# Chromosomal Aberration in Human Lymphocytes Culture

**Induced by Crude Extract of Datura innoxia**

## El- Garabulli F. R.1 and EL- Oami M. M.2

1Genetic Department, Faculty of Science, Misurata University

2Zoology Department, Faculty of Science, Benghazi University

**Abstract:**

In this study cytological and genotoxical effect of Datura innaxin water extract was investigated by aberration bioassay in human cells. Obtained result revealed mitodepressive action of Datura extract that led to significantly decrease in mitotic index of divided tested cells. The appearance of abnormality in chromosome behaviour and structure at low and high concentration of crude extract indicate the genotoxicity of Datura innaxin.

**Keywords**: Aberration assay, genotoxicity, mitodepressive, Datura innaxi.

**Introduction**

The increasing reliance on medicinal plant is not confine to the poorest developing countries; the developed countries with the sophisticated pharmaceutical industry are also rediscovering the usefulness of medicinal plants. Medicinal plants contain one or more endogenous substances, which can be use directly for medicinal purposes or in semi synthesis of preparation. WHO continue to promote the development and application of analytical methods that can be used to evaluate the safety and efficacy of various element of traditional medicine (Akerele, 1984). Among the medicinal plants, species of the genus Datura, family solanaceae, were popularly use in folk medicine to relief pain and for treated of skin disease, mental disease and organ phosphorus toxicity (Abena et al, 2004). Several workers (Gerber et al., 2006; El-Torki, 2006; Defrates et al 2004; Berkov et al, 2004) have demonstrated the toxicological, physiological and anticholinergic effects of Datura extracts on animal systems and the other employments of different parts of these plants. However, studies of the mutagenic and carcinogenic potentials of Datura aqueous crude extract on cell division and chromosomes behavior in relation to their action on the nucleic acids have not been sufficiently carry out. The present study aimed to investigate the cytological and the mutagenic potentialities of Datura innoxia leaves aqueous crude extract using human white blood cells to underline the mechanism of its genotoxicity action.

**Material and Methods**

Datura inoxia Plants D. inoxia: leaves collected from botany area and in waste area nearby Benghazi city.

**Plant extract preparation:**

Medium sized normally looking leaves were collect, washed with water and dried in well-ventilated room. 20 g of D.innoxia tiny cuts was add to 200 ml distilled water in 500 ml flask and shacked by shaker over night at room temperature before filtrated by what man 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 40-45C˚.The aqueous crude extract was transferred in to a stopped glass container and its volume was adjusted to 20ml before stored in freezer at -18C˚. Four different concentrations (0.1, 0.01, 0.001, and 0.0001mg/ml) were prepared by serial dilution method.

**Human Chromosomes Technique:** Human lymphocyte culture was undertaken following the technique of Jain and Sethi, 1991; Ribas, 1996 with minor modification.

**Cell Culture:** Venous blood was take using pre-heparinized syringes. Lymphocyte culture were setup by adding 0.5 ml of whole blood to 5 ml of RPMI-1640 medium with 1 ml human AB serum, 0.2 ml phytohemagglutinin (PHA) and 1500 U/ml penicillin. All cultures bottles incubated at 37C˚ for 72 hrs in shaker incubator

**Treatment:** The cultures treated with different concentrations of D. innoxia aqueous extract (0.1, 0.01, 0.001, 0.0001 and 0.0001 mg / ml) for 4 hrs except the control. 0.1ml of colchicines solution (0.05mg/ml) were add to each culture for 3 hrs prior harvesting and re-incubated at 37C˚.

**Harvesting:** After incubation, the cells collected by centrifugation for 10 min at 2000 rpm. The supernatant removed and the cells resuspended in 5ml per-warmed 37C˚ hypotonic solution (0.075M KCL) for 25-60 min, then centrifuged for 10 min at 2000 rpm. The supernatant was then removed and the cells resuspended in 5 ml freshly prepared cold fixative (3 methanol: 1 glacial acetic acid v/v) drop by drop and kept in the fridge at 4C˚ for 30 min to be fixed.

**Slide Preparation:** The supernatant was replaced twice with cold fixative then centrifuged and resuspended in 0.5 ml of cold fixative to make a cloudy suspension, then by the pipette from a height about 8 inches, drop 4-5 drops of fluid on to each slide for well spread metaphases. The slide left for few minutes to dry on a hot plate and stained with 4% Gimsa stain for 20 min. Stained slides then washed with distilled water and air-dried. The slides were scan with light microscope under oil immersion 100 x objectives for cell examination at each concentration and treatment period. The experiment was repeated three times and the incidence of aberrations like chromosome break, chromatid break, ring chromosome and chromosome stickiness were determined, while the mitotic index (MI) was evaluated by counting out of 1000 lymphocytes per experiment and the dividing cells were scored. MI = (Number of dividing cells) / (Total number of scored cells) x 100. The data were statistically analyze by one-way analysis of variance. Multiple comparisons were performed by least significant difference (LSD) using statistical package for social science (SPSS), with significance level less than 0.05.

**RESULTS AND DISCUSSION**

The effect of D.innoxia crude water extract on the genetic material during cell division in human lymphocyte culture was investigate by measuring the value of mitotic index (MI) and chromosome aberrations. Obtained results of MI were Illustrated in figure (1). Statistical analysis of variance of MI values showed highly significant effects of concentration (p < 0.001). MI values decreased continuously (17.01, 14.84, 12.17, 11.50 and 7.12 %) with increased in extract concentrations (0.00001, 0.0001, 0.001, 0.01 and 0.1 µg/ml respectively) comparing to control. Data showed also a Significant Differences between different concentrations.

The effect of D.innoxia crude water extract on the genetic material during cell division in human lymphocyte culture investigated by measuring the value of mitotic index (MI). Obtained results were represented in figure [1]. Statistical analysis of variance in MI values showed that there were highly significant effects of concentration (p < 0.001). MI values were decreased (17.01, 14.84, 12.17, 11.50 and 7.12 % with increased in concentrations (0.00001, 0.0001, 0.001, 0.01 and 0.1 µg/ml respectively) comparing to the control.



**Figure (1)** Effect of different concentrations of Datuea innoxia

aqueous extract on mitotic index (MI) of cultured human lymphocytes

\*\*\*= significant at p<0.001, Different litter= significant, Similar litters= non-significant

The effect of D. inoxia leaves aqueous extract on frequency and types of mitotic aberrations in human lymphocytes also investigated. Table (1) and figure (2) showed the result of treated human lymphocytes with different concentrations of Datura crude water extract and their effect on chromosomal structure in c-metaphase. One-way analysis of variance (ANOVA) showed Significant Differences between treatments (p < 0.01). However, the percentage of abnormalities increased gradually (37.445, 38.796, 38.933 and 41.994 %) with the increase in D.innoxia concentration (0.00001, 0.0001, 0.001, and 0.01 µg/ml, respectively), except the high concentration, 0.1 which revealed a decrease in abnormalities value (33.797 %) compared to the control. D.innoxia extract revealed physiological abnormalities in treated cells such as chromosome stickiness. However, clastogenic abnormalities such as chromosome break, chromatid break and ring chromosome were also observed).

**Table (1)** Type and % of abnormalities in human blood cells treated with different concentrations of D. innoxia aqueous extract.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Conc.**  **mg/ml** | **Mean of M.C.** | **% of**  **AB ± SE** | **Type of abnormality** | | | | | | | |
|  | **Clastogenic** | | | **Physiological** | | | | |
| **%** | **%Sti.** | **%HP.** | **%** | **%Ch.Br.** | **%Ctd.Br.** | **%p.red** | **%R.ch.** |
| 0 | 245 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.00001 | 124 | 37.445± 4.175 | 56.08 | 19.767 | 0.185 | 43.92 | 4.954 | 7.008 | 4.892 | 0.639 |
| 0.0001 | 131 | 38.796± 5.432 | 52.147 | 19.164 | 0.33 | 47.853 | 6.049 | 6.539 | 5.288 | 1.426 |
| 0.001 | 115 | 38.933± 5.721 | 5.816 | 19.511 | 1.483 | 47.184 | 7.127 | 6.351 | 4.712 | 0.749 |
| 0.01 | 95 | 41.974+8.016 | 48.837 | 20.20 | 0 | 51.163 | 6.541 | 8.143 | 4.422 | 2.668 |
| 0.1 | 41 | 33.797+6.797 | 57.142 | 18.53 | 0.775 | 42.858 | 7.286 | 3.176 | 2.48 | 1.55 |

The physiological aberration had the highest percentage of total abnormalities 57.142 %and 56.08 % in samples treated with 0.1 and 0.01 µg/ml, respectively, whereas the clastogenic aberrations had the lowest percentage of total abnormalities 42.858 % in samples treated with 0.1µg/ml. The result showed percentage and type of chromosomal aberrations, chromosome stickiness (20.20 %) chromatic break (8.148 %)\_chromosome break (7.286 %) ploidy reduction (5.288 %) ring chromosome (2.668 %) and hyperploids (1.483 %) observed in treated cells with different concentrations (0.01, 0.01, 0.1, 0.0001, 0.01 and 0.001 µg/ml). Statistical multiple comparisons test, Least Significant Differences (LSD) showed high Significant Differences was only between control with other concentrations and no significant differences between some treatment concentrations figure (2).

 

A-Normal c-metaphase at control B-Sticky metaphase (0.1µg/ml)

****

C-Chromatid break & chromatid D- Ring chromosome (0.1µg/ml) …………deletion (0.1µg/ml)

 

E-Terminal deletion (0.001µg/ml) H-Micronuclei in lymphocytecell ……………………………………………… ……… (0.00001µg/m;)

The results also showed increased of MI value at lower concentration (0.0001 mg/ml), this indicated that substances may induce cell division in lower concentrations and cause mitotic depression at higher concentrations by extending S-phase and impaired the G1 cells from entering in to the S-phase (Saggoo et al, 1991; EL\_Garabulli and Bashasha, 2005). Mitotic index depression is evidence of the interaction of compounds in the crude extract with genetic material that responsible for the inhibition of cell division in the treated cells (Teixeira et al, 2003, El-lfy et al., 2016). The inhibition of cell division may be attributed to many factors that interrupted the cell cycle (Thybaud et el., 2007). The decreased in MI values could be due to reduction in DNA and RNA and inhibition of DNA synthesis. In this study DNA damage may explain the accumulation of treated cells in interphase which lead to the observed decrease in MI values. Similar results have been founding by other authors (Lamchouri et al, 2000; Juchimiuk et al., 2006; YadaV and Chiller, 2002; Bonassi et al, 2007; el-Alfy et al.,2016). The obtained results suggested that plant extract contain phytochemical compounds that have a negative effect on genetic materials (Saggoo et al1991; Willams et al., 1991; Berkov and Philiv, 2002; Akinboro and Bakare, 2007) that lead to mitodepressive effect of D. innoxia.

**Conclusion**

M.C: mitotic cells, AB: abnormalities, SE: standard error, Sti: stickiness, HP.: hyperploidy, Chr Br: chromosome break, Ctd.Br.: chromatid break, P.red: ploidy reduction, Rch.: Ring chromosome

**REFERENCES**

1) Abena, A; Miguel, L; Mouanga, A; Ouamba, J; Sainard, D; Thiebolt, M; Hodi-assah, T; and Diatewa, M. (2004) Neuropsychopharmacologi- Cal effects of leaves qnd seeds extracts of Datura fastuosa. Biotech-Nologym, 3(2):109-113

2) 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

3) Akerele, O. (1984) WHO´s traditional medicine program: progress and perspective. WHO Chron., 38(2):76-81.

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):470-5

5) Attia, S. M. (2007) The genotoxic and cytotoxic effects of nicotine in the

6) Berger, F; Gage, F; and Vijayaraghavan, S. (1998) Nicotinc receptor- induced apoptotic cell death of hippocampal progenitor cells. J. of neuroscience, 18(7):6871-6881

7) Berkov, S; and philipv, S. (2002) Alkaliod production in diploid and Autotetrapliod plants of Datura stramonium. Intern. J. of Pharmaco., 40(8):617-621

8) Berkov, S; and Zayed, R. (2004) Comparison of tropane alkaloid spectra between Datura innoxia grown in Egypt and Bulgaria. Naturforsch, 59(3-4):184-6

9) Berkov, S; Doncheva, T; Philipov, S; and Alexandrov, k. (2005) Ontogenetoc variation of the tropane alkaloids in Datura stramonium. Biochemical and systematic and ecolo.,33(10):1017-1029

10) Bonassi, S; Znaor, A; Ceppi, M; Lando, C; Peter, W; Holand, N; Kirsch-Volders, M; Zeiger, E; Ban Sadayuki; Bigatt, M; Bolognesi, C; Fabianova, E; Fucie, A; Hagmar, L; Joksic, G; Martilli, A; Magliore, L; Mirkova, E; Scarfi, M; Zijno, A; Norrppa, H; and Fenech, M. (2007) An increased micronucleus frequency in peripleral blood lymphocytes. Carcinogenesis. 28(3): 625- 631

11) Defrates, L; Hoehns, J; Sakornbut, E; Glascock, D; and Tew, A. (2004) Antimuscarinic intoxication resulting from the ingetion of moonflower seeds. The Annals of pharmacotherapy, 39(1):173-176

12) 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 Aqueos exract. Sci. and their appli., 1:9-19

13) El-Alfy,Nagla; Mahmoud Fathy;Amany Al qosaibi; SallyEl-Ashry (2016)Genotoxic effect of Methotrexate on bone marrow and DNA of male albino mice. The Egyptian Journal of Medicine Vol.64, Page 350-363.

14) El-Garabulli, F; and Bashasha; J. (2006) Effect of Aspirin on cell division. Sci. and their appli., 1:9-19

15) Gerber, R; Naude, T; and Kock, S. (2006) Confirmed Datura poisoning in a horse most probably due to D. ferox in contaminated tef hay. J.S. Afer Vet ASSOC.,77(2):85-89

16) Hammer, K; Carson, C; and Riley, T. (1991) Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbial., 86(6): 985-90

17) Jain, A. K; and Sethi, N. (1991) Chromosomal aberration and sister chromatid exchanges in culture human lymphocytes I. induced by crude extracts of black and green tea. Cytologia, 56:533-538

18) 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. EtcytoBio., 44(1):127-131

19) 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

20) Lamchouri, F; Settaf, A; Cherrah, Y; Hassar, M; Zemzami, M; Atif, N; Nadori, E; Zaid, A; and Lyoussi, B. (2000) In vitro cell- toxicity of peganum harmala alkaloids on cancerous cell- lines. Fitoterapia, 71(1): 50-54

21) Mouse bone marrow. Mutat. Res., 632(1-2):29-36

22) Nanda Kumar, N; Prabhakar, E and Srinivasa murthy, K (2006) Pharmacological evidence for aspasmogenic agent in Datura root extract tested on dispersed smooth muscle cells

23) Ribas, G; Surralles, J; Carbone, E; and Xamena, N. (2000) Genotoxicity of the herbicides alachlor and maleic hydrazide in cultured human lymphocytes. 11(3):221-227

24) 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

25) Sharma, A. and Gautam, D. (1991) Chromosomal aberrations induced by phosphomidon and endosulfan in the bone marrow cells of mice In vivo. Cytologia, 56:73-78

26) Steenkamp, P; Harding, N; Vanheerden, F; and Vanwyk, B. (2004) Fatal Datura poisoning: identification of atropine and scopolamine by high performance liquid chromatography photodiode array/mass spectrometry. Forensic Sci. Int., 145(1):31-39

27) Teixeira, R; Camparoto, M; Mantovani, M; and Pimentavicenini, V. (2003) Assessment of two medicational plants Psidium guajava L. and Achillea millefolium L. In vitro and In vivo assay. Gene. Mol. Boil.,26(4):13-20

28) Thybaud, V; Aardema, M; Clements, J; Dearfield, K; Galloway, S; Ohyama, M; Schuler, M; Suzuki, M; and Zeiger, E. (2007) Stratgy for genotoxicity testing: Hazard identification risk assessment in relation to In vitro testing. Mutat. Res. /gene. Toxocol. / mutagenesis, 627(1):44-58

29) Williams, P; and Rayson, G. (2003) Simultaneous multi- element detection of metal ions bound to a Datura innoxia material. J. Hazard Mater., 30(3): 277-85.