# Chromosomal Aberration in Human Lymphocytes Culture

**Induced by Crude Extract of Datura innoxia**

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**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.

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**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)

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 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

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