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Research

Cytological Effects of Chloroquine on Root Mitosis of *Allium cepa* (L.)

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Abstract

The effects of chloroquine (antimalarial drug) on the mitosis of Allium cepa L. was investigated with a view to ascertaining its mutagenic effects. Onion roots were treated with 20%, 50% and 100% concentrations of chloroquine at 6hours, 12hours and 18hours duration respectively and distilled water as control. There was a significant difference (0.05%) among the mitotic indices (2.0, 2.06 and 0.7) for 20%, 50% and 100% concentrations of chloroquine respectively compared with the control (3.8). The result further showed that chloroquine induced cell mitotic abnormalities like anaphase bridge, fault polarization of anaphase, chromosome fragmentation, disorderly anaphase, C-metaphase and clumping of chromosomes at 20%, 50% and 100% concentrations and different time of exposure. The result implicated chloroquine as a mitotic depressor and confirmed chloroquine as mutagenic to plant cell, when absorbed in high dosage.

Keywords: Mutagenesis; Mitotic index; Anaphase bridge; Chromosomes; Mitotic depressor

Introduction

The mechanism leading to the formation of daughter cells and the retention of identical chromosome numbers and other hereditary factors in the newly formed cells, following treatments with various reagents have been studied by several workers such as Shanthamurthy & Rangaswamy (1979), Okoli & Russom (1987), Okagbue (1990) and Umar (2004). The general principles and mechanism of mitosis is best and easily studied in actively growing regions of plant shoot or root apexes or flower buds. The current study is an attempt to

investigate the effects of chloroquine – a widely used antimalarial drug, on mitotic activities in *Allium cepa* root tip cell. The justification for the use of chloroquine is its popular use and outstanding record in the treatment of malaria, while *A. cepa* has been selected for its relatively low chromosome number (2n = 16). Its chromosomes are relatively large and the species is susceptible to cytological manipulations Mercykutty & Stephen (1980).

Chloroquine belongs to the 4-aminoquinoline class of antimalarial drugs with an active side chain. It is soluble in water and its chlorine atom has been shown to be crucial to its antimalarial activity Catpool (1984). The effects of various plant extracts and chemical constituents have been the subject of many investigations on root mitosis. Shanthamurthy & Rangaswamy (1979) reported a low mitotic index in *A. cepa* in strong concentration of paper mill effluent. They also concluded that an increase in concentration of this effluent with extended treatment duration also resulted in decreased mitotic index and with a resultant toxic effect on the root meristem. Shehab (1979) observed a depressed mitosis in *A. cepa* in the water extract of *Pulicerra crispa*. Shehab (1979) equally observed that water extract of *Teucrim pilosum* had a strong depressive effect on mitosis of *A. cepa* and toxic at (>10%) high concentration. Kabarity & Mallah (1980) using Khat extract observed a mitodepressive effect in the meristematic region of *A. cepa*. Misera (1982) also reported a mitodepressive effect of calcium salt on *A. cepa*, while Okoli & Russom (1987) demonstrated the effect of high concentration of *Cassia alata* extract on the mitotic index of *A. cepa* root cells.

Apart from the use of plant extracts for mitotic studies, there are several other reports on the use of inorganic chemicals. El-Bayoumi *et al.* (1979) reported high mitotic index in *A. cepa* caused by papeverine chloride. Sarbhoy (1980) noted that the germination ability of *Lens esculenta* and subsequent growth of roots were inversely proportional to the concentration duration of treatment with Paradichlorobenzoic acid (PDCB) and observed such chromosomal anormalies as fragmentation and chromosome bridges. Badr & Elkington (1982) reported the antimitotic and chromotoxic effects of Isoproturon on *A. cepa* and *Hordeum vulgare* roots. Al-Najjari & Soliman (1980) observed a significant reduction in mitotic index induced by herbicide (Vitavax-200) and pesticide (Duthane S-60), while Singh (1982) investigated the effect of Indole Acetic Acid, (IAA), Maleic acid and Colchicine on root tip mitosis and observed a decreased mitotic index. Similarly, Kim *et al.* (2000) reported that brassinosteroids (BRs) have been implicated in cell elongation, cell division, reproductive and vascular development in maize root whereas Howell *et al*. (2007) revealed that

plant steroidal hormone (24-epibrassinolide) promoted root growth at low concentrations in *A. cepa* by increasing the mitotic index. Tabur & Oney (2009) reported that ammonium sulphate and potassium sulphate have varying effects on the mitotic index of *Vicia hybrid*a seeds. At low to moderate concentrations of 1, 10, and 50µm, these chemicals increased the mitotic index of seeds but higher concentrations (100µm and above) inhibited mitotic index of seeds. Okagbue (1990) observed an anti-mitotic effect of three antimalarial crude plant extract- *Azadirachta indica, A. jusc*, *Alstonia boonei*, *Carica papaya* and two synthetic antimalaria drugs (Fansidar and Daraprim) on the root tip mitosis of *Hippeastrum equestre*.

Previous investigations including that of Nasakhare (1979) implicated most antimalarial drugs to induce some physiological and cytological abnormalities in cells of animals. The ability of chloroquine to bind to DNA and intercalates with guanine containing double stranded DNA makes it a potential mutagenic agent (Allison *et al.*, 1966). The ability of 4-quinolines to bind with DNA is enhanced by the chloride at position 7 (Ciak & Hahn, 1966). In this study, the cytological effects of chloroquine on the root tip mitosis of *A. cepa* was investigated to reveal the potency of chloroquine as a probable mutagen and establish its mechanism of action and implication on cell division and meristematic activities of the root tip cells.

Materials and Methods

Preparation of chloroquine solutions

Ten tablets of chloroquine obtained from a pharmacy were ground to powder and later on dissolved in one litre of distilled water to form a main stock of chloroquine (1500mg/cm^3) solution (100%). Each tablet of chloroquine used in this study contained 150mg of chloroquine disulphate. From the main stock (100%), 50% and 20%, concentrations were prepared in triplicate using serial dilution method, and distilled water served as control.

Experimental design and treatments

A total of 36 onion bulbs were used for this study: 12 bulbs were used for each group (time duration). Sets of three onion bulbs were used for each of the three concentration (20%, 50%and 100% and control) and for each of the three durations (6hours, 12hours and 18hours.). The onion bulbs were induced to root by placing them on beakers filled with distilled water with the base of the onion touching the surface of the water. The rooted bulbs were later placed on beakers containing the different concentrations of chloroquine solutions with the roots immersed in the solutions and for 6hours, 12hours and 18hours as shown in figure 1. Roots from the three set of bulbs placed on the beakers

containing distilled water served as the control. At the end of each treatment, sixty healthy roots were randomly selected from the three bulbs for each treatment and analyzed for mitotic activities.

Mitotic Studies and Analysis

The roots were fixed immediately after the treatments in 1:3 acetic alcohol for 24hours at room temperature and later transferred into 70% alcohol before preserving in the refrigerator. Slides were prepared for mitotic studies by squashing root tips in FLP-Orcein, following the methods described by Okoli (1983). The slides were viewed for cell count at X400 magnification, while photograph was taking at X1000 magnification under oil immersion. The number of dividing cells was scored and the mitotic indices calculated for the treatments and the control. The mitotic index was calculated by the dividing the number of cells undergoing mitosis with the total number of cells examined for each treatment (Balog, 1982). The data obtained were analyzed to determine the effects of the treatments (concentration and duration of treatment) on mitotic activities of *A. cepa* cells. A't'-test was carried out to determine whether there was any significance between treatments on the mitotic index. Photomicrographs of cells showing chromosomal aberrations as well as cells showing normal mitosis were taken using Olympus microscope.

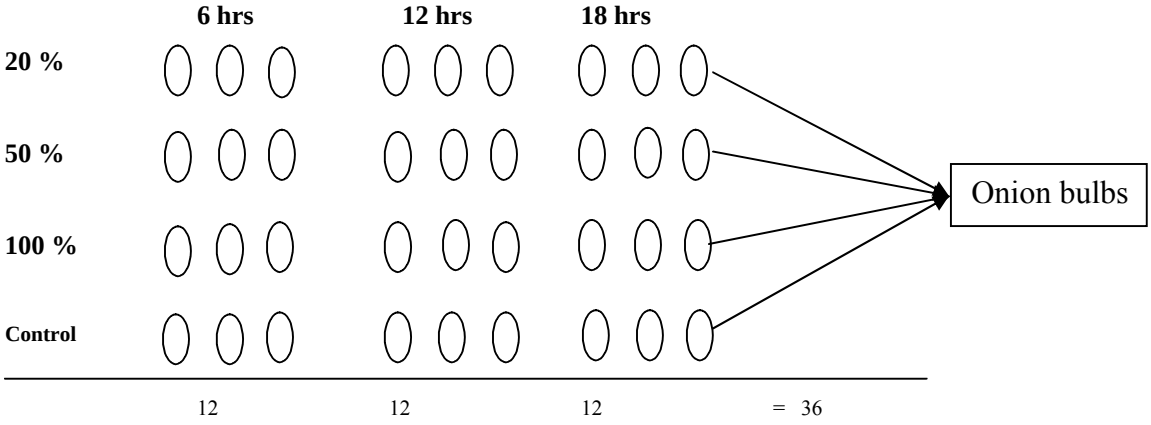

Fig. 1: Experimental layout

Results

Effect of chloroquine concentration on Mitotic index (MI) of *A. cepa*

Table 1 shows the mean mitotic index in *A. cepa* treated with different concentrations of chloroquine and at different duration. The result shows a significant ($p<0.05$) treatment effects on treatment effect on the mitotic index of *A. cepa*. However, result further revealed a non significant ($p>0.05$) duration effect on

the mitotic index of *A. cepa* root cells. The mean MI ranged between 3.8 in the control and 0.73 in the 100% chloroquine treatment. However, the treatments with 20% and 50% chloroquine showed Mitotic indices of 2.0 and 2.06 respectively. The mean MI for duration of treatment was least at 12 hours (2.02) followed by 6 hours (2.07) and highest at 18 hours (2.24) though these values were not significantly different (p>0.05) from each other. Table 2 shows the trend in mitotic index of *A. cepa* treated with different concentrations of chloquine different durations. Figure 2 shows the trend of mitotic index of *A. cepa* root cells treated with different concentrations of chloroquine at different duration. The result shows similarity in trend in the mitotic indices of control and 20% chloroquine treatment. In both the control and 20% chloroquine treatments the mitotic indices started at 6 hours and maintained a steady increase at both 12hours and highest at 18hours. For 50% chloroquine treatment, the mitotic index suffered a drop after 6hours duration (12hours) and picked up slightly at 18hours but with mitotic index below the value at 6hours. Meanwhile for 100% chloroquine treatment the mitotic index rose a little above the value at 6hours of treatment but dropped significantly below the starting value at 6hours. Table 2: shows the effect of chloroquine on the mitotic interphase of *A. cepa*. The result shows that the mean percentage of interphase was significantly highest with 100% chloroquine (92.15) treatment and least in the control (61.49) the 20% and 50% chloroquine concentrations accounted for 79.51% and 78.56% interphase respectively.

Table 1: Mean values of the mitotic index in *A. cepa* at different durations and concentrations of chloroquine

	Control	Concentration			Time		
		20%	50%	100%	6hours	12hours	18hours
MEAN	3.8	2.0	2.06	0.73	2.07	2.02	2.42
SD	0.95	0.42	0.53	0.31	1.03	1.06	1.88
SE	0.55	0.24	0.31	0.178	0.51	0.53	0.94
MEAN Difference		1.80	1.74	3.07	1.73	1.78	1.38
SED	0	0.47	0.51	0.44	0.76	0.77	0.94
t-CAL	0	3.87	3.41	5.16	2.28	2.31	1.47
t-tab (0.95)	0	2.77			2.57		

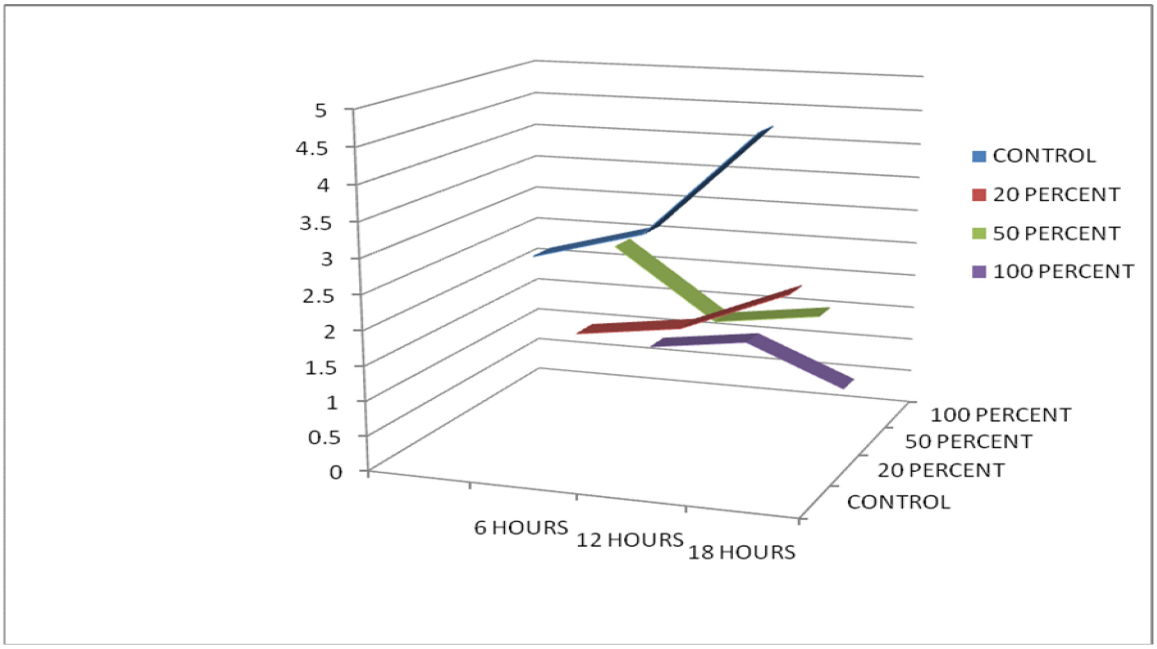

Fig. 2: mitotic index of *A. cepa* treated with different concentration of chloroquine and different durations

Table 2: Frequency of Chromosomal Aberrations Induced by Chloroquine in *A. cepa*

Chloroquine				% Abnormal Cell Observed					
Conc.	Duration	No of Cells Exam	Mitotic Index	N	I	P	M	A	T
20%	6HRS	687	1.72	35	0	20	0	80	0
50%	"	516	2.67	7	0	0	71	29	0
100%	"	518	0.8	6	0	0	100	0	0
Control	"	602	3.1	0	0	0	0	0	0
20%	12HRS	617	1.93	6	0	0	100	0	0
50%	"	716	1.66	13	0	25.6	74.4	0	0
100%	"	736	1.16	10	0.5	0	74.5	25	0
Control	"	885	3.5	0	0	0	0	0	0
20%	18HRS	612	2.5	25	4.6	0	70.4	0	25
50%	"	490	1.86	7	0	0	0	100	0
100%	"	842	0.4	4	0	0	100	0	0
Control	"	453	4.9	0	0	0	0	0	0

N= total number of abnormal cells, I= Interphase, P=Prophase, M=Metaphase, A=Anaphase, T=Telophase

Effect of Chloroquine on the chromosome behavior in *Allium cepa* root cells

Table 2 shows the percentage chromosomal aberrations induced by chloroquine in *A. cepa* root cells. The results reveal that most of the aberrations observed in chromosomes were at metaphase and anaphase, and very few at prophase and telophase. The aberrations also increased with duration of treatment. At 6hours 20% chloroquine treatment produced 80% anaphase chromosomal aberration and 20% prophase chromosomal aberrations, whereas 50% chloroquine at 6hours produced 71% metaphase aberrations and 29% prophase aberrations. Meanwhile 100% chloroquine at 6hours induced only metaphase aberrations.

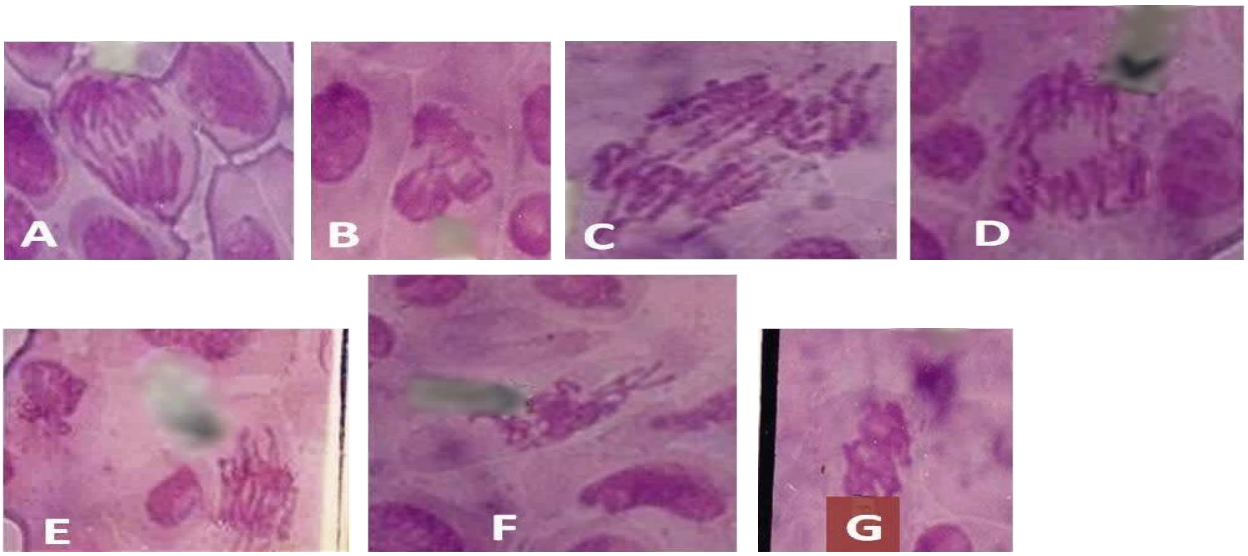

Fig. 3: chromosomal abnormalities in *A. cepa* treated with different concentrations of chloroquine and at different duration: A. Anaphase bridge; B. Fault polarization; C. Chromosome fragmentation; D. Disorderly anaphase; E. Adherent chromosome; F. Clumping of chromosome; G. Abnormal metaphase and H. C-metaphase.

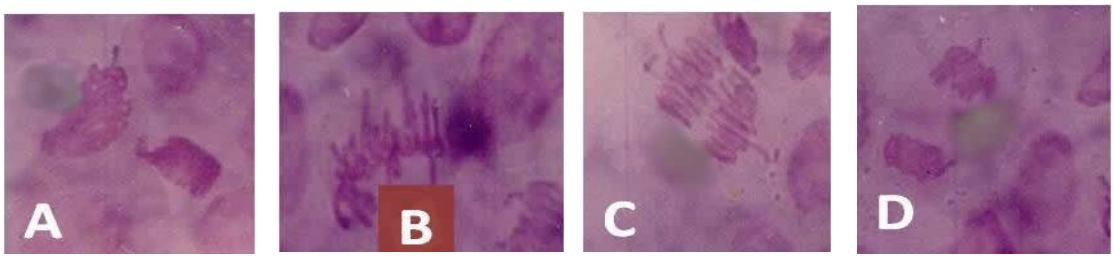

Figure 4: Normal mitosis in *A. cepa* cells with control at 18hours

A. Normal prophase; B. Normal metaphase; C. Normal anaphase; D. Normal telophase

Similarly at 12hours 20% chloroquine treatment, all aberrations in chromosome were observed at metaphase, whereas 50% chloroquine treatment induced 25.27% prophase and 74.43% metaphase aberrations respectively. 100% chloroquine treatment at 12hours induced substantial chromosomal aberrations at metaphase (74.5%) and anaphase (25%). At 18hours duration 20% chloroquine induced 70.4% metaphase and 25% telophase aberrations whereas 50% chloroquine treatment induced only anaphase aberrations. Similarly, 100% chloroquine induced only metaphase aberrations. Figure 3 shows chromosomal aberrations in *A. cepa* root cells treated with different concentrations of chloroquine at different durations. The abnormalities observed were more at anaphase and includes anaphase bridges, fault polarization, chromosome fragmentation and disorderly anaphase at 20% and 100% chloroquine concentration, Figure 3(a-d), whereas metaphase abnormalities include adherent chromosomes, clumping of chromosome, c-metaphase, abnormal metaphase with 20 %, 50% and 100% chloroquine treatment at 12hours, and 6hours, 18hours respectively (e-h). Figure 4 (a-d) shows the normal mitosis with distilled water treatment of *A. cepa* roots.

Discussion

The results obtained showed a lowering of the mitotic index and development of some chromosome abnormalities especially in cells treated with high concentrations of chloroquine. This suggests an inhibitory effect of chloroquine on DNA biosynthesis. This may further imply an inhibitory effect of chloroquine on *A. cepa* cell growth. The mitotic index can also be used to quantify differences in cell division when an environmental parameter is changed (Darbelley *et al.*, 1989) and is determined by the ration of dividing cells to the total number of cells examined for each treatment (Balog, 1982). The various abnormalities observed further gave credence to the negative effect of chloroquine. The higher percentage of interphase cells as compared to the control as observed in this experiment is an indication that chloroquine inhibited cell division considerably and agrees with the work of Shehab (1979), in respect of the effect of *Teucrium pilosum* extract on the root mitosis of *A. cepa* as well as agrees with report of Tabor & Oney (2009) on their work on the effect of artificial fertilizers on mitotic index in *Vicia hybrid.* For a group of cells that rarely complete the cell cycle, a high proportion of cells to be in the resting stage of the cell cycle is expected (Darbelley *et al.*, 1989).

The various chromosomal aberrations observed in cells treated with chloroquine even at the lowest concentration (20%) used as opposed to the control may suggest that chloroquine can be used in mutagenic studies in cells. The type of

chromosomal aberrations observed at metaphase were similar to that reported by Badr & Eikington(1982), Umar (2004) and Oyelana & Ogunwenmo (2006), while the aberrations observed at anaphase was similar to those reported by Tabor & Oney (2009). The induction of highly spiralized chromosomes from interference of chloroquine with spindle formation has been described as c-mitosis by Levan (1938). The c-metaphase with unusual constriction of chromosomes as observed has earlier been suggested to be due to inhibition of protein biosynthesis during mitosis (Mercykutty & Stephen, 1980). The nuclear dissolution is similar to those reported by Mercykutty & Stephen (1980) for *A. cepa* and they linked this phenomenon to acute fragmentation of the chromosomes resulting from displacement of nucleotide by the action of chloroquine. The disintegration of nucleus in the100% concentration may be due to the stripping of the protein covering on DNA as suggested by Stephen (1984).

This study has shown that chloroquine is a strong mitotic inhibitor and could give rise to mitotic abnormalities with increase in concentration. Their accumulation in cells may be inhibitory to cell growth. It has further revealed that chloroquine, though used as an antimalarial drug, can likely be used in plant mutagenic studies since it has the tendency of interfering with DNA biosynthesis. There is however need to extend investigation using much lower concentrations of chloroquine as well as other antimalarial drugs in order to ascertain of their effect on mitotic index and chromosomal behavior.

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