Cytological Assay of C-mitotic Potency of Colchicine Obtained from *Gloriosa superba* L.

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Since the discovery of colchicine as polyploidy inducing agent (Blakeslee 1937) there has been much demand for this alkaloid which stimulated a search for other chemicals, possessing similar effects, preferably less costly and easier to produce. As a result of this, over the years many chemicals (Levan and Östergren 1943, Kihlman and Levan 1949, D'Amato 1959, Mäkinen 1963) and several antimitotic agents (Bowen and Wilson 1954) have been tested for polyploidizing potentialities. However, colchicine is still the only alkaloid that fulfills the different requirements as an effective polyploidizing agent.

The source of colchicine is *Colchicum autumnale* L. a native of European countries. An allied species *C. luteum* L. grows in North-West Himalayas and much of its range falls in West Pakistan and its colchicine content is also lower (0.3%) than the *C. autumnale* (0.7%). (Mehra and Khoshoo 1951).

Clewer *et al.* (1915) for the first time isolated colchicine from the rhizomes of *G. superba*. Thereafter, numerous reports (Subbratnam 1952, 1954, Santavy *et al.* 1957, Fell and Ramsden 1967) have also confirmed the presence of colchicine in rhizomes and seeds of *G. superba*. Eigsti and Dustin (1955) have listed a number of plants which contain colchicine along with *G. superba* (0.23-0.3%) and suggested latter as a notable substitute for *Colchicum*. Due to the easy availability of *G. superba*, it can be a cheaper source of colchicine (Parthasarthy 1941, Mehra and Khoshoo 1951, Watt and Bryer-Brandwijk 1962, Narain and Khoshoo 1967).

Though colchicine induces C-mitotic reactions and polyploidy, but the effectiveness of the chemical obtained from different sources may vary. Therefore, it was thought desirable to have simple assay to test the differential potentialities and C-mitotic potency of the colchicine obtained from *C. autumnale* and *G. superba*.

Materials and methods

Cytological assay of the mitostatic quality of the crude drug and the colchicine, derived from *Gloriosa* was made from the dried powdered rhizomes and colchicine obtained through the courtesy of Messers Mehta Pharmaceuticals (Pvt.) Ltd., Amritsar, India. For comparison, standard colchicine from *Colchicum autumnale* L. obtained from Messers Russel of France, was used. The effect was tested on the root tips of *Allium sativum* L. which were treated with 100, 75, 50 and 25 ppm colchicine solutions and crude extracts from 2.5, 5, 10, 15 and 20 per cent dried rhizome powder. Control experiments were run by treating with tap water. All

experiments were run at 29°C. The root tips were fixed at an interval of 4, 8, 24, 48, 72, 96 and 120 hrs after treatment. After each fixation, the solution was changed regularly and cytological details were analysed from acetic lacmoid squashes. Results were computed according to the system proposed by Greenberg *et al.* (1966).

Results

Levan (1938) was first to notice that when root tips of growing plants are treated with the colchicine, mitotic division of the dividing tissues is arrested at metaphase. The chromosomes form a typical 'x' shaped configuration with their chromatids well spread and widely scattered in the cell. He introduced the term C-mitosis for such a reaction.

Root tips after four hours treatment in 100, 75 and 50 ppm solutions of colchicine obtained from *G. superba* showed C-mitotic reactions in the form of spindle dissolution and 'x' shaped chromosome configurations at metaphase. Furthermore, chromosomes were shortened and distributed randomly at metaphase plates (Fig. 2).

Table 1. C-mitotic potency of the colchicine and obtained from *G. superba* and *C. autumnale*

Strength/type of colchicine	2 hours	4 hours	8 hours
100 ppm			
Standard colchicine	1.37	2.8	4.20
<i>Gloriosa</i> colchicine	1.80	3.13	4.20
75 ppm			
Standard colchicine	1.08	2.0	4.44
<i>Gloriosa</i> colchicine	0.41	1.59	3.54
50 ppm			
Standard colchicine	1.0	1.31	2.90
<i>Gloriosa</i> colchicine	Not effective	1.10	2.65
25 ppm			
Standard colchicine	0.62	0.91	0.81
<i>Gloriosa</i> colchicine	Not effective	Not effective	Not effective

Root tips treated with lower concentrations (25 ppm) contained both normal and C-mitotic cells. The latter were not very conspicuous even after 96 hours. However, 5, 10, 15 and 20 per cent crude extracts of *Gloriosa* rhizome powder were lethal and the chromosomes were clumped (Figs. 4-5). At lower concentration (2.5%; of dried rhizome powder) neither lethality nor poison effects could be observed, however, cells showed positive C-mitotic activities (Fig. 3).

Macroscopic effects

Colchicine is also noted for its macroscopic effects, in the form of C-tumors as shown by Levan (1938) in *Allium* roots.

After treatment for 48 hours, a small swelling at the tip of roots was noticed in the three sets of experiments (Table 2).

There was further increase after 72 hours in swelling in all the cases but no

further swelling was found after 96 hours although the treatment was continued for a longer period. The tumors that developed in crude extract were less pronounced than those in colchicine solutions. Cytological analysis of the C-tumor showed a

Figs. 1-5. 1, somatic chromosomes of *Allium sativum* ($2n=16$) treated with 100 ppm colchicine from *C. autumnale* for 4 hours. 2, somatic chromosomes of *A. sativum* ($2n=16$) treated with 100 ppm colchicine from *G. superba* for 4 hours. 3-5, somatic chromosomes of *A. sativum* ($2n=16$) treated with 2.5% (Fig. 3), 5% (Fig. 4) and 10% (Fig. 5) crude extracts of *G. superba* rhizome for 4 hours. Figs. 1-3 $\times 900$. Figs. 4-5 $\times 600$.

Table 2. Chronology of tumor formation induced by colchicine in *Allium* roots

Colchicine	24 hours	48 hours	72 hours	96 hours	120 hours
100 ppm					
Standard colchicine	—	Formed	—	—	—
<i>Gloriosa</i> colchicine	—	Formed	—	—	—
75 ppm					
Standard colchicine	—	—	Formed	—	—
<i>Gloriosa</i> colchicine	—	—	Formed	—	—
50 ppm					
Standard colchicine	—	—	—	Formed	—
<i>Gloriosa</i> colchicine	—	—	—	Formed	—
25 ppm					
Standard colchicine	—	—	—	—	Formed
<i>Gloriosa</i> colchicine	—	—	—	—	—

high percentage (58.8%) of polyploid cells.

Recovery process

After treatment with colchicine and crude extract solutions, bulblets were transferred to tap water to see the recovery. Growth was resumed gradually and completely after 70–72 hours in roots treated with colchicine obtained from *Gloriosa* and *Colchicum* while there was no sign of recovery, in roots treated with crude extract which eventually died.

Discussion

It is a well known fact that colchicine does not inhibit mitotic initiation and normal course of mitosis but dissolves or aborts spindle, while other phases of nuclear development continue. Due to lack of spindle, the anaphases block but splitting of C-metaphase chromosomes automatically take place due to repulsion. Since each chromosome has divided before the nucleus was reconstituted, the tetraploid nuclei are formed. This is how tetraploid cells result after treatments with C-mitotic agents.

A series of concentrations of *Gloriosa* colchicine and standard colchicine were tested and it was observed that 100, 75 and 50 ppm solutions of *Gloriosa* colchicine induce C-mitotic (Table 4) and C-tumor (Table 2) reactions in *Allium* roots.

As is apparent from Table 1 and Fig. 6 (A–D) that *Gloriosa* colchicine has a C-mitotic potency of 1.8, while standard colchicine has 1.37, after 2 hours of treatment in 100 ppm solutions. The value obtained from *Gloriosa* colchicine is higher than from standard colchicine. The present results are in full agreement with those of Greenberg *et al.* (1966), who suggested that a standard sample of colchicine should not have a C-mitotic potency less than 1.7, after two hours of treatment in colchicine solution. The values of standard colchicine used in the present investigation are far lower than those of standard sample. The deterioration may be due to defective storage of chemical (Greenberg *et al.* 1966). *Gloriosa* colchicine at lower concentration (25 ppm) was less effective as compared to standard colchicine. A

continued treatment, however, was found effective but values were lesser than the standard colchicine (see Table 1 and Fig. 6D). Since *Gloriosa* colchicine at 50 ppm concentration is effective to induce C-mitotic and C-tumor reactions in *Allium* roots, therefore, it may also be considered its threshold value to induce C-mitotic and C-tumor reactions in plants.

Fig. 6. Graphs depicting C-mitotic potency of colchicine obtained from *Gloriosa superba* and *C. autumnale*. A: 100 ppm solution, B: 75 ppm solution, C: 50 ppm solution, D: 25 ppm solution.

A close look at the C-mitosis and C-tumor activities produced from *Gloriosa* colchicine and standard colchicine clearly indicate that there is great similarity

both at micro (Table 1 and Fig. 6) and macroscopic levels (Table 2). The crude extract when used at 5, 10, 15 and 20 per cent was lethal, and prevented C-mitoses and normal metabolic initiation of the mitosis but at lower concentration (2.5%) was a C-mitotic agent.

The initial effect of crude extract (at higher concentrations) on chromosomes was to induce clumping and bridges at anaphases (see Fig. 4) which later degenerated. Such toxic effects may be considered due to the presence and interactions of other compounds which also appear in the residue while extract is made. Evidently crude extract at higher dosage is a prophase poison, otherwise acts as C-mitotic substance.

Cytological analysis of the C-tumor has indicated that swelling at the ends of growing regions of roots, was due to occurrence of aberrant polyploidy in cells which consequently increased the cell size. Due to accumulation of such aberrant cells in growing region or meristematic tissues, there was swelling and consequently tumor formation.

Bulblets with swollen roots, when transferred to water, restored their further growth. This clearly indicates that long exposure of root to aqueous solutions of colchicine induces C-tumor reactions but has no permanent effects on growth, which is resumed as soon as favourable conditions are available.

Parthasarthy (1941) and Khoshoo (unpublished) are among the first to have studied the effects of crude extract on maize and *Pisum sativum* respectively and showed that crude extract has an effect on cell division much like that produced by colchicine. Parthasarthy (1941) also induced autotetraploidy in maize plants. Moreover, in this laboratory, a series of concentrations of *Gloriosa* colchicine and crude extracts were tried for induction of tetraploidy in several plants like *Impatiens*, *Bougainvillea*, and *Verbena*, while crude extract did not yield polyploids *Gloriosa* colchicine gave positive results.

Summary

C-mitotic activity of colchicine and crude extract obtained from *G. superba* was tested and C-mitotic potency was scored according to the system proposed by Greenberg *et al.* (1966). It was observed that 100, 75 and 50 ppm solutions of *Gloriosa* colchicine induced C-mitotic and C. tumor reactions. While crude extract at lower concentration (2.5% of dried rhizome powder) gave very promising results in inducing C-mitosis but higher dosages (5, 10, 15 and 20% of dried powder) were lethal and prevented mitotic initiations and normal course of mitoses in the dividing tissue. The roots with C-tumors when returned to water resumed their further growth. Therefore, it is concluded that *Gloriosa* colchicine has similar micro and macroscopic effects to that produced from colchicine obtained from *Colchicum autumnale* Linn.

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