

Cytotoxic and Genotoxic Potentials of *Datura innoxia* Leaves Aqueous Extract on *Allium cepa* Meristemic Cells



Marwa M. El Omami¹, Suliman M. Hussein², Muna Salem M. Solyman³, Abeer H. Amer^{4*}, Fauzia R. El-Garabulli⁵

¹Zoology Department, Faculty of Science, Benghazi University, Benghazi, Libya

²Botany Department, Faculty of Science, Benghazi University, Benghazi, Libya

³Department of Molecular Diagnostic, Faculty of Biomedical Sciences, University of Benghazi, Benghazi, Libya.

⁴ Department of Histology, Faculty of Medicine, University of Benghazi, Benghazi, Libya

⁵ Genetic Department, Faculty of Science, Misurata University, Misurata, Libya

ABSTRACT: Medicinal plants are still vitally important in the preparation of pharmaceutical products. Plants have been used both for their prophylactic effects and for the treatment of illnesses and diseases. According to the World Health Organization (WHO) about 80% of developing countries use traditional folk medicines, 85% of which are plant extracts. *Datura* plant was used medicinally over the world in historical times for treatment of colds, nervous conditions, and as ointment for cuts or wounds and to relieve pain during the setting of bones. The present study aimed to investigate the mutagenic potential of *Datura innoxia* water extract using *Allium cepa* root tip cells as biological systems they were used to find out the effect of *Datura* extract on chromosomal structure and behavior during cell division. Therefore, the mitotic index and chromosome aberration in treated cells were recorded. The root meristem cells were treated with different concentrations of *D. innoxia* at different times, cytological analysis revealed significant depression in cell division, this was seen noticeably in all used concentrations especially at high ones. The interaction of *D. innoxia* extract with the genetic material led to DNA damage and decreased cell number depending concentration and time.

KEYWORDS: *D. innoxia*, mitotic index, chromosome aberration, DNA damage

INTRODUCTION

Today, plants continue to be a major source of medicines as they have been throughout human history. Medicinal plants contain one or more, endogenous substances which can be used directly for medicinal purposes or in semisynthesis of preparation. The medicinal plants contain a large kinds of natural chemical products, these chemicals exhibit diverse biological activities including antimutagenic, anticarcinogenic, antitumor, antimicrobial and others. However, natural chemicals of such activities are alkaloids, glycosides, steroids saponins and flavonoids insecticidal. (Gocze, 2000; Gananamani *et al*, 2004; Uzun *et al*, 2004 and Pramod *et al* 2009).

Medicinal plants, species of the genus *Datura*, family solanaceae, were popularly used in folk medicine to relief pain and for treated of skin disease, mental disease and organophosphorus toxicity (Abnea *et al*, 2003; Kuete, 2014). *Datura* leaves were smoked as cigarettes to relives asthma. (Hussen, 1985). *Datura* plant was used medicinally over the world in historical times; the Chinese used *Datura* to treat colds and nervous conditions. Whereas, Zuni Indians and Mexican people used *Datura* as ointment for cuts or wounds and to relieve pain during the setting of bones. Plants produce variety of substances that can induce alterations in genetic materials. Therefore, toxic compounds in these plants may favor mutational events in somatic or germ cells, such events possibly leading to developing of diseases or teratogenic and carcinogenic effects. Accordingly, some substances produced by medicinal plants have been studied, but insufficient toxicological, genotoxicological studies have been done, although it is very reasonable to direct the focus on possible genotoxic, cytological effects of those plants which used therapeutically. This paper aimed to investigate the cytological and the mutagenic potentialities of *Datura innoxia* leaves aqueous crude extract using *Allium*

Cytotoxic and Genotoxic Potentials of *Datura innoxia* Leaves Aqueous Extract on *Allium cepa* Meristemic Cells

cepa root tip cells to underline the mechanism of its genotoxicity action, and to find out the effect of *Datura* extract on chromosomal structure and behavior during cell division.

MATERIALS AND METHODS

Experimental Models

Datura innoxia Plants

D. innoxia leaves were collected from botany area and in waste area nearby Benghazi city.

Onion seeds

Dry healthy onion seeds (*Allium cepa* –Texas Grano 502 PRR, Enkhuizen, Holand).

Preparation of the Extract

Normally looking leaves were collected, washed, dried. 20 g of *D. innoxia* tiny cuts was added to 200 ml distilled water in 500 ml flask, the content of the flask was shaken and left at room temperature overnight and filtrated with Whatman No.1 filter paper. The filtrate was, then reduced in volume by mean of thin film evaporator (Gallen Kamp, Germany) with water bath temperature within a range of 40-45°C. The aqueous crude extract was transferred in to a stopped glass container and its volume was adjusted to 20ml before stored in freezer at -18°C. At this step, any specific volume of the extract represented a crude aqueous extract of a specific weight of the dry leaves. The water extract now is ready to be used as a stock solution and four different concentrations (0.1, 0.01, 0.001, and 0.0001mg/ml) were prepared by serial dilution method (El-torki *et al*, 2005 and Iranbakhsh *et al*, 2010).

Procedure of Plant Chromosomes Technique

The preparation of plant chromosome was done as per standard methods described in (Grant, 1982 and Soltan & Ata 2008).

Onion Seeds Germination

Dry *Allium cepa* seeds were grown on wet filter paper in Petri dishes and distilled water was added. Germination was carried out at constant temperature of 20±2°C in darkness for 2-3 days. When the primary root tips are 0.5-1.5 cm long, they were treated with different concentrations of *D. innoxia* water extract (0.1, 0.01, 0.001 and 0.0001 mg/ml) for 4, 12, 24 and 48 hours, while control roots were treated with distilled water only. Nine root tips were examined for each treatment.

Fixation and Slide Preparation

For cytological preparation, the root tips were fixed in 95% ethanol / glacial acetic acid 3:1 (v/v) for 1-24 hrs. (root tips can be stored in 70% ethanol in the refrigerator until the procedure resumed). Slides were prepared using the aceto-orcein squash technique, by hydrolyzing the root tips in 1N HCL at 60C for 12 min and stained with aceto orcein stain for 45min. The meristematic region of the root was removed and squashed with flattened glass rod in 45% acetic acid and mounted with cover slip and temporary sealed with clear finger nail polish. Slides from each treatment and control were examined by light microscope under 40x and 100x (oil immersion lens) to find out the mitotic aberrations, then photographed by install video camera on the microscope and transfer the picture to the computer. The mitotic index MI was calculated as the percentage of dividing cells to the total number of cells examined, and the same slides were analyzed for the percentage and type of the chromosomal abnormalities in dividing cells.

RESULTS

1-The effect of *D. innoxia* leaves aqueous extract on Mitotic Index of *A. cepa* meristematic cells.

The obtained data in **table (1)** and **figure (1)**, showed highly significant differences within treatments and with the control ($p < 0.001$). In short hours' treatments (4 hours), the mitotic index values were concentration depending, its values were decreased (6.923, 4.726, 3.764 and 2.423 %) as concentrations of *D. innoxia* extract were increased (0.0001, 0.001, 0.01 and 0.1 mg/ml, respectively), comparing to the control which was 8.668 %. After 12-hour exposure time, MI which increased by decrease the concentration at same time (12 hours), they were 0.755, 3.05, 4.374 and 5.55 % in samples treated with 0.1, 0.01, 0.001 and 0.0001 mg/ml respectively where the control was (8.922 %).

At 24 hours, there was more cell death and MI value was 0.32% at highest concentration. After 48-hours, a slightly increase in MI values was observed in cells treated with the low concentration (0.0001) to 5.123 % then decreased by increasing concentration. Then strongly decreased to 0% at high concentration (0.1), compared with control (6.916 %). The statistical analysis of variance test showed that there were highly significant effects of concentrations and times ($p < 0.001$). There were also significant differences in effect of concentrations with times ($p < 0.05$). Least Significant Differences (LSD) test showed there were highly significant differences ($p < 0.001$) between the concentrations including the control. There were also high significant differences between the effect of 4-hours treatment with all other treatments ($p < 0.01$), and between 48-hours and other times of treatments. **Fig. (2)**, showed there is an interaction between the time and concentrations (0.01 and 0.001 mg/ml).

Cytotoxic and Genotoxic Potentials of *Datura innoxia* Leaves Aqueous Extract on *Allium cepa* Meristemic Cells

2-The effect of *D. innoxia* water extract on the mitotic aberrations.

The resulted data were presented in **table (2)** and illustrated in **figure (3)**. All treatments induced highly significant ($p < 0.001$) percentage of abnormal dividing cells compared to the control. Its values increased with increase of concentration and time of treatment. They increased from 28.603 (0.0001mg/ml at 4 hrs. treatment) to 51.795 and 45.736 (0.1 at 12 hrs. and 24 hrs. respectively). At control treatment samples no abnormal cells were observed at all applied times. At 48 hrs. showed, a decline in cell abnormalities to zero was observed at 48 hr. treatment as the result of cell death or inactivation (no mitotic cells).

Types of chromosomal abnormalities induced by *D. innoxia* were classified into physiological and clastogenic type of aberration. The first class of abnormalities such as early condensation chromosome in prophase, lagging chromosome in metaphase, anaphase and telophase and binucleate cells. The second class includes a chromosomal fragmentation and bridges in anaphase and telophase and micronuclei formation (**Fig. 4**).

The physiological type of aberration was the dominant type of mitotic cell abnormality in all treated samples at short and long treated time. The maximum percentage value is (88.04%) was observed in samples treated with 0.1 mg/ml for 12 hrs. exposure time, Clastogenic type occur in all treated samples with lower level than physiological type of aberration, its maximum value was observed at 0.001mg/ml for 48 hours' treatment and the lowest value was recorded at 0.1 mg/ml for 48 hours also. The total percentages of abnormalities were decreased by increase the time of treatment and decrease *D. innoxia* extract concentration.

Statistical analysis of variance showed highly significant effects of concentration, times and concentration with times ($p < 0.001$). However, LSD test showed highly significant differences between the effect of 0.01mg/ml with other concentrations, and between concentrations 0.001mg/ml with 0.0001mg/ml. LSD test also showed highly significant differences between the long time treatment (48 hrs) with all times that were applied, and no significant different between the 4 and 12 hours of treatments. **Figure (5)** showed there is an interaction between the time and concentrations (0.01 ,0.001 and 0.000).

Table 1: Mitotic Index (MI) in *A. cepa* root tip cells treated with different concentrations of *D. innoxia* leaves aqueous extract for different exposure times.

Concentration mg/ml	4 hours			12 hours			24 hours			48 hours		
	T.C.E	M.C	M.I±SD	T.C.E	M.C	M.I±SD	T.C.E	M.C.	M.I±SD	T.C.E	M.C.	M.I±SD
C	6058	525	8.666 (0.471 6)	7157	641	8.955 (0.334 3)	7090	638	9.012 (0.3075)	7070	490	6.93 (0.624)
0.1	4427	100	2.423 (0.560 4)	6832	55	0.805 (0.429 1)	4637	15	0.323 (0.5007)	0	0	0
0.01	7598	286	3.764 (0.325 3)	8351	270	3.233 (0.717 2)	12183	534	4.383 (0.5259)	11420	406	3.555 (0.9 825)
0.001	7887	378	4.805 (0.87)	8766	388	4.425 (0.071 3)	10033	415	4.136 (0.9796)	9933	398	4.006 (0.719 2)
0.0001	7763	533	6.865 (0.805 3)	10283	576	5.601 (0.746 5)	10866	279	4.845 (0.5457)	11066	569	5.141 (0.597)

Cytotoxic and Genotoxic Potentials of *Datura innoxia* Leaves Aqueous Extract on *Allium cepa* Meristemic Cells

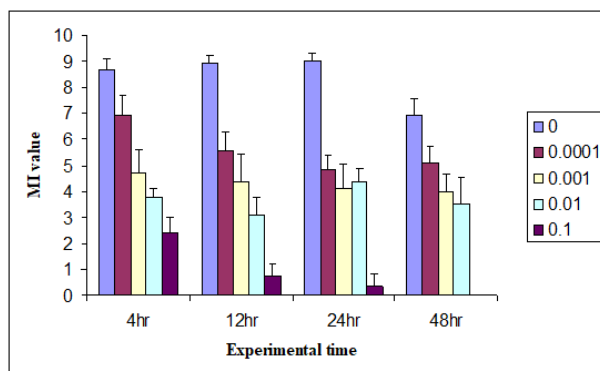


Figure (1): Mitotic index (M.I) in *Allium cepa* root tip cells treated with different concentrations.

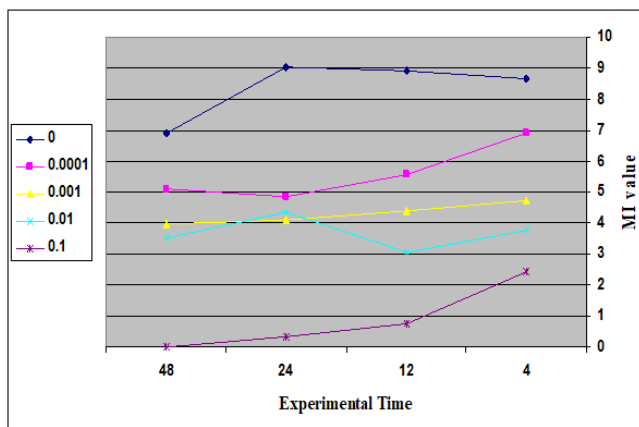


Figure (2): Mitotic index (MI) considering time and concentration interaction

Table 2: Types and percentage of abnormalities in *A. cepa* root tip cells treated with different concentrations of *D. innoxia* leaves aqueous extract for different exposure times.

Concentration mg/ml	4 hours			12 hours			24 hours			48 hours		
	(Sd) AB%	AB Type (%)		(Sd) AB%	AB Type (%)		(Sd) AB%	AB Type (%)		(Sd) AB%	AB Type (%)	
		P	Clst		Ph	Clst		Ph	Clst		Ph	Cl
		hys.	og.		ys.	og.		ys.	og.		ys.	stog.
C	0	0	0	0	0	0	0	0	0	0	0	0
0.0001	28.60 (0.368)	81.7 13	18. 287	29.443 (2.06)	76.719 23.	23. 281	22.266 (1.076)	71.502 28.498	28.498	30.026 (0.317)	73.743	26.25 7
0.001	33.62 (1.836)	74.5 61	25. 439	30.714 (1.88)	78.282	21.718	27.29 (2.113)	74.90 25.10	25.10	28.971 (0.67)	67.419	32.58 1
0.01	40.392 (2.862)	69.3 84	30.616	28.147 (4.464)	81.402	18.598	26.65 (1.296)	73.424 26.576	26.576	28.214 (7.747)	79.134	20.86 6
0.1	38.603 (5.795)	81.5 82	18.148	51.759 (15.345)	88.041	11.959	45.736 (7.384)	60.416 39.584	39.584	0	0	0

AB: Abnormality AB%: Percentage of abnormalities Pys.: physiological Clastog.: clastogenic C: control

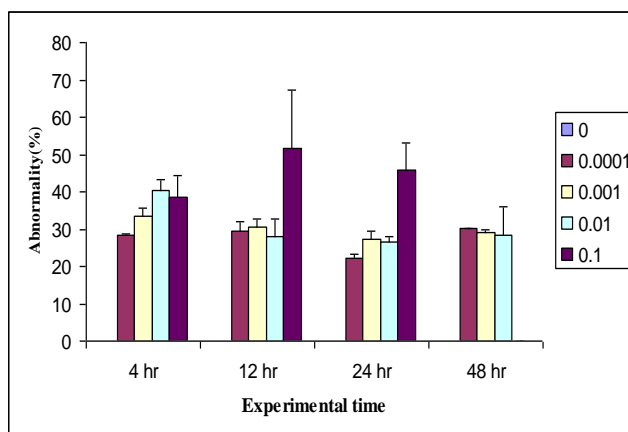


Figure (3): Percentage of abnormalities (AB%) in *Allium cepa* root tip cells treated with different concentrations of *D. innoxia* water extract for different exposure times.

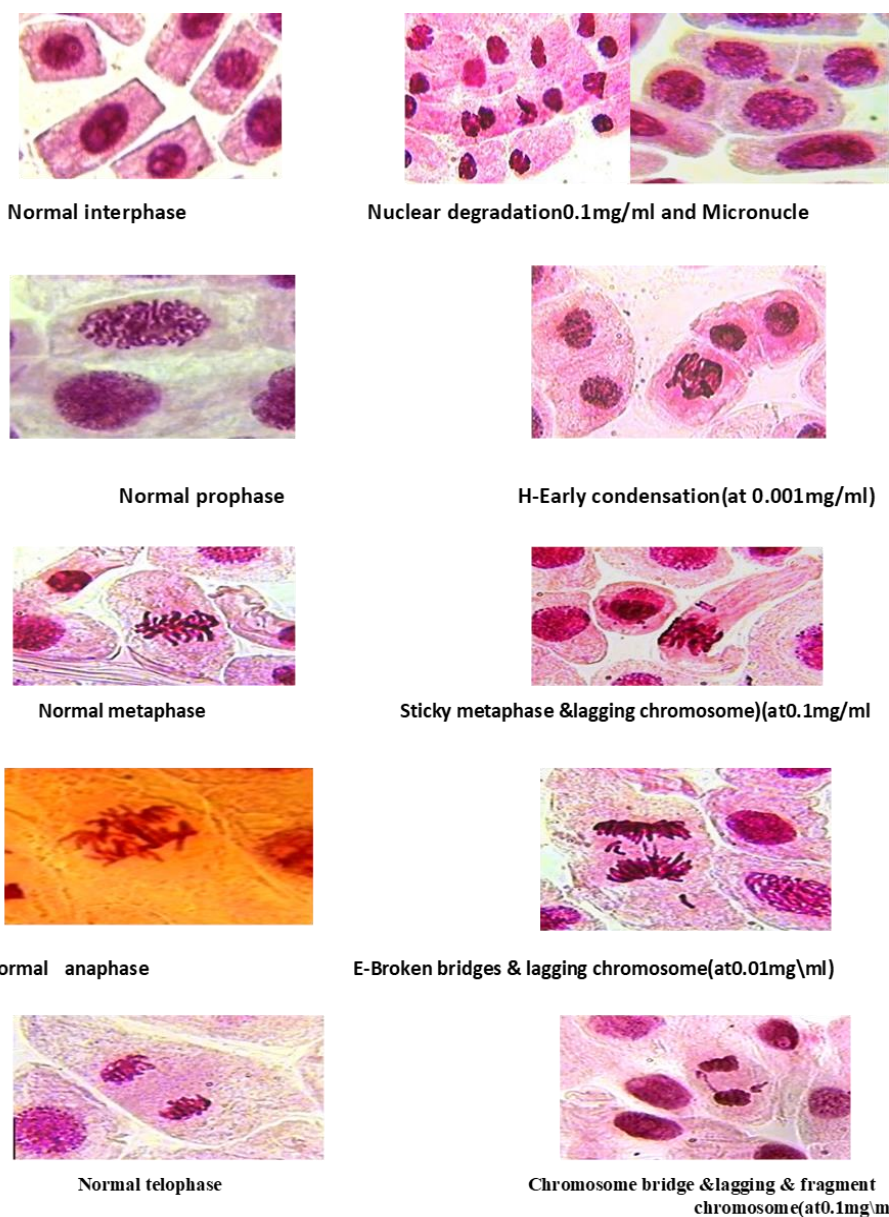


Figure (4): Abnormal mitotic phases of *A. cepa* root tip cells after different treatments of *Datura innoxia* water extract comparing to normal cells.

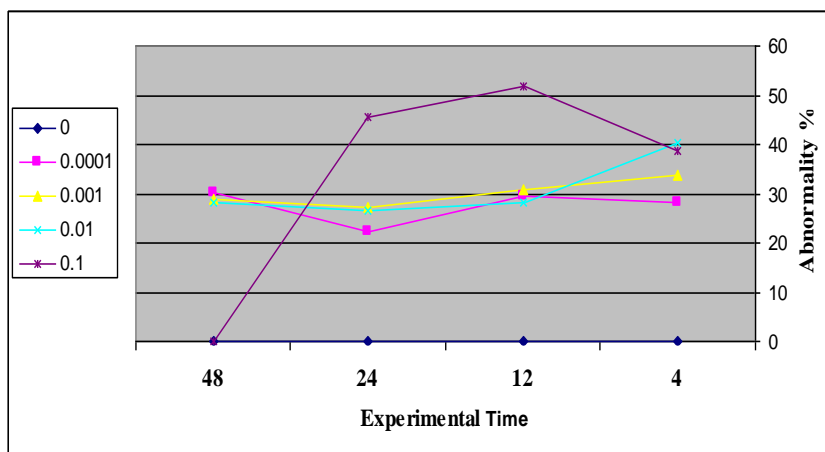


Figure (5): Percentage of abnormalities according to time concentration interaction

DISCUSSION

1- Effect of *D. innoxia* Leaves Aqueous Extract on Mitotic Index of *Allium cepa* Meristemic Cell

The data revealed that the mitodepressive effect of *D.innoxia* water was concentration and time dependent. The inhibition of cell division was observed in *Allium cepa* root tips treated with highest concentration (0.1 mg/ml) at (4, 12, 24 and 48 hr). However, the same effect was observed in samples treated with (0.01, 0.001 and 0.0001) at (12, 24 and 48 hours) that was attributed to a number of factors interrupted the cell cycle. and may be due to interference of *D. innoxia* ingredients in the normal process of mitosis by reducing the number of dividing cells (Shobana, & Nandhin, 2022 and Carballo et al; 2006). Such mitotic inhibition could result of the inhibition of DNA synthesis which is considered as one of major prerequisites for a cell to divide. However, the reduction of mitotic activity after treatment could be associated with a reduction in the amount of both DNA and RNA (Solanke et al ,2008 and Soliman and Ghonium, 2004). Many other investigators attributed the depression in mitotic index values to the inhibition of protein synthesis, or due to inhibition of certain types of nuclear proteins essential in mitotic cycle (EL-Nahas, 2000 and El-Garabulli & Bashasha, 2006)

Mitotic index values were decreased with increase of *D. innoxia* concentration and exposure times, especially at high concentration (0.1 mg/ml). However, the complete inhibition in cell division was observed at 48 hrs. of exposure time. This inhibition of cell division was due to reduction in DNA and RNA or due to a deficiency of DNA in nucleus as a result of inhibition of DNA synthesis (Solanke et al, 2008). The inhibition of DNA and RNA synthesis were associated with reduction of oxidative phosphorylation in plant (Uzun 2004). The obtained results is in agreement with Adam and Farah (1989) who reported that *D. innoxia* water extract proved had a mitodepressive effect on *A. cepa* meristemic cells, that significantly reduced mitotic index after 24 and 48 hours of treatment that may be ascribed to partial blockage of DNA synthesis, thus minimizing the number of cells entering mitosis rather than hindering spindle formation. Other investigations attributed the mitodepressive effect on cell division to the disturbance and interaction with microtubules and tubulin which lead to blocking in mitosis and induced cell death by apoptosis (Akinboro & Bakare, 2007). The rate of cell division (MI) decreased with the increased of *D. innoxia* extract concentration. This result is in agreement with action of other plant extract proved to be mitodepressive such as (Soltan & Ata 2008; Mondakl et al, 2006; Abderrhman, 1997; Saggoo ,1991 and Solanke et al, 2008). Similar negative effect on MI has been found by other chemical treatments (El-Garabulli & Bashasha, 2006 and Gantayat et al, 2017). The mitodepressive was visible in roots treated with higher concentrations of *D. innoxia* extract Whereas, mitoactivation and increased MI were observed in roots treated with lower doses comparing with higher doses. These phenomena may be due to induction of cell division in the differentiated cells. According to Saggoo et al, 1991, the substances which induce cell division in lower concentrations cause mitotic depression at higher concentrations by extending S phase and impairing the G1 cells from entering in to S phase. The decreased cell number was evidence in most treated samples which was concentration and time dependent. Our data also indicated the interaction of *D. innoxia* extract with the genetic material which led to DNA damage. This interaction may explain the accumulation of treated cells in interphase, and depression of MI in the treated root tip cells. The decrease in MI can be also explained by the induced accumulation of chromosomal abnormality during mitotic phases of treated cells which led to mitodepressive effect on the rate of cell division. This data may reflect the expected genotoxicity of *D. innoxia* and suggested that *D. innoxia* extract contains phytochemical compounds that act as inhibitor agent to mitotic index (Adam and Farah, 1989 and Eleyowo, 2018)

Cytotoxic and Genotoxic Potentials of *Datura innoxia* Leaves Aqueous Extract on *Allium cepa* Meristemic Cells

2- Effect of *D. innoxia* Leaves Aqueous Extract on Frequency and Types of Mitotic Aberrations.

The present experiment showed that all doses (0.1, 0.01, 0.001 and 0.0001 mg/ml) of leaf extract induced mitotic abnormalities. The percentage of chromosomal anomalies was decreased at lower extract concentrations but it found to be increase with increase of the concentration. The highest value (51.76%) for chromosomal anomalies was observed at 0.1 mg/ml and the lowest 22.26% one was found in the least dilution (0.0001 mg/ml). Such results agree with those obtained by Ige *et al* (2022), who tested water extract of *D. stramonium* on *A. cepa* root tip cells. They found that the mitodepressive effect of plant extract on the rate of cell division and chromosome abnormalities were increased with increase the concentration and exposure time. The highest concentration (0.1 mg/ml) had more deleterious effect on cell division and induced more mitotic abnormalities with increase of treatment duration. In contrast with high concentration, low concentration induced less percentage of abnormalities at the same time of treatment. However, prolonged exposure time (48 hrs.), at same concentration there were no mitotic abnormality. This observation may be due to mitodepressive effect of *D. innoxia* extract lead to complete inhibition of cell division. The ability to stop cell division by mitodepressive agent present in leaves extract was suggested in this work. The action of these agents increased with increase of extract concentration. These results are in agreement with the finding of Adam and Farah (1989). *D. innoxia* extract clastogenicity was dose and time dependent, it exhibited significant clastogenic action at higher concentrations (0.1 and 0.01 mg/ml) that may lead to observed decline in MI. Similar results have been reported by other workers such as Mondal *et al*, 2006 and Eleyowo *et al*, 2018.

The presence of the chromosomal aberration might be induced by the following ways: First, chemical compounds directly affect DNA and lead to chromosomal aberration. Second, chemical compounds could disturb the synthesis of DNA and protein, or the translation of RNA, so that no materials relating to the chromosomal movement could be formed, and the chromosomal aberration occurred eventually. Third, prevent the re-establishment of the chromosome under normal conditions through interfering with normal repairing of damages, such as rearrangement of chromosomal bridge, loops and fragments (Xiao-wei, 2004). The treatment of *A. cepa* root tip cells with different doses of *D. innoxia* water extract induced clastogenic as well as physiological types of chromosomal abnormalities. The percentage of physiological aberration was increased by increasing of *D. innoxia* extract concentrations and exposure times, these observations agreed with other reports (Gantayat *et al*, 2017 and Solanke *et al*, 2008). However, the presence of high percentage of laggard chromosome, stickiness and c-metaphase were recorded in high frequency and they are evidence of spindle microtubules poisoning. Thus the primary mechanism of genotoxic action of *D. innoxia* at the protein level was suggested (Mondal *et al*, 2006). Early chromosomal condensation at prophase was also appeared frequently at all concentration of *D. innoxia* which support the action of *D. innoxia* on the protein level (Hallak *et al*, 1999). The most common type of physiological abnormality observed in all the concentrations and periods of treatment was, chromosomal stickiness at metaphase, this even may be due to alteration of chromosomal proteins resulting in change in surface nucleoprotein configuration or improper folding in chromosome fiber (Saggoo *et al*, 1991). Chromosomal stickiness was appeared due to adhesion or clumping of chromosome. In addition, stickiness could be due to depolarization of nucleic acid caused by mutagenic treatment which lead to partial dissociation and altered pattern of organization of nucleoprotein (Juchimiuk *et al*, 2006). Chromosomal stickiness that covers all chromosome complement leading to the appearance of chromatin masses were the general appearance of chromosome is lost in treated cells (Ige *et al*, 2022 and Iranbakhsh, *et al*, 2010). Stickiness interpreted as a result of improper folding of chromosome fibers in to chromatids and there is an intermingling of the fibers so that chromosomes become attached to each other by sub chromatid bridges. Among aberrations that appeared frequently in *A. cepa* root tip cells after treatment with *D. innoxia* extract were lagging chromosomes at metaphase, anaphase and telophase. The induction of laggard could be to the failure of the spindle apparatus to organize and function in a normal way rather than inhibition of these spindle fibers and this lead to irregular orientation of chromosomes (Cai, 2012). The reasons responsible for lagging chromosomes or failure to reach the equatorial plane probably lay in the formation of spindle fiber and destruction of its function by *D. innoxia* extract or the movement regulations of chromosomes per second are interfered so that it can not reach the equatorial plane on time (Soliman and Ghoneam, 2004). Similar physiological aberrations have been reported to be induced by medicinal plant and chemical agents (Saggoo *et al*, 1991 and Adam and Farah, 1989 and El-Garabulli, & Bashasha, 2006). At c-metaphase, chromosomes are appearing scattered in the cytoplasm. The metaphase chromosomes are shorter, thick and show no equator orientation. The presence of such type of anomalies is an indication of the action of *D. innoxia* contents on the inhibition of spindle fiber formation. Inhibition of ATPases might be the cause of spindle disorganization (Carballo, *et al* 2006 and Gocze & Freeman, 2000). The presence of colchicines metaphases indicate that *Datura* probably has effect similar to colchicines, depolymerizing the micro tubular proteins and blocking fiber formation during the mitotic state (Hallak *et al*, 1999). However, the induction of physiological aberrations may arrest cell cycle at c-metaphase and lead to MI inhibition (Soltan, & Ata, 2008) which observed in treated cells. The clastogenic effects were noticed in the form of chromatin bridges at anaphase and telophase and chromosomal fragmentation. Bridges observed at anaphase may be formed due to stickiness of chromosomes or due to formation of dicentric

Cytotoxic and Genotoxic Potentials of *Datura innoxia* Leaves Aqueous Extract on *Allium cepa* Meristemic Cells

chromosome as a result of breakage and reunion (Adam and Farah, 1989). Bridges may be due to breakage and reunion of chromatid or sub chromatid (Solanke *et al*, 2008). The stickiness makes their separation and free movement incomplete and thus may remain connected by bridges. This may also due to defective formation of spindle apparatus (Ashiru *et al*, 2020). Induction of chromosomal and chromatin bridges at anaphase and telophase stages were also observed after treatment of *D.innoxia* extract, which may result from chromosome stickiness. Due to such stickiness the separation of daughter chromosomes becomes incomplete even in the presence of spindle fibers and thus they remain connected by chromatin bridges (Soliman and Ghonium, 2004). Fragmentation of chromosomes might have arisen due to stickiness of chromosomes and consequent failure of separation of chromatids to poles. Fragments may be acentric chromosome that is formed as a result of inversion or chromosome deletion (Ashiru *et al*, 2020). In addition to the different type of chromosomal abnormalities induces in divided cells, micronuclei were also observed in the interphase cells as well as different mitotic stages. Micronuclei formed as result of exclusion of a centric fragment of chromosomes out of the nuclear envelope during the completion of mitosis (Hallak *et al*, 1999). Micronuclei forms in two ways: one is, the chromosomal fragments found in the last G2 could not act in phase with normal chromosomes, and are rejected to the outside of nuclei in interphase. The other is the occurrence of various forms of lagged chromosome, and the chromosomal grouping (Xiao-wei, 2004). The induction of micronuclei is usually caused by chromosome breaks or fragment or spindle poisoning, which is an anomalous disjunction of chromosomes during anaphase (Ige *et al*, 2022). Bi and multinucleated cells were recorded in few percentages; that may be result of a preceding of multipolar mitosis the failure of cell plate formation following mitosis. *D.innoxia* extract recorded as inducer of cytokinesis abnormalities due to presence of the binucleated cells in the treated samples, which suggested the cytoskeleton proteins could be a cell target for *D.innoxia* content (Carballo *et al*, 2006). Different types of chromosomal abnormalities such laggards, bridges, stickiness and c-mitaphase were observed in all *A.cepa* root tip cells after treatment with *D.innoxia* water extract in this investigation (plates 2,3,4 and 5), these results indicated the potentiality of the *D.innoxia* constituents to induce mitotic irregularities, that agree with the finding of (Soltan & Ata 2008 and Eleyowo *et al*, 2018). The accumulation of chromosomal abnormalities inhibition the cell division and decreased the MI were observed in cells treated with high concentration (0.1mg/ml) and with prolonged time of treatment (48 hrs.) such results agreed with that mentioned by Keshaw (2000).

CONCLUSION

The negative influences of *D. innoxia* extract on the cell division of *Allium cepa* cells was evidenced. The present results revealed mitodepressive action led to decrease in cell number. Therefore, inhibition mitotic index of *Allium cepa* cells. The appearance of abnormalities at low and high concentrations at different exposure time indicates the genotoxicity of *Datura innoxia* leaves water extract.

REFERENCES

- 1) Abena, A; Miguel, L; Mouanga, A; Ouamba, J; Sainard, D; Thiebolt, M; Hodi-assah, T; and Diatwa, M. (2003) Evaluation of analgesic effect of *Datura fastuosa* leaves and seeds extracts, *Fitoterapia*, 74:486-488.
- 2) Abderrhman, S. (1997) effect of *Peganum hamala* extract on root tips of *Allium cepa*. *Cytobios.*, 90(363):171-174
- 3) Adam, Z; and Farah, O. (1989) Cytological effects of water extracts of medicinal plants in Egypt, mitotic disturbances induced by water extract of *Cymbopogon proximus* (Halfa barr) on *Vicia faba*. *Cytologia*, 54:489-492 .
- 4) Akinboro, A; and Bakare, A.(2007) Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. *J. Ethnopharmacol.*, 112(3):470-507.
- 5) Ashiru, O. M; Sifau, M. O. and Oluwatontloba, O. (2020) Cytogenotoxicity Of Aqueous Extracts Of Leaves an Seeds Of Gegemu (*Datura Stramonium*) On *Allium Cepa*, *Annals of West University of Timișoara, Biology*, 23 (1),.11-20 .
- 6) Cai ShiLin C. S. ; Mu XiaoQian M. X.Q.; Mao LiYan M. L.; Ran JunXiang R. J. X. and, Xu Ning X. N. (2012) Young root growth and cell division of soybean stressed by aqueous leachate of *Datura stramonium* leaves. *J. Acta Botanica Boreali-Occidentalia Sinica*, 32, (1), 117-123.
- 7) Carballo, J; Pincheina, J. and De LA Torre, C. (2006) the G2check point activated by DNA damage does not prevent genome instability in plant cells. *Biol, Res*; 39(2); 331-340.
- 8) Eleyowo, O. O.; Amusa, O. D. ; Omotayo , M. A. and Akpan, U. U. (2018) An Investigation Of Phytochemicals Antioxidant and Genotoxic Potential Of *Datura Metel* Inn. *Annals of West University of Timișoara, ser. Biology*, 21 (2), pp.133-142.
- 9) El-Garabulli, F; and Bashasha; J. (2006) Effect of Aspirin on cell division. *Sci. and their appli.*, 1:9-19.
- 10) El-Nahas, A; Khalifa, S; and Abu El-Enein, M. (2000) Karyological studies on the solanaceae. *Pakis. J. of biolo.sci.*, 3(5):717-720

Cytotoxic and Genotoxic Potentials of *Datura innoxia* Leaves Aqueous Extract on *Allium cepa* Meristemic Cells

- 11) El-Torki, M, Mehdi, A. W; Mohammed, A; and Al- Jiboori, N. (2006) Liver and kidney functions in adult male rats following multiple subcutaneous injection of non- lethal doses of *Datura metel* leaves Aqueous extract. *Sci. and their appli.*, 1:9-19 .
- 12) Grant, W, F. (1982) Chromosome aberration assay in *Allium cepa*; Areport of the U.S. Environmental Protection Agency Gene –Tox Program. *Mutation Research*. 99: 273-291.
- 13) Gocze, P; and Freeman, D. (2000) Cytotoxic effects of cigarette smoke alkaloids inhibit the progesterone production and cell growth of cultured MA-10 leydig tumor cells, *Eur. J. Obstet Gynecol. Repord Biol.*, 93(1):77-83
- 14) Gnanamani, A; Gopal, R; Babu, M; and Sadulla, S. (2004) A study on scientific validation of the Indian traditional wound healing agent. *Wound repair and regene.*, 12(2): A39-A39
- 15) Gantayat, S. Mania, S. Pradhan, C. and Das, B, A. (2017) Ionic Stress Induced Cytotoxic Effect of Cadmium and Nickel Ions on Roots of *Allium cepa* L. ; *Cytologia*, 83(2)143-148.
- 16) Hussein, K. (1985) Medicinal plants in Libya. Arab Encylopedia house, Tripoli. pp392-396
- 17) Hallak, A, M; Davide, L, C and Souza, I, F (1999) Effects of *Sorghum (sorghum bicolor L.)* root exudates on cell cycle of the bean plant (*Phaseolus Vulgaris L.*) root. *Genet. Mol. Biol.* 22(1): 11-18.
- 18) Ige J. K; Christianah A. D; Mayowa O.; Akintude O. A., Elizabeth A. F.; Matthew O. and Oluokun O. O. (2022), Cytotoxic and Genotoxic Potentials of the Extracts and Fraction of the Leaf of *Datura metel* (Lnn.) *NJBMB* , 37(1), 32-40.
- 19) Iranbakhsh, A; Ebadi, M and Bayat, M. (2010) The inhibitory effect of plant methanolic extract of *Datura Stramonium L.* and leaf Explant Callus Against Bacteria and Fungi. *Global Veterinria*, 4(2)149-155
- 20) Juchimiuk, J; Gnys, A; and Maluszynska, J. (2006) DNA damage induced by mutagens in plant and human cell nuclei in a cellular comet assay. *Folla Histoche. EtyctoBio.*, 44(1):127-131
- 21) Keshaw, K. (2000) Effect of female oral contraceptive on human chromosomes and its role in behavioural changes in offsprings. *J. Anat. Soc.*, 49(1):40-212.
- 22) Kuete, V. (2014) 21 - Health Effects of Alkaloids from African Medicinal Plants, *Toxicological Survey of African Medicinal Plants*, PP : 611-633.
- 23) Mondakl, A; Kabir, G; Yasmin, N; Alam, A, M. and Khatun, H, A. (2006) Mitotic Effect of Water Extract of Different *L. pomoea* species on *Allium cepa* L. *Pakistan Journal of Biological sciences*, 9 |(6)' 1116-1120.
- 24) Parmod, K; Singh, S. and Jayahaaskaran, C, (2009) Expression of hyoscyamine 6b-hydroxylase in the root pericycle cells and accumulation of its product scopolamine in leaf and stem tissues of *Datura metel l.*. *Plant Science*, 178(2): 202-208.
- 25) Saggoo. M. I; Kumari, S; and Bindu (1991) Cytological effects of Indian medicinal plants I- Mitotic effects of leaf homogenate of *Tylophora indica L.* on *Allium cepa*. *Cytologia*, 65:633-637.
- 26) Shobana, M. K. and Nandhin, G. (2022) ArticlInfluence of phytochemicals with iron oxide nanoparticles for biomedical applications: a review, 80, pages 11715–11758.
- 27) Solanke, P; Singh, M; Rthore, H. S; Sharma, A; Makwana, M; and Shrivastava, S. (2008) An evaluation of the genotoxic effects of seed Decoction of *Cassia tora L. (Legumin osae)* in *Allium cepa* model. *Gene. and Mol. Bio.*, 23(2):497-507
- 28) Soliman, M; and Ghoneam, G. (2004) The mutagenic potentialities of some herbicides using *Vicia faba* as a biological system. *Biotechnology*, 3(2):140-154
- 29) Soltan, H. and Ata, A. (2008) Studies on Genetical and Cytological Effects of Some Agrochemicals on Different Biological Systems, Athesis for master degree at Minia University, pp29-37.
- 30) Uzun, E; Sariyar, G; Adersen, A; Karakoc, B; Otuk, G; Oktayoglu, E; and Pirildar, S (2004) Traditional medicine in sakarya province (Turkey) and antimicrobial activities of selected species of Ethnopharmacology, 95(2-3); 287-296.
- 31) Xiao-Wei, Q. (2004) Mutagenic effect of chromoium trioxide on root tip cells of *vicia faba*. *J of Zhejiang university of scince*; 5(2): 1570-1576.



There is an Open Access article, distributed under the term of the Creative Commons Attribution – Non Commercial 4.0 International (CC BY-NC 4.0) (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits remixing, adapting and building upon the work for non-commercial use, provided the original work is properly cited.