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CYTOGENOTOXICITY OF AQUEOUS EXTRACTS OF LEAVES AND SEEDS OF GEGEMU (DATURA STRAMONIUM) ON ALLIUM CEPA

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ABSTRACT
Cytogenotoxicity effects of aqueous extract of Datura stramonium leaves and seeds were investigated on root cells of Allium cepa by employing Allium cepa assay. A. cepa roots were treated with the aqueous extracts of both the leaves and seeds at five different concentrations (12.5, 25, 50, 75, and 100 p.p.m) each for 72h. Exposure of aqueous extracts of both the leaves and seeds increased mitotic index, and total chromosomal aberrations by the Allium test. While chromosome laggards, stickiness, disturbed anaphase–telophase and anaphase bridges were observed in anaphase–telophase cells, c-metaphase and binuclear cells were observed in other cells. These results indicate that aqueous extracts of both the leaves and seeds exhibits genotoxic activity in A. cepa root meristematic cells.
KEY WORDS: *cytogenotoxicity, Datura, mitotic index, aberrations*

INTRODUCTION

Datura stramonium is a wild growing flowering plant which belongs to the family Solanaceae which includes some 2,400 species in total. According to Bonde (1997) the genus *Datura* contains about ten different herbaceous species and the most important ones are *D. stramonium, D. anoxia,* and *D. ceratocaula*. However, *D. anoxia* and *D. stramonium* are the most important drug plants that are found in wide distribution and are often found in foodstuff causing most incidences in humans (Soni *et al*., 2012). It is commonly called 'Jimson weed' or 'Thorn apple' but locally called 'Gegemu' by the South western people of Nigeria and generally referred to as 'troublesome weed'. *D. stramonium* is a medicinal plant with high antinociceptive, antioxidant, anti-inflammatory, anti-rheumatoid and hypoglycemic properties (Abdollahi *et al*., 2003; Ahmad *et al*., 2009).

Datura is an annual herb (occasionally short-lived perennial) that attains a height up to 2 m. This herb has 10- to 20-cm-long and 5- to 18-cm-wide lobed alternate leaves. The other distinguishing feature is the erect 5- to 20-cm-long trumpet-shaped white to pale purple flowers. The 4- to 10-cm-long and 2- to 6-cm-broad fruit of *Datura* has a spiny capsule, hence the name thorn apple. The ripe fruit splits open, dispersing numerous kidney-shaped seeds over pastures, fields, or wastelands (Kachan & Atreya 2016). Datura (devil's trumpet) has structural resemblance to the genus *Brugmansia* (angel's trumpet). Taxonomically distinct woody shrubs *Brugmansia*

(formerly included in *Datura*) are identified by their pendulous flowers and the absence of spines on the fruit capsule (Kachan & Atreya 2016).

The poisonous incidence as well as medicinal uses of *D. stramonium* has been reported (Ahmad *et al*. 2009; Devi *et al*., 2011) among adolescents. Consumption of any part of *D. stramonium*, may result in severe anticholinergic reaction that may as well lead to toxicities and often times make diagnosis very difficult (Devi *et al*., 2011; Soni *et al*., 2012; Tranca *et al*., 2017). Amongst its medicinal uses are for the treatment of inflammation, stimulation of the central nervous system (CNS), respiratory decongestion, treatment of dental and toothache as well as alopecia (Devi *et al*., 2011; Al-Snafi 2017). According to Adekomi *et al*. (2011), the alkaloid content of *D. stramonium* has been emphasized by the phytochemical investigators dealing with the biochemical composition of various parts of the plant. Atropine, hyoscyamine and scopolamine (hyoscine) are the tropane alkaloids of all species of the genus *Datura* and their concentrations showed variations depending on species and on the part of the plant. Ahmad *et al* (2009) reported that treatment of aqueous leaves extracts of *D. stramonium* on different human cancer cell lines resulted in significant reduction in cell survival at 24 and 48 hrs of exposure including increase in redox sensitive enzymes an indication of high oxidative stress induction by the extract.

This study is aimed at determining and comparing the cytogenotoxic effects of both the leaves and seed aqueous extracts of *D. stramonium*.

MATERIALS AND METHODS

Plant collection. The flowers of *D. stramonium* were collected from a home garden located in Agiliti community of Mile 12 Lagos, Kosofe Local Government Area of Lagos state with and GPS (geographic position system) reading recorded.

Preparation of the aqueous extracts of *D. stramonium* leaves and seeds. Plant material (leaves and seeds) was dried at room temperature in the dark and ground finely using blender. The powder was placed in small plastic bags (100 g each) and stored at 4˚C until use. Weighted dried ground leaves and seeds were boiled in distilled water for 10 min and, cooled to room temperature for 20 min. Thereafter, the extract was filtered through a filter paper to remove particulate matter. Stock solution was diluted with distilled water to 1.0. 2.5, 5.0, 10.0, 25.0, 50.0 and 100mg/ ml concentrations for both seeds and leaves and tap water were used as positive control. The concentrations were prepared by serial dilution.

***Allium cepa* test.** Onions bulbs were commercially obtained from a local supermarket. Before use, they were dried and the dried outer scales of the bulbs were removed away without destroying the root primordial. These were used for the bioassay according to standard procedures. For the root growth inhibition, three concentrations of each extracts, viz: 1.0. 2.5, 5.0, 10.0, 25.0, 50.0 and 100mg/ ml were considered for both seeds and leaves. A series of three bulbs were placed in tap water

for 48h to germinate at room temperature 25°C for each concentration of each extract and the control (tap water). After the newly roots (1-2cm in length) were emerged, then onion roots were treated with the roots extracts for 24h, 72h. After end of 24h, several of root tips were then cut from each bulb for chromosomal analysis. Then the bulbs were returned to water for a recovery period of 24 hours. So, Roots were collected three times from each bulb: before treatment (control), after 24h of treatment in aqueous solution (treatment) and after 24h of recovery in water (recovery). In order to study cytogenotoxic effect, after 24h of exposure for each treatment, several root tips were removed from each concentration, fixed in 3:1 (v/v) ethanol: glacial acetic acid and stored overnight at 4◦C. The next day they were placed in 70% (v/v) aqueous alcohol and refrigerated until used. An average of five slides was made for each bulb using five root tips which hydrolyzed in 1N hydrochloric acid (HCl) for 3 min and microscope slides were prepared by squashing the stained root tips in 0.5% (w/v) toluidine blue. Five slides were prepared per treatment and control, and each slide was examined using Olympus BX51 at a total magnification of 40×10. The following parameters such as the mitotic index (the ratio between the number of mitotic cells and the total number of cells scored and expressed as percentage) and cytogenetic effects were scored in interphase cells per 6000 cells were used for determination of cytotoxicity and genotoxicity.The effect of each sample on the morphology of growing roots was also examined.

Maceration of the root tips and preparation for microscopy. After the exposition, the plant with the poorest root growth was excluded. Two onion bulbs were utilized from each water samples for chromosomal preparation. About 5 root tips per plants were cut using forceps at a length of 10 mm and placed into a petri-dish with 2 ml acetic acid and Hydrochloric acid solution. The roots tips were then heated for 5 minutes at 50^0C. Hereby, the root cells become fixated and macerated. The heated root tips were placed on a petri-dish saturated with aceto-orcein solution for staining procedure and takes about 5-10 minutes. Thereafter, the saturated root tips were removed and placed on glass slides covered with a covers lip. The root tips were then squashed by pressing slightly down with a thumb and ready for microscopy.

Statistical analyses. Data obtained were subjected to statistical package for social science (SPSS) version 22.0.

RESULTS AND DISCUSSIONS

Effect of *Datura* treatment on *A. cepa* root cell proliferation. Mitotic index (MI) varies with concentrations of *Datura* seed and leaf extract with duration in the study. At 24 hr, mitotic index was higher in 1%, 2.5% and 50% treatment concentrations of the leaf than the seed. Seed treatment concentrations of 25% and 100% show a higher mitotic index than those leaf concentrations. However, there was

no difference in mitotic index of both seed and leaf treatments at 5% and 10% concentrations (Figure 1).

At 48 hr, there was a gradual increase in mitotic index with increase in *Datura* leaf treatment concentrations between 1% and 10%, then a gradual decline between 10% and 100% of *Datura* leaf treatment. Mitotic index was highest in 10% leaf treatment. Mitotic index observed in *Datura* seed treatments did not show any pattern with increasing treatment concentrations. The study observed that *Datura* leaf extracts had higher mitotic index than seed treatments at 1%, 5% and 10% concentrations while vice versa for 2.5%, 25%, 50% and 100% treatment concentrations (Figure 2)

At 72 hr, mitotic index was highest in 10% treatment of *Datura* seed. there was a gradual increase in mitotic index with increase in *Datura* leaf treatment concentrations between2.5% and 10%, then a gradual decline between 10% and 100% of *Datura* leaf treatment. Mitotic index was highest in 10% leaf treatment. Mitotic index observed in *Datura* seed treatments did not show any pattern with increasing treatment concentrations. The study observed that *Datura* leaf extracts had higher mitotic index than seed treatments in all treatment concentrations evaluated (Figure 3).

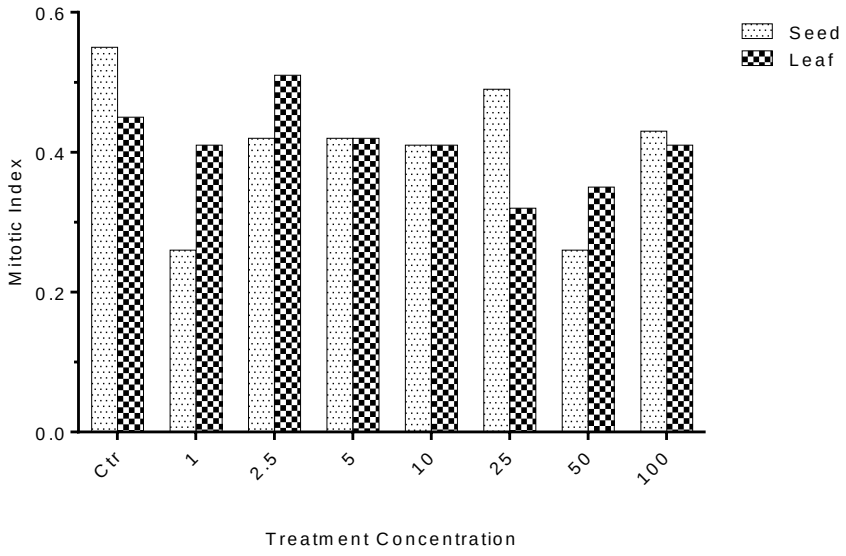

FIGURE 1. Mitotic index comparison between seed and leaf of *Datura* at 24 hr

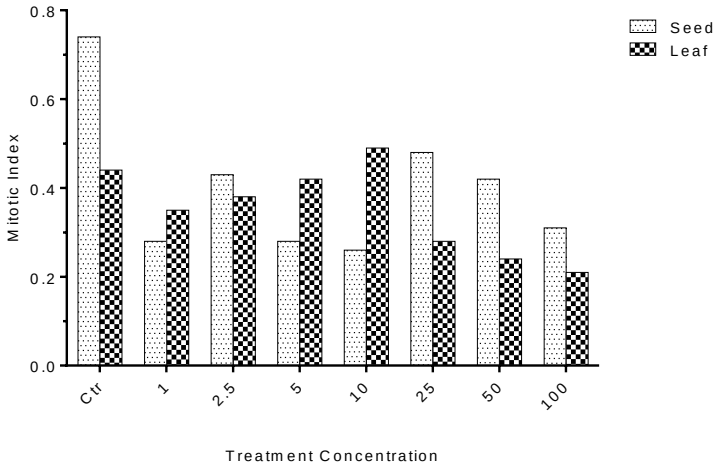

FIGURE 2. Mitotic index comparison between seed and leaf of *Datur*a at 48 hr

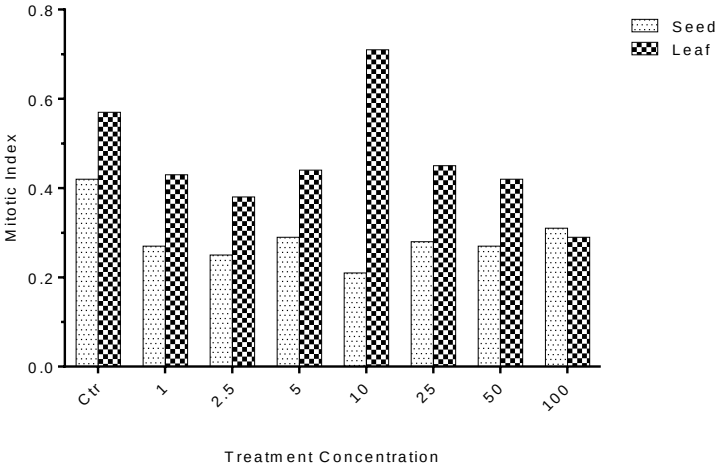

FIGURE 3. Mitotic index comparison between seed and leaf of *Datura* at 72 hr

Evaluation of aberrations with *Datura* treatments. At 24 hr, there was a gradual increase in percentage aberration with increase *Datura* leaf concentration between 1% and 25% while percentage aberration increase with increase *Datura* seed concentration between 2.5-10% and 25-100%. The study also observed that percentage aberration was higher in *Datura* seed treatment concentrations than treatments with

Datura leaf, with *Datura* seed treatment at 100% having the highest percentage aberration (figure 4).

The study also observed that percentage aberration was higher in Datura leaf treatment concentrations than treatments with *Datura* seed, with *Datura* leaf treatment at 50% having the highest percentage aberration at 48 hr after treatment (Figure 5). A gradual increase in percentage aberration was observed with *Datura* leaf treatment between 2.5% and 10% while a decline in percentage aberration was observed with *Datura* seed treatment between 10% and 50% (Figure 5).

At 72 hr, percentage aberration was higher in treatments with *Datura* leaf than treatments with *Datura* seed for all concentrations evaluated except at 100% treatment concentration where *Datura* seed treatment has more percentage aberrations than treatment with *Datura* leaf at 100% concentration. There was a gradual increase in percentage aberration with increasing *Datura* seed concentration between 1 – 5% and 10 – 100% (Figure 6).

Allium cepa test has been recognized as an excellent *in vivo* model for cytogenotoxicity tests due to the fact that the roots have direct contact with the substance of interest (Ciobanu, 2019; Datcu *et al,* 2020). Different structural and numerical chromosomal alterations are important for adequate and better understanding of the actions of any substance. *A.cepa* test ranks among the few tests with direct approach for measuring impairment and subsequent evaluation of the effects of the damages from potential mutagens, and carcinogens in systems that are exposed to the said damages. In this study analysis of mitotic index, types of chromosomal aberrations and their frequencies were examined in order to establish the cytogenotoxic effects of aqueous extracts of seeds and leaves of *D. stramonium*. The mitodepressive effects of both aqueous extracts were obvious irrespective of the concentration of aqueous solutions used for immersions of adventitious roots formed by the onion bulbs.

The results above showed that all concentrations (high to low) of both aqueous leaves and seeds extracts of *D. stramonium* used in the present study induced important abnormalities of *A.cepa* during divisions when compared to control and between leaves and seeds tested.

The study observed an increase in root length in all concentration of both aqueous extracts of leaves and seeds of *D. stramonium* and corresponding increase in aberrations indicating the cytogenotoxic effects that *D. stramonium* exhibited on the *A.cepa* roots at high concentration. Strikingly there were similarities between the types of aberration observed in both aqueous leaves and seed extracts from *D. stramonium* treatment. Similar results were obtained with root length inhibitions (mitotic index). Furthermore, there were also an increase in mitotic index which is an indication of probable uncontrolled proliferation of cells; a precursor to tumor development. The

inhibitions observed could also be as a result of intracellular stress, including DNA damage which prevents cells from entering into mitosis.

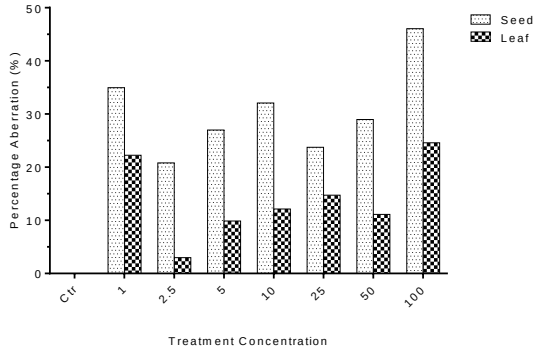

FIGURE 4. Percentage aberration comparison between seed and leaf of *Datura* at 24 hr

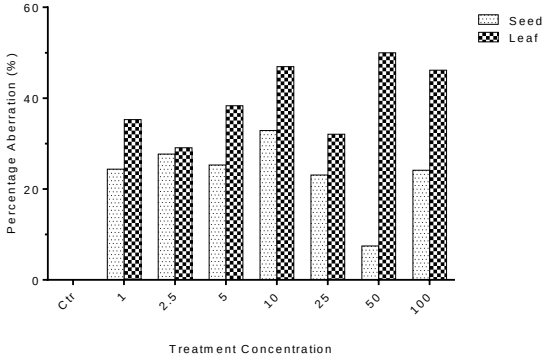

FIGURE 5. Percentage aberration comparison between seed and leaf of *Datura* at 48 hr

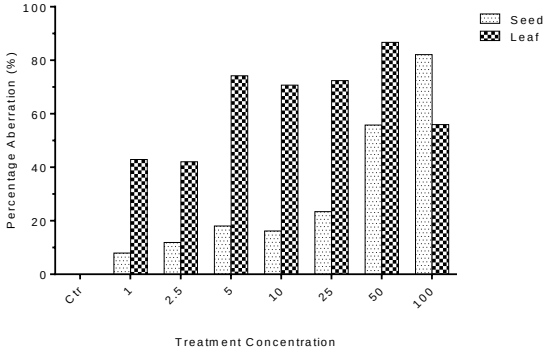

FIGURE 6. Percentage aberration comparison between seed and leaf of *Datura* at 72 hr

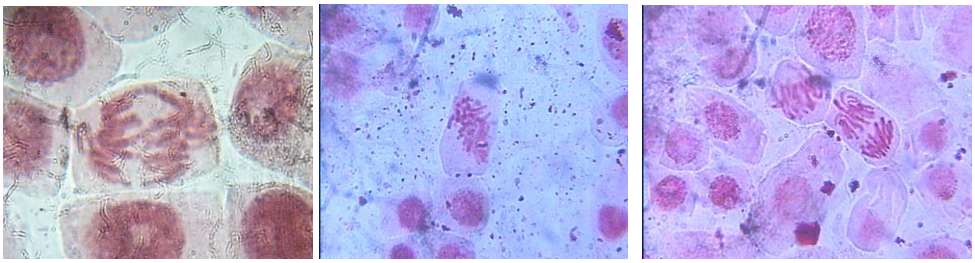

FIGURE 7. Aberrations observed in *Allium cepa* meristematic cells exposed to aqueous extracts of *Datura* seeds and leaves. (a) Anaphase with chromosome bridge (b) sticky chromosomes at metaphase; (c) sticky anaphase Magnification 1000.

Despite the pharmacological and traditional uses of the plant, *D. stramonium* poisoning had been reported, however, its anti-mitotic effects could be exploited in the therapeutic treatment of cancer. A dose of 0.05-0.10g of *D stramonium* was reportedly used for cure of cancer. On a contrary the aberrations observed in these studies also could further lead to other form of ailment when the above recommended dose is exceeded. The results obtained in this study are similar to the conclusion of the author with regards to the safety of *D. stramonium* at very low concentration. With respect to aqueous leaves and seed extracts we obtained an effective concentration (EC_{50}) of 6.68% concentration. According to Prasad & Sudedi (2013), *D. stramonium* is generally administered at a dose of 60to 185 mg powder for leaf and 60 to 120 mg powder for seed.

The varieties of alkaloids including atropine, hyoscamine and scopolamine present in both leaves and seeds aqueous extracts including other sixty four alkaloids detected could be employed for the treatment of cancer and ageing. Kaymack & Goc-Rasgele (2009) and Radic *et al.* (2010) affirmed that MI is considered to reliably identify the presence of toxicants in food and drink consumables as well as in the environment. There were similarities in the MI and the aberrations of both leaves and seed extracts from *D. stramonium* observed herein. Several studies have also reported inhibition of mitosis by heavy metals, and alkaloids present in medicinal and other food substances. Therefore, this mechanism could be an appropriate explanation for the inhibitory effects of the tested aqueous leaves and seed extracts of *D. stramonium* on *A.cepa* plant system in the study. In the, aqueous leaves and seed extracts of *D. stramonium,* a strong positive correlation between the mean root length and the time of exposure which implies that the highest effect was observed at 72hours and the toxic elements in the aqueous extracts were the causative agents for the reduction in root growth.

CONCLUSIONS

Atropine and anticholinergic compounds present in *D. stramonium* has been found to be useful remedy in ancient traditional medicine practices. Results from the above observation it is evident that ingestion of high concentration of *D. stramonium* are potential genotoxic agents for human consumption. The abnormalities manifested may be due to the action of variety of toxic tropane alkaloids such as atropine, hyoscamine, and scopolamine present in the extracts that can also cause cytological damages.

Almost all the parts of *D. stramonium* are reported to have toxic effects, and the toxicity of this plant is mainly due to the tropane alkaloids. Each part varies in the concentrations of alkaloids and other active substances. For this reason, it is very important for individuals, especially young people, to be aware of the toxicity and potential risks associated with the "recreational" use of this plant. *D. stramonium* in the form of a paste or solution of very low concentration as that reported in this and other studies to relieve local pain may not have a deleterious effect; however, oral and systemic administration of the *D. stramonium* may lead to severe anticholinergic symptoms. Various cases of toxic delirium and psychiatric symptoms have been reported after ingestion of *D. stramonium* indicating the necessity of extreme precaution while using this plant. The results from the study established that high concentration of aqueous extracts of *D. stramonium* are connected with high personal and public health risks which may result from direct ingestion or smoking of the tropine alkaloids. Both aqueous extracts were found to inhibit root growth proliferation and induced cytogenotoxicity at the chromosomal level in *A.cepa*.

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