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CYTOGENETICAL STUDIES ON THE EFFECT OF OMNACORTIL ON ROOT TIP CELLS OF *ALLIUM CEPA* L.

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Abstract: Omnacortil is steroid, an allopathic drug commonly used as analgesic and suppressant in various diseases. In the present work, onion root tips were subjected with a series of omnacortil concentrations, ranging from 0.2 µg/ml, 0.4 µg/ml, 0.6 µg/ml, 0.8 µg/ml and 1.0 µg/ml for 24 hours for studying its effect on root mitosis in Allium cepa L. The roots were examined in permanent root tip squash preparations stained by the aceto-orcein. This work has confirmed that omnacortil have various effects on chromosomes and induced different mitotic abnormalities and structural aberration of chromosomes. Mitotic index (MI), relative division rate (RDR), relative abnormality rate (RAR) of treated material has been determined. Obtained results showed that all the concentrations of omnacortil showed higher MI and RDR, lower concentrations such as 0.2 µg and 0.4 µg showed higher values over rest of the concentrations and control. Among the subjected concentrations 0.8 µg concentration is most effective and showed highest RAR. Low concentrations of steroids act as stimulants while higher concentrations induced various chromosomal aberrations such as clumping and stickiness, fragmentation, C-mitotic effect, anaphase bridge, chromosomal condensation and contraction, dissolution of chromosome, which clearly showed the mutagenic, clastogenic, antimitotic and cytotoxic effect of omnacortil. This is the first report detailing the effects of omnacortil on chromosomes.

Key words: Omnacortil, *Allium cepa*, Antimitotic effect

INTRODUCTION

Omnacortil is one of the corticosteroid containing prednisolon. The corticosteroid is amongst the most widely employed therapeutic agent used as anti-inflammatory and immunosuppressant. However, they do not prevent the progress of underlying disease and only offer a symptomatic relief. Prolonged administration of steroids necessarily leads to adverse effects viz, adrenocortical failure, immunosuppressant, appearance of red spot on the body, dizziness, cardiovascular diseases and cushing's syndrome [1]. Nevertheless, scanty reports are available on clastogenic, mutagenic and carcinogenic properties of such drugs [1]. According to Grant [2] cytological aberrations in plants serve as an excellent monitoring system for the detection of effect of chemicals that may pose a genetic hazard. That plant systems are sensitive indicators of cytological aberrations is clear from studies by Fernandez-Gomez [3] and Fernandez-Gomez et al. [4] on the C-mitotic effect of the four isomers (a, 3,By, 8) of hexachlor-ocyclohexane. In view of this background, the present study was undertaken to analyse the mutagenic and clastogenic effects of the omnacortil on root tip mitosis of *Allium cepa* L.

MATERIALS AND METHODS

Allium cepa (2n=16) root tips were used as an experimental material. Healthy onion bulbs of cultivar Nashik red were allow to germinate roots by placing them in beaker containing water at a constant temperature 25 ^{0}C. After 72 hours roots were developed 2.0 to 2.5 cm, they were transferred to beaker containing test solution of omnacortil.

$$MI = \frac{\text{Total number of dividing cell}}{\text{Total number of cell examined}} \times 100$$

$$RDR = \frac{\text{Percentage of dividing cell in treated root tips - Percentage of dividing cell in control root tips}}{\text{100 - Percentage of dividing cell in control root tips}} \times 100$$

$$RAR = \frac{\text{Percentage of abnormal cell in treated root tips – Percentage of dividing cell in control root tips}}{\text{100 - Percentage of dividing cell in control root tips.}} \times 100$$

Aqueous test solutions of omnacortil were prepared as a 0.2 µg/ml, 0.4 µg/ml, 0.6 µg/ml, 0.8 µg/ml and 1.0 µg/ml. Roots from water serve as a control. Each treatment was lasted for 24 hours. Treated root tips were fixed in carnoy's fixative (3 parts ethanol: 1 part glacial acetic acid) and hydrolyzed for 10 minutes in 1 N HCl at 60^0, stained with 2% aceto-orcein and squashes in 45% acetic acid [5] for each treatment and slides were examined for mitotic abnormalities. For the calculation of mitotic index (MI), relative division rate (RDR) and relative abnormality rate (RAR) the above formulae were used.

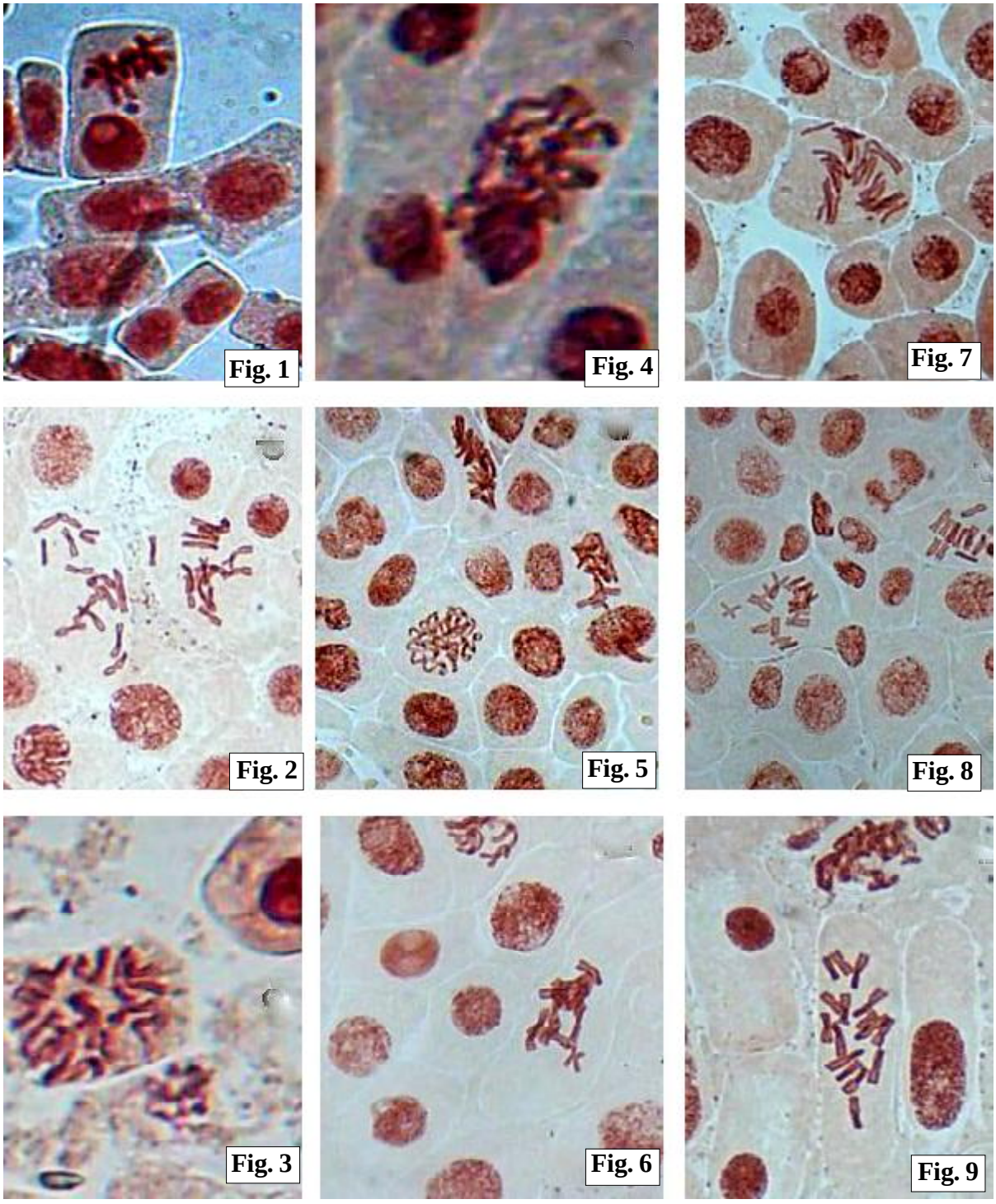
RESULTS

Treatment of different concentrations of steroids (omnacortil) induced different types of chromosomal aberration, viz. clumping and stickiness of chromosome, binucleate cell, micronuclei, anaphase bridge, pairing of chromosomes, chromosome condensation and contraction, separation of chromosome and C-mitotic effect (Fig 1-9). Total number of cells was higher in control than the cells appeared in treated root tips. The MI in all concentrations of omnacortil were quite higher (61.19, 64.46, 31.11, 42.45 and 32.23) than control (11.47). Lower concentration of omnacortil 0.2 µg and 0.4µg causes higher R.D.R. (56.16 and 59.85) due to its stimulatory activity (Table 1). While higher concentrations (0.6 µg/ml, 0.8 µg/ml and 1.0 µg/ml) exhibited higher RAR. (22.18, 34.49 and 23.42). This clearly indicated the stimulatory activity of lower concen-tration and cytotoxic effect of higher of omnacortil. Among the treated concentrations viz, 0.6 µg/ml, 0.8 µg/ml and 1.0 µg/ml concentration was most effective for the induction of various types of chromosomal abnormalities.

DISCUSSION

Cell division is one of the most important phenomenons, which control the growth of the organism. Behavior of the chromosomes during cell division is one of the unique features of the cell division. Effect of various drugs depends on the cell cycle of the organism. Cell treated in early interphase (G_1), affects synthetic process, while latter anaphase causes unequal distribution of the chromatids.

The chromosomal aberration causes changes in the gene combinations, which affects the morphological traits or metabolism of the organism. Prime objective of the work was to study the effect of omnacortil on the mammalian cell or human cell and create awareness among the people towards the excess or unnecessary use of steroids. For the present study *Allium cepa* was used as an experimental material because, chromosomal aberration appeared in a plant cells shows resemblances with the aberration produced in the mammalian cells [2]. Plant system serve as a first-tier bioassay system for the detection of the possible genetic damage [2]. The use of plant tissue primarily root tip for studying the induction of chromosomal aberration is one of the oldest, simplest, most reliable and inexpensive method. Perhaps the most serious disadvantage of a plant system for the detection of genetic risks to man is the lack of similarity between vegetative and mammalian metabolism. Nevertheless, the positive correlation which has been noted between aberrations induced by the same chemical in plant root-tip cells and in cultured mammalian cells indicates that a plant root-tip system must be recognized as an appropriate first-tier assay system [2].



Figs 1 to 9 are mitotic abnormalities induced by Omnacortil in root tip cells of *Allium cepa*. **Fig. 1:** Binucleate Cells, **Figs. 2,3:** C-Mitotic effect, **Figs. 4,5:** Clumping of chromosomes, **Fig. 6:** anaphase bridge, **Fig. 7:** chromosome pairing and **Figs. 8,9:** chromosome spreading.

Table 1: Effect of omnacortil on cell division of *Allium cepa* var. The values are the mean of the 10 observations. MI -Mitotic index, RDR -Relative division rate, RAR- Relative abnormality rate

Variables	Control	Omnacortil (Steroid) µg/ml				
		0.2	0.4	0.6	0.8	1.0
Total no of cells	610	506.6	403.3	450	353.3	496.6
No. of dividing cells	70	310	260	140	150	160
Abnormalities %						
Prophase	0.92	-	3.95	6.66	5.66	-
Metaphase	1.85	3.15	2.43	6.66	25.47	4.07
Anaphase -Telophase	3.70	1.38	2.72	4.44	-	10.0
Micronuclei	-	-	4.95	-	5.66	2.013
Binucleate cell	-	11.84	2.87	2.22	5.66	2.013
Clumping & Stickiness	-	3.94	3.95	11.11	-	6.041
Bridges	-	-	3.95	-	8.49	8.05
Fragmentation & pairing	-	-	-	4.44	-	8.05
Dissolution	-	-	-	-	5.66	-
Condensation & Contraction	-	1.97	2.47	-	-	-
Total Abnormality %	6.47	22.28	27.29	35.53	56.6	40.24
MI	11.47	61.19	64.46	31.11	42.45	32.21
RDR	-	56.16	59.85	22.18	34.99	23.42
RAR	-	12.21	17.86	27.17	50.97	32.49

Different concentrations of omnacortil induced various chromosomal aberrations viz., clumping and stickiness, fragmentation, C-mitotic effect, anaphase bridge, chromosomal condensation and contraction, dissolution of chromosome. Higher MI and RDR of treated material over the control indicated that the omnacortil exhibited both stimulatory activity and mutagenic agent since it causes various chromosomal abnormalities.

Colchicine mitosis defines as an inactivation of the spindle followed by a random scattering of the chromosomes over the cell [6]. Different concentrations of the omnacortil retarded growth of the roots by arresting cell division. Omnacortil causes spindle abnormality and produces C-mitotic effect. C-metaphase and lagging chromosomes even causes blockage of metaphase [7]. Similar results were obtained by Meenakumari and Stephen [8]. This has reveled the antimitotic property of the drug. Chromosomes clumping arise from improper folding of the chromosome fibers [9], which causes intermingling of the fibers due to which chromosome becomes attached to each other by means of subchromatid bridges. Chromosome dissolution refers to complete breakdown in chromosome structure resulting in the formation of long thin chromatin threads which possibly arises from complete dispiralization of chromosome [2]. The long chromatin threads form bridges between chromosomal materials [10].

Binucleate cells arise as consequences of inhibition of cell plate formation. This forms a distinct subpopulation of easily detected cells. Failure of cell plate formation in binucleate cells may give rise to multinucleate condition. Mitotic irregularities, such as incomplete anaphases or unequal distribution of chromosome to the daughter cells can result in aneuploid or even euploid cell [11]. In Anaphase, when the 2 sets of chromosomes move to opposite poles, the section of chromatin between the centromere is stretched across between the poles, hindering separation into new daughter cells. The pycnoticnuclei formation indicate the mitotic arrest at prophase and metaphase [12]. Arrest of cytokinensis leading to the formation of binuclate or multinucleate cells. Steriods act like a clastogenic compounds [13] and terbagenes compounds. Binucleate cell and multinucleate cell also reported in cancer cell [14].

Chromosome stickiness and clumping causes inactivation of cell. According to Gaulden [15] chromosome stickiness results from changes in specific non-histone proteins (topoisomerase II and the peripheral proteins) that are integral components of the chromosome and whose function is necessary

for separation and segregation of chromatids, the changes being caused either by mutation in structural genes for the proteins (heritable stickiness) or by direct action of mutagens on the proteins (induced stickiness); causes chromosome aberrations by the physical stretching and breaking of chromatids at the sticky sites; hence the breakage resulting from stickiness is a secondary effect that requires anaphase movement, in contrast to breakage resulting from direct action of mutagens on DNA.

According to Wagennar [16] during early prophase and late telophases of mitotic cells, chromosome attach end to end and form chain like or ring like chromosome association and cause is premature chromosome splitting.

Michael et al. [17] proposed that the singular phosphorylation of the amino-terminus of histone H3 involved to regulate protein-protein interactions to promote binding of trans-acting factors that "drive" chromatin condensation as cells enter M-phase and coordinate chromatin decondensation. In general, there is a precise spatial and temporal correlation between H3 phosphorylation and initial stages of chromatin condensation. Coordinate chromatin decondensation associated with M-phase.

According to Michelle and Michael [18] origin recognition complex (ORC) play some role in chromosomal folding. ORC is a subunit complex required for eukaryotic DNA replication initiation and silencing of the heterochromatic region. Guacci et al. [19] identified and analyze the *mcd1–1* mutant in budding yeast and showed that Mcd1p (mitotic chromosome determinant) is a chromosomal protein required for sister chromatid cohesion and condensation. They also suggested that Mcd1p work as a nexus between cohesion and condensation.

In the treated root tip cells, endopolyploidy was another serious abnormility which was due to endo-reduplication and C-metaphase. These are common cytological feature of cancer cell [6]. Obtained results coincide with those reported by Wong and Li [20], who pointed out a reduction of the growth, induced by vanadium in soybean.

Our results are supported by the work of Babu and Stephen [12], Meenakumari and Stephen [8] and Kirtane et al. [21] who studied the effect of Palgin, Analgin, tribhuvan kirti and Ultragin respectively on root tip of *Allium cepa*. Since large numbers of new drugs are introduced in the market without following the instructions insisted by World Health Organization (WHO), hence there is urgent need to study the harmful effect of such drugs. The present study concludes that, a higher doses of omnacotril causes hazardous effects due to its mutagenic, clastogenic and carcinogenic effects on cell system and suggested to avoid prolong use of steroids.

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