arrest of one or more mitotic phases, or the slowing of the rate of cell progression through mitosis (Khanna and Sharma, 2013).

The chromosome aberrations observed at all concentrations of the treatment in the present study were chromosome stickiness, bridges, c-mitosis and vagrant chromosomes. These aberrations might also due to the effect of the extract on the spindle formation and thus resulted in cell division disturbances. Chromosome bridges demonstrate signs of clastogenic effects caused by chromosome breaks, and vagrant chromosomes and c-metaphases, which increased the risk for aneuploidy (Olorunfemi *et al*., 2015). The remarkably observed stickiness in prophase and metaphase cells and the bridges in anaphase and telophase cells might result from improper folding of chromosome fibers which makes the chromatids connected by means of subchromatid bridges. Sticky chromosomes indicated a highly toxic, irreversible effect, probably leading to cell death. The bridges noticed in the cells were probably formed by breakage and fusion of chromatids or subchromatids (Dragoeva *et al*., 2015). Vagrant chromosomes that were not organized to a specific stage of the mitotic division might be due to unequal distribution of chromosomes with paired chromatids which resulted from nondisjunction of chromatids in anaphase (Firbas and Amon, 2013).

The reduction in DNA concentrations observed could be due to the deleterious effect elicited by the allelochemicals in the plant extract on the nuclear materials of *A. cepa* roots. This could be connected to growth inhibitory potential of the extract and fraction observe in this study. It has been widely documented that some plant secondary metabolites are known to block cell division, vesicle transport and microtubules (Chikezie *et al.*, 2015). The extract might destroy or disrupt the structure of nucleic acid thereby blocking cell division and elongation which in turn could affect the growth of the plant.

The reduction in nucleic acid concentrations could also be connected to the increase in chromosomal aberrations observed in the study.

The result of the study also revealed a decrease in total soluble protein concentrations of the roots of *A. cepa*. This reduction in protein concentrations could be due to the inhibition of protein biosynthesis caused by phytotoxic compounds in the extract and could be a reflection of reduction in nucleic acid concentrations. Proteins are essential macromolecules which perform important roles in growth, development and biochemical reactions in plant. It

also facilitates transportation of materials across cell membrane (Zagorchev *et al*., 2014).

CONCLUSION

The findings suggest that the mechanism by which the plant affects other plant is through the disruption of cell division and the extracts and fraction of the leaf of *D. metel* could be of benefit in development of natural herbicide. Further study can be done to identify and isolate the exact constituent responsible for cytotoxic and genotoxic potentials of the leaf of *D. metel*.

AUTHORS' CONTRIBUTIONS

Authors IJK and OOO design the work. Authors IJK, MOg and AOA did the benchwork, IJK, CAD and EAF analysed the data. Author MOz carried out photomicrograph and interpretation, IJK wrote the first draft of the manuscript. All the authors reviewed the manuscript.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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