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Research Article

Evaluation of Cytotoxic Potential of Oxytocin in *Allium cepa* L. Root Tip Cells

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ABSTRACT
The effects of oxytocin on mitotic cell division of *Allium cepa* L. were investigated to ascertain its mutagenic potential. Onion roots were treated with 0.1%, 0.2% and 0.3% concentrations of oxytocin and compared with control. Significant differences ($p<0.05$) were observed in mitotic indices (31.28±1.0, 31.17±0.8, 31.70±1.1) when treated with 0.1%, 0.2% and 0.3% concentrations of oxytocin respectively. Oxytocin induced cell mitotic aberration like polyploidy, stickiness, chromatin bridges, failure of cell-plate formation, chromosome breaks, shortening and contraction of chromosomes, vacuolization, anaphase disturbance, tumor, multipolar spindle, anaphase with laggard, telophasic bridge and chromosomal loss were also seen. We concluded from the present investigation that oxytocin is a mitotic depressor as well as mutagenic as observed in plant cell.

Keywords: Oxytocin, Onion roots, Mitotic aberration, Chromosome breaks, Mitotic depressor, Mutagenic.

INTRODUCTION

Allium is the largest genus of petaloid monocotyledons, containing hundreds of species distributed naturally in temperate climates of the northern hemisphere (1). It has been used by many researchers to investigate physical and chemical mutagenesis, pollutant agents, plant extracts and similar active material's cytogenetic effects in mitotic cell division. It is stated that the Allium test exhibits similar results with mammalian test systems (2, 3). Induction of mitotic abnormalities on root tip cells of plants may cause a decrease in mitotic index (4-6).

Oxytocin is a nine amino acid neuropeptide that helps in contraction of uterine smooth muscles (7, 8). The cyclic nonapeptide oxytocin and its structurally related peptides facilitate the reproduction in all vertebrates at several levels. The effects of oxytocin containing medicinal plant extracts on root tip mitosis of *A. cepa* have already been studied (9).

Some chemicals accumulated within food chain to a toxic level then these chemicals affect directly the public health (10). Generally, toxic effects of environmental pollutants cause genetic damage on plant cells but toxicity is not always correlated with genotoxicity (4, 10). The present study was designed to examine the effect of oxytocin on mitotic cell division in root tip cells of *Allium cepa* L. commonly known as onion.

MATERIALS AND METHODS

Plant Material: The plant used as test material was *Allium cepa* L. (2n= 16). Fresh onion bulbs of same variety approximately 2 cm in diameter were purchased from registered authentic seed seller from Bhagalpur, Bihar. About 72 onion bulbs having weight 45-50 gm were selected for the study. Twelve onions were used as control and twelve for each of the experimental group. The basal root plates were scraped by means of sharp scalpel. Earthen pots were filled with filtered sand and onion base was placed on the wetted sand (half portion of the onion pressed on the sand). Ten clean and healthy bulbs of *A. cepa* were chosen for each treatment group.

Treatment with oxytocin: Plant materials were treated with different concentrations of oxytocin (0.1%, 0.2%, 0.3% and normal) dissolved in distilled water when the roots reached to 1.5-2 cm in length. Controls were also treated with distilled water for the same time periods. The concentrations were chosen according to their dose of application in agricultural field.

Fixation, hydrolysis and dyeing*:* The root tip cells were fixed, stained and examined using a compound microscope. The treated roots were rinsed in distilled water and cut into segments of 1-2 cm length from the tips and fixed in pure glacial acetic acid (45%) for 30 min at room temperature before rinsed twice in ethanol (70%) for 5 min. The specimens were then transferred into cases containing 70% ethanol and sealed with stretch film and kept at +4 °C until use. For mitotic studies, the root tips of *A. cepa* were fixed in mixture of 1:3 acetic acid and ethyl alcohol for overnight followed by 5-7 minutes treatment with 45% acetic acid. The root tips were then hydrolyzed in 1N HCl for 5 minutes followed by staining with 2% aceto-orcein following the methods (11). After proper fixation and staining, appropriate squash preparations were made for each of the treatment and control. Effect of oxytocin treatment and control on

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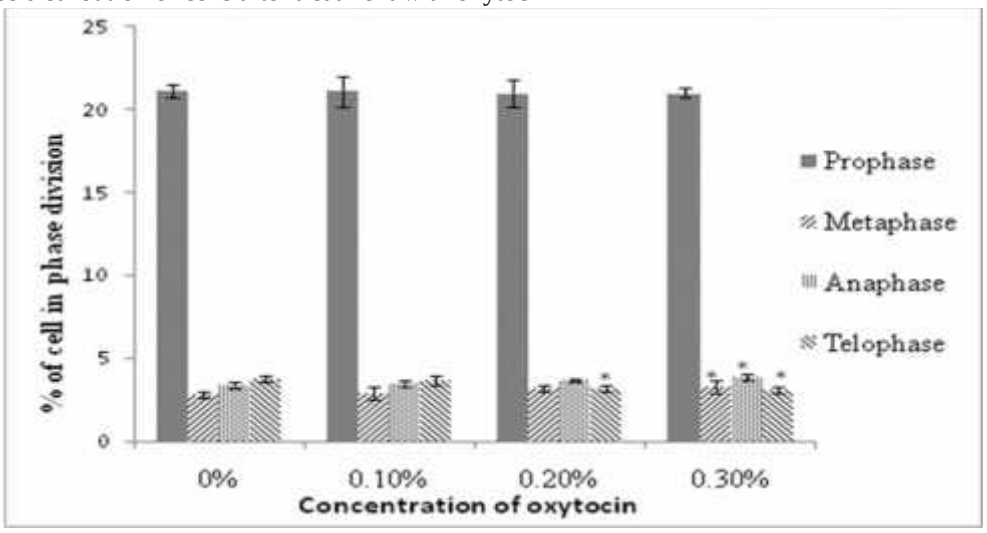
Table 1: Schedule for oxytocin spraying with different concentration and collection of root-tips

Round no.	Concentration of oxytocin	First spray	Second spray	Collection of root-tips
I	0.1%, 0.2%, 0.3%, Normal	Day 1	Day 3	Day 6
II	0.1%, 0.2%, 0.3%, Normal	Day 7	Day 11	Day 15

Figure 1: Phase distribution of cells after treatment with oxytocin



*Values are expressed as mean ± SEM of 6 observations. **p<0.05 when the value are compared with 0% of concentration of oxytocin.*

Table: 2 Mitotic index after treatment with oxytocin

Concentration	Cell Observed	Cell in division	Mitotic Index (MI)
Normal (control)	3580	1135	31.70 ± 1.1
0.10%	3420	1070	31.28 ± 1.0
0.20%	3460	1080	31.21 ± 0.5
0.30%	3560	1110	31.17 ± 0.8*

*Values are expressed as mean ± SEM. *Values in same column with different superscripts are significantly different at p<0.05.*

different chromosome plates were observed under light microscope. To determine the effects of this chemical in control group and in each treated group mitotic index (MI) were calculated.

Microscopic investigation: Mitotic index was calculated as percentage of dividing cells. Slides were scanned to investigate the different stages of mitosis. Approximately 3500 cells were scanned for each group of onion. The prepared slide was observed under a compound light microscope to study the different phases of mitosis.

For experimental group 0.1%, 0.2% and 0.3% concentration of oxytocin were prepared whereas in control group 100 ml of water was sprayed. The prepared concentration was sprayed in two rounds on day 1, 3, 7 and 11 (Table 1). The date of sowing onion bulbs were taken as day 01 and collection of root tips were done on day 6 for the first round and day 15 for the second round.

RESULTS AND DISCUSSION

Oxytocin has been reported to modulate social distance between males and females (12). It produces negative chronotropic effect even at low concentrations (90-125 nmol/L). It has numerous biochemical effects after binding to its membrane receptors, including the modulation of mitotic activity of various cell lines. *Allium cepa* has been selected for its relatively low chromosome no. (2n=16). Its chromosomes are relatively large and the species is susceptible to cytological manipulations (13). Allium test involved growing onion roots in the presence of oxytocin at various concentrations (14). The primary purpose was to remove the growing onion root tip and to prepare microscopic slides of the meristematic region in order to gather quantitative data of the number of cells undergoing mitosis relative to the number of cells in interphase (15).

The results showed lowering of the mitotic index and development of some chromosomal abnormalities in cells treated with 0.1%, 0.2% and 0.3% concentrations of oxytocin (Fig 1). This may further imply an inhibitory effect of oxytocin on *Allium cepa* cell growth. The various abnormalities in different stages like metaphase, anaphase, telophase etc. had been observed that further gave credence to the negative effect of oxytocin. These abnormalities cause genetic disturbances in the cell leading to mutagenic effects. It has been observed that rapid cell division at certain concentrations that lead to the splitting of root-tips in some onion bulbs.

Cytological effects of water extracts of *Pulicaria crispa* and *Teucrium pilosum* on *A. cepa* root tip mitosis showed depressive effect on both mitotic index and percentage of mitotic phases as well as an increase in the percentage of abnormal cells (16). It was found that inhibition of MI increased with increase in extract concentrations and duration of treatment. Mitodepressive effects of alcoholic extracts of five molluscicidal plants on root tip mitosis of *A. cepa* were also observed (17). It was reported that extracts of some medicinal plants, *Borreria filiformis* and *Vinca rosea* had a mutagenic effect (18). A common effect of medicinal plant extracts containing oxytocin on root tip mitosis of *A. cepa* is an inhibition of spindle mechanism leading to the scattering of the chromosomes, stickiness of chromosomes, anaphase bridge and diverse kinds of abnormalities (19, 20).

Table 2 shows the MI values and cell in division among the treatment with oxytocin at different concentrations. A lowered cell division index suggests an inhibitory effect at the interphase stage (20). The rate of mitosis was closely related to the resultant level of ATP (21, 22). The low mitotic index among treatments and decrease in other phases suggest that the treatments interfered with the respiratory pathways resulting in low ATP level. Treatments and concentrations had significant ($p<0.05$) effect on mitotic phases when compared to the control. Overdose of oxytocin concentration had significantly ($p<0.05$) higher depressive effects on all the stages of mitosis on the mitotic progress of *A. cepa* root tip. Prophase accumulation has been reported by other workers while treating *A. cepa* root tip cells with various extracts of medicinal plants especially at low concentrations (16, 17). The prophase accumulation has been attributed to delay in the breakdown of the nuclear membrane (23). It could also occur as a result of disturbance or breakdown of spindle apparatus (24).

Microscopic examination of squashed *Allium cepa* L. root tip meristem cells showed that oxytocin treatments induced a number of mitotic abnormalities when compared with control. The increase of mitotic abnormalities was dependent on the increasing concentrations. The most common chromosomal abnormalities were stickiness, laggards, c-mitosis, bridges, multipolarity, picnosis, star-anaphase, star-telophase, shortening and contraction of chromosomes, stickiness, chromatid bridges, chromosome breaks, failure of cell plate formation, polyploidy, anaphase disturbance, vacuolization, multipolar spindle, splitting of root-tips, clumping and fragmentation. Oxytocin caused a decrease in MI at all the treatment groups (Table 2).

It has been shown by many investigators that several other fungicides induce chromosomal abnormalities in different plants (25-29). In this study, the most common abnormalities were stickiness, laggards, c-mitosis, bridges, multipolarity, picnosis, star-anaphase, star-telophase, clumping and fragmentations in cell division. Disturbed metaphase, anaphase and telophase may be due to disturbance of spindle apparatus which allows the chromosomes to spread irregularly over the cell; results c-mitosis, star-anaphase and star-telophase respectively (30). Similar results on *A. cepa* cells were observed when maleic hydrazide in 10-3 M and 10-6 M for 2 and 24 h periods, were applied respectively (31). All our findings are in agreement with the other studies (32,33).

Thus, our study showed that oxytocin exhibits cytotoxic properties like lowering of the mitotic index and development of some chromosomal abnormalities in cells. However, the mechanism for such an effect needs further evaluation.

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