
Cytological studies on vicia faba and allium cepa plants as affected by chemical extracts of some naturally growing plants in Egypt

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The present study represents the first that comprises the cytological effects of the phytochemically analysed plant extracts of *Cleome droserifolia* and *Portulaca oleracea* on *Allium cepa* root meristem and *Vicia faba* flowerbuds, as observed by light microscopy. In addition, tracing those effects on the ultramicroscopic level was carried out. The results of the work can be summarized in the following :

- Phytochemical screening of the aerial parts of *C. droserifolia* and *P. oleracea* plants, and the selection of a substance from each plant to isolate, purify and test.

The experiments tested for its cytological effects. The results showed that both plants contain tannins, sterols, terpenes, glycosides, and carbohydrates, alkaloids and mucilage; *C. droserifolia* contains flavonoids and saponins in addition. A mucilaginous substance was isolated in a pure form from each plant; Compound (1) ($C_{12}H_{20}O_{10}$) isolated from *C. droserifolia* and Compound (2) ($C_{10}H_{16}O_5$) from *P. oleracea*. Their structures were confirmed by paper chromatography, infra red and mass spectrum analyses. Light microscopic examination of *A. cepa* root tip cells to investigate effects of the tested material on the mitotic activity, percentages of phases, induction of abnormalities, the nucleoplasmic index, and the recovering ability of the induced effects after 24 hours immersion of the treated tissue in water. *Cleome* extract was tested at concentrations of 0.25%, 0.5%, 1% and 3%, while *Portulaca* extract at concentrations of 1%, 5% and 100%; treatment durations were for 4, 8 and 24 hours. The isolated compounds, Compound (1) and Compound (2), were applied as aqueous solutions of 100, 300 and 500 μ g/ml. In all the conducted cytological experiments control treatments using tap water, instead of the tested solution, were carried out. The observations proved the following :

- a- Both of *Cleome* and *Portulaca* extracts and the isolated compounds induced statistically significant, dose-dependent reductions in the mitotic index. These reductions were recoverable in all the extracts treatments but those of the highest concentrations and longest durations, where complete arrest at interphase was induced.
- b- Very weak or no effect of the plant extracts and the isolated chemicals on the percentages of the mitotic phases was observed.
- c- Effects on the percentage of total abnormalities induced by the two extracts were highly significant, with more recovery after treatment with *Portulaca* extract than *Cleome* extract. The most affected stage after direct treatment with either extract seemed to be

prophase. Treatment with Compound (1) and Compound (2) resulted in highly significant, dose-dependent increase in the percentage of total abnormalities. Anaphase and telophase seemed to be the most affected stage by treatment with either compound. Stickiness of chromosomes was induced by all the tested materials with very high percentages amounting to 100% of the induced abnormalities in some treatments. Much lower percentages of spindle disturbance, chromosome bridge, irregular prophase and chromosome despiralization were induced by direct and recovery treatment of Cleome and Portulaca extracts. In addition to stickiness, Compound (1) and Compound (2) induced very low percentages of bridges and spindle disturbance, respectively.

d- Statistically significant reductions in the nucleoplasmic index were induced by Cleome and Portulaca extracts, specially after high concentration and long duration treatments, which could not recover after treatment with Portulaca extract. This may reflect the existence of a relation between mitotic index decline and nuclear volume reduction.

Light microscopic examination of pollen mother cells and pollen grains in *V. faba* flower buds to test effects of the two plants extracts on the induction potential of meiotic irregularities (their total percentages, distribution in the 1st and 2nd divisions, and types), in addition to effects on pollen grain viability. Cleome extract solutions were applied at concentrations of 1% and 3% and those of Portulaca at 2.5% and 10%. Flower buds were covered with cotton wool pieces moistened with the extract solution for 3 hours, then removed. The flower buds were left intact and two groups were gathered after 24 and 48 hours of treatment. The data showed that :

- a- The two extracts induced highly significant increases in the percentage of total abnormalities which were negatively correlated to the treatment concentrations.
- b- The induced percentages of total abnormalities were diminished after elapse of 24-48 hours following treatments, meanwhile percentages of abnormalities of the 1st division were higher than those of the 2nd one. These observations point to recovery of pollen mother cells from the induced aberrations.
- c- Five types of abnormalities were induced by both plant extracts; namely, chromosome stickiness (which was the dominant type as in mitosis), bridges, un-oriented chromatin material and chromosome lagging, spindle disturbance, and multinucleate cells. It is obvious that most of the scored abnormalities in meiosis resemble those observed in mitotic cells.
- d- Aberrant spore tetrads were abnormally arranged after treatment with Cleome extract, while after Portulaca extract treatments they were sticky or deformed. Aberrant pollen grains were deformed after Cleome extract treatments, whereas those induced by Portulaca extract were either deformed, unstained or sticky. It was suggested that the induced abnormalities may negatively affect pollen grains viability.

Electron microscopy of *V. faba* root meristem treated with 0.5%, 1% or 3% Cleome extract, or 1%, 5% or 10% Portulaca extract, to investigate ultrastructural changes induced in the cytoplasm. The study showed the following changes in :

- a- Mitochondria : which appeared disrupted after Cleome extract treatments and slightly deformed after those of Portulaca, thus disturbance of the cells energetics could be expected.
- b- Dictyosomes : appeared with enlarged vesicles after Cleome extract treatments, whereas Portulaca extract treatments caused decrease in volume of the vesicles accompanied by increases in number, length and electron-density of dictyosomal cisternae, specially after high

concentration treatments. c- Most treatments of Cleome or Portulaca extracts induced the formation of autophagic vacuoles, which points to injury of the treated cells. d- The endoplasmic reticulum cisternae appeared destructed after Cleome extract treatments, while those of Portulaca extract induced the formation of whorls which may include some cytoplasmic organelles and acting as autophagic areas. From the obtained results it was concluded that four main effects were induced by the tested materials, these are : [1] Reduction of the mitotic activity, [2] high percentages of abnormal mitotic and meiotic figures, [3] diminution of the nuclear volume, and [4] disturbed metabolic energetics expressed as malformed mitochondria, dictyosomes and endoplasmic reticulum.