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Research Article

Cytotoxic/Genotoxic Effects of the Antibiotic Streptomycin on Root Meristem Cells of *Allium cepa* L.

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ABSTRACT

Antibiotics such as streptomycin are used not only to treat human and animal diseases but also for the control of plant diseases. The effect of the antibiotic streptomycin on plant root meristem cells was investigated with the aim of studying its cyto-genotoxicity using the *Allium cepa* assay. Onion root tips were treated with different concentrations of streptomycin for 3-,5- and 24 hours duration, respectively, and distilled water as control. For statistical analysis one way analysis of variance (ANOVA) with Tukey's post hoc multiple comparison tests at significance level $p < 0.05$ was performed. The results showed that streptomycin reduced mitotic index and induced chromosomal aberrations in root tip cells like c-metaphase, vagrant chromosomes, anaphase bridge, contraction of chromosomes, chromosome fragmentation, disorderly anaphase and clumping of chromosomes at different concentrations and different duration of exposure. Thus, the results implicated streptomycin as an inducer of mitotic abnormalities and mutagenic to plant cells. This clearly indicates that streptomycin should not be the antibiotic of choice for control of diseases.

Keywords: Antibiotic, root tips, cytotoxicity, genotoxicity, mitotic index, chromosomal aberrations.

INTRODUCTION

Antibiotics or antibacterial compounds are the anti-microbial drugs used for the prevention (growth inhibition) and treatment (killing of bacteria) of bacterial infections. Antibiotics can be classified by their activity against a spectrum of microorganisms. The more the species of organisms that are killed, the broader is the spectrum of activity. Narrow-spectrum antibiotics target a few types of bacteria. Broad-spectrum antibiotics target many types of bacteria. Both types work well to treat infections. Using broad-spectrum antibiotics when they are not needed can create antibiotic-resistant bacteria that are hard to treat. Antibiotic resistance mostly develops in bacteria by mutation. Once acquired, the resistance can be transferred to other bacteria horizontally or to their progeny vertically[1]. New lethal strains of bacteria can evolve due to indiscriminate use of antibiotics. These new strains may be more lethal than the parent strain, posing a major environmental and health concern.

The antibiotics used for treating livestock animals are not metabolized and mostly excreted in manure and thus not only contaminate the environment but also affect the soil microbiome. These pose a serious risk of spreading antibiotic resistance genes in soil microorganisms[2,3]. The antibiotics can be taken up by plants which grow in manure enriched soil[4]. Besides their use for treating human and animal diseases, antibiotics are also sprayed on plants to control plant diseases. Bacteria and other prokaryotic microbes (e.g., phytoplasmas) are known to cause several diseases in plants of commercial importance, for example bacterial soft rots of fruits and vegetables, citrus canker caused by *Xanthomonas campestris* pv. *citri* and fire blight of apple and pear caused by *Erwinia amylovora.* Now-a-days antibiotics (for example, streptomycin, gentamycin, oxytetracycline, oxolinic acid) are also used directly on fruit crops like apple, pear and peach[5]. From a trial of about 40 antibiotics tested for their efficacy in controlling plant diseases, less than ten were brought into commercial use out of which only streptomycin is being used on a global scale[6]. Specifically, plant diseases like fire blight of pear and apple, flower and fruit infection of pear and apple trees caused by *Pseudomonas syringae*, and bacterial spot of pepper and tomato caused by *Xanthomonas campestris* are presently being managed by using streptomycin[7].

The antibiotics enter groundwater due to irrigation and rainfall[4]. Most water resources are gradually becoming polluted due to antibiotics and their metabolites coming from household and industrial waste, animal farms and agricultural run offs directly without treatment. The use of this water for irrigation and its uptake by plant roots may affect the vegetation, more importantly the agricultural crops. Therefore, recurrent, excessive and prolonged use of antibiotics may affect the physiology of plants by inducing harmful cytotoxic and genotoxic effects.

Tanaka and Satô[8] reported the mutagenic effect of streptomycin on the cells of *Tradescantia paludosa* undergoing mitosis pointing to its ability to cause minor genic changes such as recombination as well as major aberrations such as polyploidy and translocation. Nwangburuka and Oyelana[9] studied cytological effects of chloroquine on root mitosis of *A. cepa.* This study showed that chloroquine is a strong mitotic inhibitor and could give

rise to mitotic abnormalities with increase in concentration. The accumulation of chloroquine in the cells may be inhibitory to cell growth. The study further revealed that chloroquine, though used as antimalarial drug, can likely be used in plant mutagenic studies.

Streptomycin is a broad-spectrum aminoglycoside antibiotic derived from actinomycetes *Streptomyces griseus.* Streptomycin belongs to the class of organic compounds known as amino cyclitol glycosides. These are organic compounds containing an amino cyclitol moiety linked to a carbohydrate moiety.

The reason for the use of streptomycin in this study is its common use for controlling plant as well as livestock diseases. *Allium cepa* has been chosen for this study because of its large chromosomes which can easily be counted (2n = 16). Its chromosomes are relatively large and the species is amenable to cytological manipulations. The *Allium cepa* test was developed by Levan[10] and is considered to be a sensitive assay for monitoring toxic effects of various chemicals on mitotic division[11-16]. The harmful effects of toxins can be investigated by screening root meristematic cells for inhibition of mitosis and incidence of chromosomal aberrations.

MATERIALS AND METHODS

Preparation of different concentrations of streptomycin

Powdered streptomycin was used for preparation of different concentrations in sterile distilled water. The different concentrations prepared were 50 ppm, 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm.

Experimental design and treatment

Onion bulbs were induced to root by placing them on coplin jars filled with tap water with the base of the onion touching the surface of water. The rooted bulbs were transferred to coplin jars containing streptomycin solution of different concentrations (50 ppm, 100ppm, 200 ppm, 400 ppm, 600 ppm, 800ppm) with the roots immersed in the solution. The coplin jars with different concentrations of streptomycin were placed in an incubator for a duration of 3h, 5h and 24h, respectively. Roots from the bulbs placed on coplin jars containing distilled water served as the control. At the end of each treatment, healthy roots were randomly selected from bulbs for each treatment and their root tips were cut and transferred into vials containing fixative.

Mitotic studies and analysis

The root tips were fixed immediately after the antibiotic treatment in acetic alcohol (45% acetic acid: ethanol, 1:3) in the vials for 24h. The root tips were then transferred to 70% ethanol and stored in the refrigerator. For analysis, root tips were softened by treatment with a mixture of 1N HCl: 45% acetic acid (3:1). Squash preparations of root tips either stained with 2% Acetoorcein or Feulgen stain were made as suggested by Sharma and Sharma[17] and observed under the microscope. Observations were recorded from ten random optical fields for each treatment. The parameters used to assess cytogenotoxicity of streptomycin were Mitotic Index (MI) and Chromosomal Aberration frequency (CA) which were calculated by the equations used by Verdes-Teodor et al.[18] as given below:

$$MI = \frac{\text{number of dividing cells}}{\text{total number of cells}}$$

$$CA = \frac{\text{number of cells with chromosomal aberrations}}{\text{number of dividing cells}}$$

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests at significance level $p < 0.05$ were performed to analyse the means for MI as well as CA values with the IBM SPSS Statistics-21.

RESULTS AND DISCUSSION

The values for MI of root meristem cells of *A. cepa* are shown as the mean ± standard error (SE) in Table 1. The average MI value for control was 0.106, while that for all streptomycin treated roots was much lower. The decrease in mean MI observed in streptomycin treated roots in comparison to the control was significant at $p < 0.05$ (Figure 1) for all durations of exposure to each concentration.

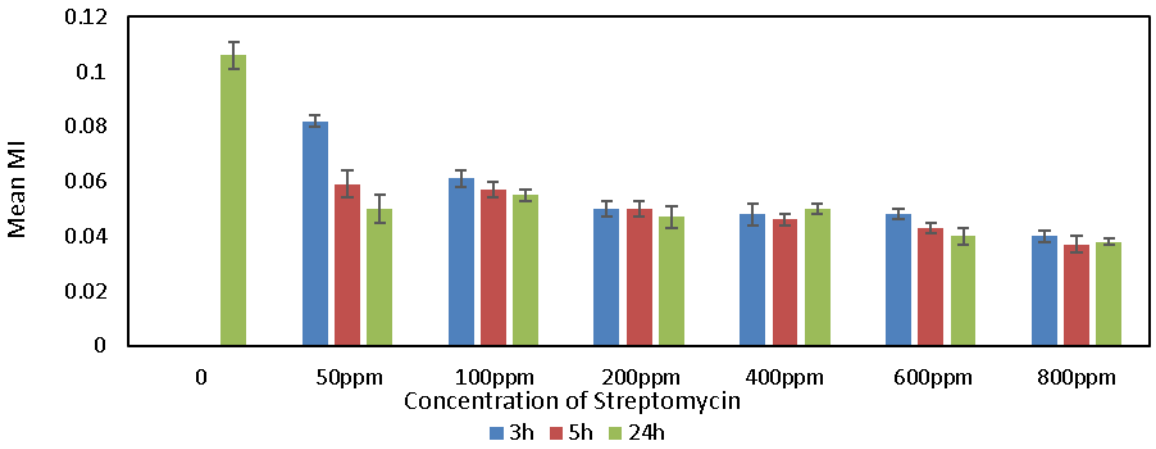

Figure 1: Effect of exposure to different concentrations of streptomycin for different durations on mean MI of *A. cepa* root cells. Error bars represent standard error of the mean.

Table 1: Mitotic index, chromosomal aberration frequency (CA) of root tip cells of *A. cepa* following treatment with different concentrations of streptomycin for different durations.

Streptomycin Concentration (ppm)	Duration of treatment	Mean Mitotic Index ± SE	Mean CA ± SE
Control		0.1060 ± 0.00499	0.0000
50	3h	0·0820 ± 0.00200 [a]	0.2850 ± 0.03998 [b]
	5h	0.0750 ± 0.00401[a]	0.3860 ± 0.03959 [b]
	24h	0.0650 ± 0.00543 [a]	0.4790 ± 0.03900 [b]
100	3h	0.0610 ± 0.00277 [a]	0.3300 ± 0.02463 [b]
	5h	0.0570 ± 0.00260 [a]	0.4430 ± 0.02071[b]
	24h	0.0550 ± 0.00167 [a]	0.4980 ± 0.02653 [b]
200	3h	0.0500 ± 0.00298 [a]	0.4440 ± 0.04306 [b]
	5h	0.0500 ± 0.00258 [a]	0.4560 ± 0.03513 [b]
	24h	0.0470 ± 0.00367 [a]	0.6120 ± 0.03901 [b]
400	3h	0.0480 ± 0.00359 [a]	0.4520 ± 0.02342 [b]
	5h	0.0460 ± 0.00163 [a]	0.4610 ± 0.03851 [b]
	24h	0.0500 ± 0.00211 [a]	0.6280 ± 0.03133 [b]
600	3h	0.0480 ± 0.00249 [a]	0.4850 ± 0.01968 [b]
	5h	0.0430 ± 0.00213 [a]	0.5730 ± 0.02329 [b]
	24h	0.0400 ± 0.00298 [a]	0.6650 ± 0.02272 [b]
800	3h	0.0370 ± 0.00213 [a]	0.6310 ± 0.02073 [b]
	5h	0.0350 ± 0.00307 [a]	0.6500 ± 0.02777 [b]
	24h	0.0350 ± 0.00167 [a]	0.8070 ± 0.02385 [b]

[a, b] Values show significant difference from control at $p < 0.05$

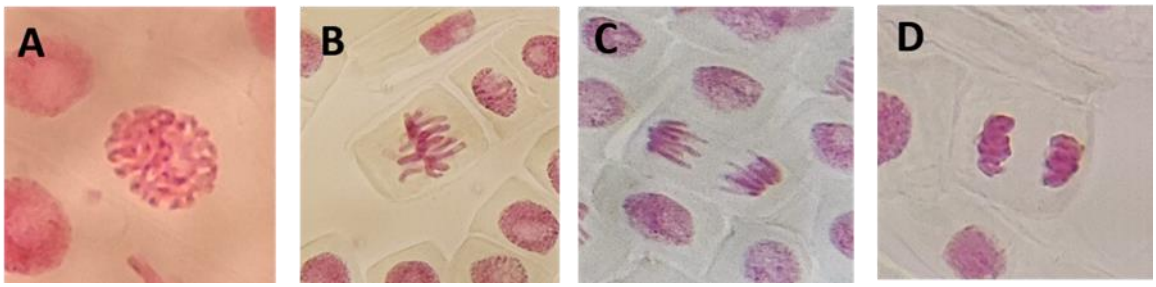

Figure 2: Normal stages of mitosis observed in control roots of *Allium cepa*. A. Prophase, B. Metaphase, C. Anaphase and D. Telophase (images at X 400).

However, the difference between mean MI for exposure to some concentrations of streptomycin for some durations was not significant. Thus, the mean MI values for 3h, 5h and 24h duration of exposure to 50 ppm streptomycin were significantly different from MI values for the three durations of 800 ppm treatment. On the other hand, the difference between means of MI of 50 ppm treatment and 100ppm, 200 ppm, 400 ppm and 600 ppm, respectively, did not differ significantly for all durations of exposure.

Mitosis in control roots of *A. cepa* was normal (Figure 2). The results obtained showed development of chromosomal abnormalities in cells of all roots in response to streptomycin treatment especially in those treated with high concentrations of streptomycin.

Although the streptomycin concentration ranged from 50 ppm, 100 ppm, 200 ppm, 400 ppm, 600 ppm to 800 ppm, it induced various types of chromosomal aberrations. The earliest chromosomal responses to streptomycin treatment were contraction, stickiness and clumping of the chromosomes. However, at higher concentration more types of chromosomal aberrations were observed. The mitotic abnormalities which were observed in the current study, carried out with different concentrations combined with different duration of treatment, are presented in Table 2. While the prophase stage seemed largely unaffected except for a few cells, all other stages showed occurrence of abnormalities which include sticky chromosomes, c-metaphase, irregular anaphase, chromosomal bridges in anaphase and telophase, and vagrant chromosomes. Another abnormality was the reductional grouping of chromosomes (Figures 3 and 4).

Table 2: Chromosomal abnormalities observed in the root tip cells of *Allium cepa* L. exposed to different concentrations of streptomycin for different durations.

Concentration of Streptomycin	Duration of treatment		
	3 h	5 h	24 h
50 ppm	Stickiness, contraction, irregular metaphase and anaphase, vagrant chromosomes	Stickiness, contraction, c-metaphase, irregular anaphase, vagrant chromosomes	Stickiness, contraction, irregular anaphase, vagrant chromosomes
100 ppm	Contraction, irregular metaphase and anaphase, vagrant chromosomes	Contraction, irregular metaphase and anaphase, vagrant chromosomes	Clumping, contraction, stickiness, irregular metaphase and delayed metaphase
200 ppm	c-metaphase, fragmentation, reductional grouping, stickiness, clumping	c-metaphase, clumping, stickiness, contraction	c-metaphase, clumping and contraction
400 ppm	Stickiness, contraction, clumping, irregular metaphase and anaphase	Clumping, c-metaphase, reductional grouping	Clumping, contraction, c-metaphase, reductional grouping
600 ppm	Contraction, clumping, stickiness, fragmentation, c-metaphase, delayed metaphase, chromosomal bridges, vagrant chromosomes	Contraction, clumping, stickiness, fragmentation, c-metaphase	Contraction, clumping, reductional grouping
800 ppm	Contraction, clumping, stickiness, vagrant chromosomes, telophase bridges	Clumping, stickiness, reductional grouping, fragmentation, c-metaphase	Clumping, reductional grouping

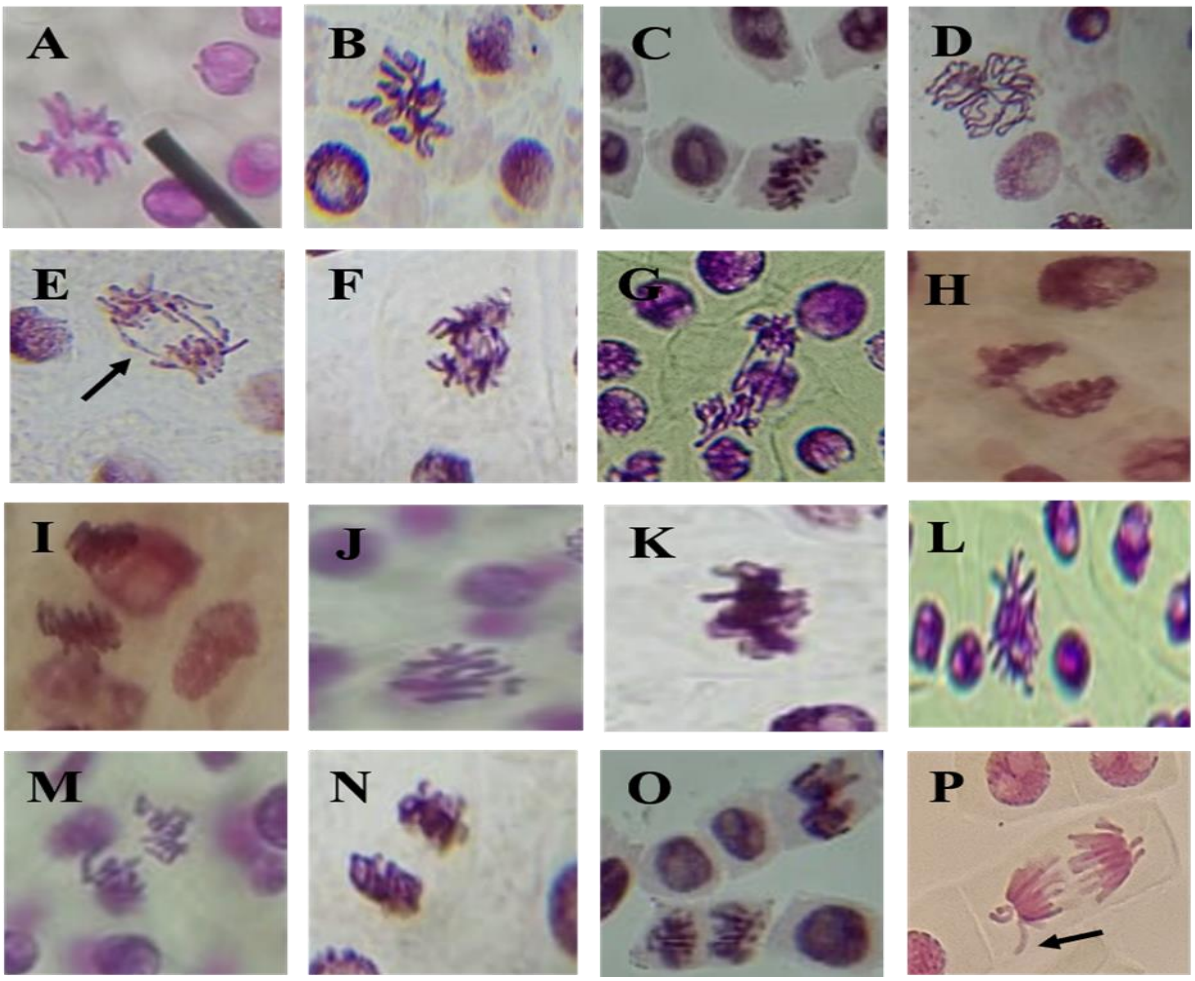

Figure 3: Contraction of chromosomes (A-C): A. 100 ppm, 3h; B. 400 ppm, 3h; C. 600 ppm, 5h; Delayed metaphase D. 800 ppm, 3h; Chromosomal fragmentation and bridges E. 400 ppm, 3h; Stickiness of chromosomes and multipolar anaphase F. 600 ppm, 5h; Stickiness of chromosomes (G-H, K): G. 800 ppm, 3h (note chromosomal bridges also); H. 800 ppm, 5h; Clumping (I-L): I. 200 ppm, 3h; J. 400 ppm, 3h; K. 400 ppm, 5h; L. 800 ppm, 3h; Reductional grouping (M-P): M. 200 ppm, 3h; N. 400 ppm, 5h; O. 800 ppm, 5h; Vagrant chromosomes P. 800 ppm, 24h (images at X 400).

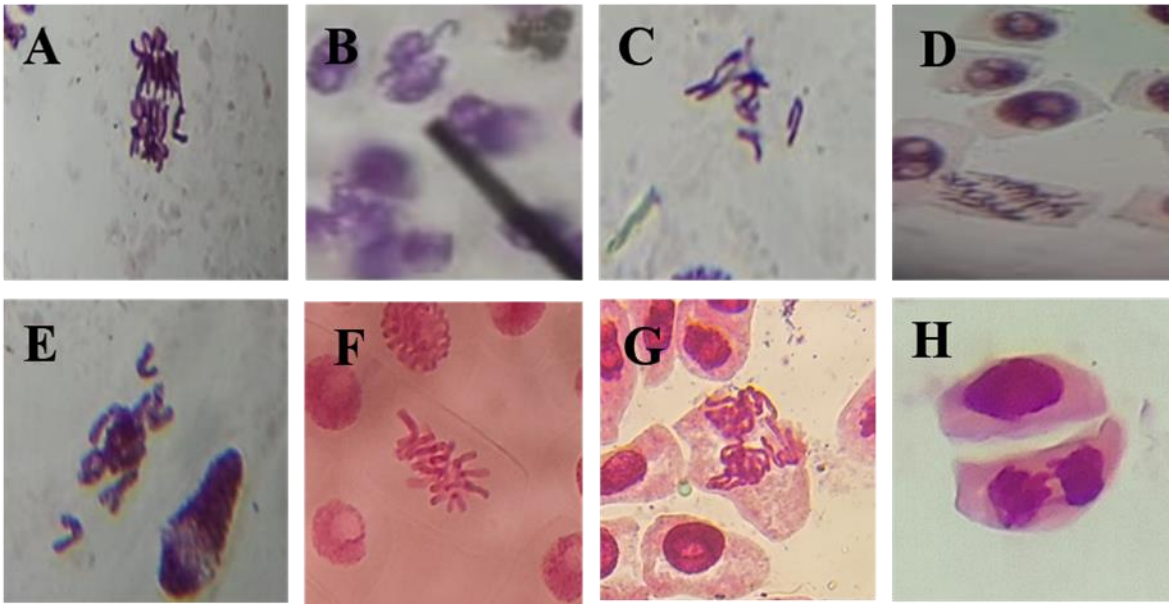

Figure 4: Chromosome fragmentation (A-D): A. 200ppm, 3h B. 600ppm, 5h C. 600ppm, 5h, D. 600ppm, 5h; c-metaphase (E-F): E. 800ppm, 5h; F. 600ppm, 3h; multipolar anaphase with chromosomal bridge G. 800ppm, 24h and telophase bridges H. 800ppm, 3h (images at X 400).

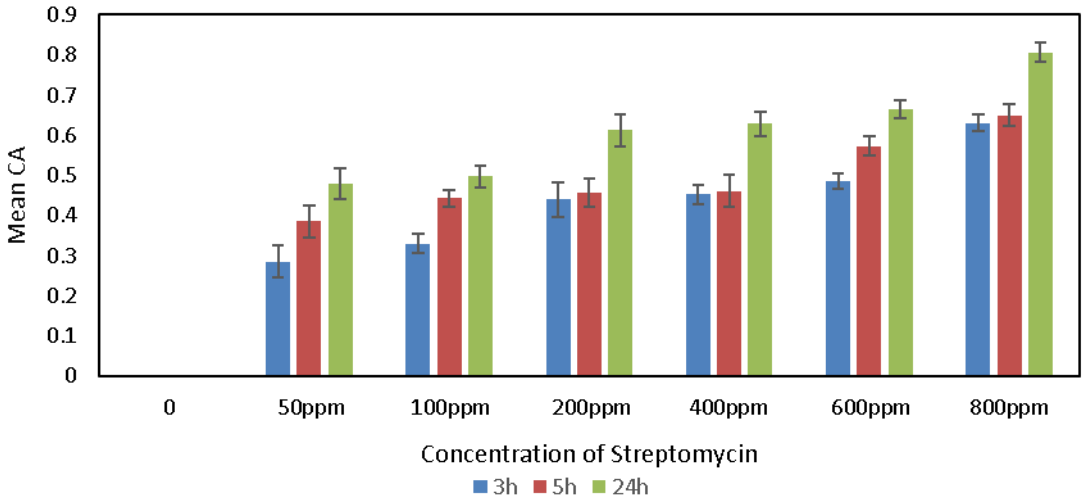

Figure 5: Effect of treatment with different concentrations of streptomycin for different durations on mean CA in *A. cepa* root cells. Error bars represent standard error of the mean.

The mean CA value for control was zero. All streptomycin treated roots showed presence of chromosomal aberrations. The CA values for all roots exposed to treatment with different concentrations of streptomycin are summarized in Table 1. An increase in CA of streptomycin treated roots was observed with increase in concentration which was significant at $p < 0.05$ (Figure 5). Such an upward trend was not so obvious with increase in duration of treatment. The difference between mean CA of 3h and 5h, respectively, and 24h was very obvious. Nevertheless, the differences between means of all groups were significant.

The results suggest that streptomycin causes chromosomal aberrations and disruption of mitosis in *A. cepa* root tip cells. Several investigators have used reduction in MI and induction of chromosomal aberrations as an indicator of cytogenotoxicity of chemical environmental pollutants[19-24]. The decrease in MI could probably be caused by the arrest of cells in G1 or G2 phase of the cell cycle, or suppression of synthesis of DNA during S phase[25-27]. Lowered MI has also been attributed by de Oliveira *et al.*[28] to incidence of c-metaphase since it prevents division of the sister chromatids and nucleus. Treatment with several chemicals causes chromosomal aberrations in a number of plants[29-35]. Antibiotics have been shown to have a disruptive effect on plant root mitosis in some studies[8,9, 36-39].

In the present study, stickiness, clumping and super-contraction of chromosomes; reductional grouping; c-metaphase; chromosome fragmentation and chromosomal bridge formation have been observed in the root tip cells undergoing mitosis in response to streptomycin treatment of *A. cepa* roots. Wilson too reported that in *A. cepa* roots streptomycin has a toxic effect on the mitotic apparatus, causing irregular anaphase and reductional grouping in

prophase[38]. In 1951, Wilson & Bowen observed stickiness of chromosomes and clumping in dividing cells of *A. cepa* root tips treated with streptomycin[39]. The authors of the present study report not only many other chromosomal aberrations induced by streptomycin such as c-metaphase, vagrant chromosomes, chromosomal bridge and multipolar anaphase but also lowering of MI. Mercykutty and Stephen studied the effect of Adriamycin treatment on *A. cepa* roots and reported stickiness at prometaphase and metaphase, failure of spindle formation resulting in c-metaphase, and chromosomal aberrations in the form of chromosome fragmentation and chromosomal bridges at anaphase[37]. Similar chromosomal aberrations have also been reported in mitotic cells of *A. cepa* root tips treated with the antibiotic chloroquine[9].

Stickiness is the tendency of chromosome arms to stick together. Stickiness of chromosomes which sometimes follows the chromosomal bridge ("sticky bridge"), is one of the most recurrent responses when mitotic cells are exposed to either ionizing radiations or chemicals, especially those having mutagenic action. Evans suggested that partial dissociation of nucleoproteins and alteration in the pattern of chromosome organization is the cause of stickiness[40]. On the other hand, Nefic et al., attributed stickiness to excessive formation of nucleoproteins and inappropriate protein-protein interaction which results in interchromosomal linkages of sub-chromatid strands[35]. According to Gaulden, stickiness could be the result of alteration in the functioning of specific non-histone proteins which may possibly be due to mutations in the structural genes encoding them (hereditary stickiness)[41].

Mercykutty and Stephen consider c-metaphase with unusual contraction of chromosomes to be due to inhibition of protein biosynthesis during mitosis[37].

Reductional grouping appears to be due to failure of chromatids to separate after metaphase and the subsequent segregation of chromosomes just like in anaphase I of meiosis[37]. Vagrant chromosomes, which move ahead of other chromosomes towards the pole of the cell during anaphase, are believed to be the outcome of non-disjunction of chromatids or unequal chromosome distribution[21]. Multipolar anaphase is likely to be caused by the formation of multipolar spindle and can result in aneuploidy and cell death in the subsequent cell cycles[42].

The chromosomal bridges observed in the cells are probably formed by breakage and fusion of chromosomes or chromatids[43]. Chromatid or chromosome breakage and fusion can lead to loss of genetic material and thus is of genetic significance, in causing the genic changes such as recombination and deletion of genes. Any mutagenic exposure may lead to random breakage and subsequent reunion of chromosomes which take place in the early prophase or interphase.

CONCLUSION

The action of streptomycin upon the mitotic cells of *Allium cepa* L. roots was studied cytologically using different concentrations of antibiotic streptomycin ranging from 50 ppm to 800 ppm. As per the observations recorded, several types of chromosomal aberrations including contraction of chromosomes, chromosomal stickiness, c-metaphase, fragmentation of chromosomes, vagrant chromosomes, anaphase or telophase bridge formation were caused by streptomycin. Therefore, it seems likely that effects of streptomycin upon mitotic cells are genotoxic or mutagenic. Thus, the use of streptomycin for control of diseases should be avoided.

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Conflict of interest

The authors declare that they have no conflict of interest.

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