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Suppression of Mitotic Process Associated with Metaphase Arrest of *Allium cepa* L. Roots Using: I. *Rosmarinus officinalis* L. Water Extract

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ABSTRACT

Experiments were carried out to investigate the potential for using extract from *Rosmarinus officinalis* L. as a mitostatic and metaphase arrest agent. *Allium cepa* L. meristem was used as a biological system in this investigation. Different concentrations were made to establish the optimum dose response. The drug was concentration- dependent. Metaphase arrest with complete inhibition in spindle assembly was common feature. The metaphase to anaphase transition was blocked leading to discontinuous mitotic activity. Meanwhile, the drastic inhibition in protein synthesis in spite of accumulation of protein synthesis devices investigated by electron microscopy, gesticulate the mode of action of the drug which has been discussed.

Key Words: Mitosis; Metaphase arrest; Mitotic index; Inhibition in total protein; *Rosmarinus officinalis* L.

INTRODUCTION

Prosperous cell division demands the cell cycle events to occur in the correct and sequential order. In mitosis, metaphase remains undistinguishable until the chromosomes are condensed during prophase and the nuclear envelope breaks down during prometaphase (Gelfand & Scholey, 1992). Anaphase is not initiated until mitotic spindle is fully assembled, and the cytokinesis does not commence until anaphase is completed. The checkpoint mechanism blocks the onset of events until preceding events are complete (Hartwell & Weinert, 1989; Elledge, 1996).

Reports in literature indicate to involvement of various types of polypeptides in regulation of cellular processes. These polypeptides are classified as checkpoint proteins that may constitute a signalling complex (Kulberg & Morgan, 1999). In the metaphase to anaphase transition, the duplicated sister chromatids segregate to the two newly formed cells and any mistake in the process may be deleterious on both cells; this process is a point of no return. The anaphase inhibitor must be degraded, so that this transition can take place (Holloway *et al.*, 1993).

The metaphase to anaphase transition is controlled by the ubiquitin-dependent proteolytic destruction of two classes of regulatory molecules i) anaphase inhibitors ii) mitotic cyclins (Murray, 1995; Yamamoto *et al.,* 1996; Morgan, 1999). These regulatory proteins are finally transferred to the substrate itself (Hersko, 1997). The molecular roles of these regulatory proteins in the checkpoint response remains unclear as mentioned by Farr and Cohen-Fix (1999), and still equivocal.

Hence, ultrastructure investigation of the sub-cellular alteration induced as a result of chemical treatments provides changes in several compartments of the cell which processed basic biochemical systems. Organelles involved in protein synthesis including ribosomes, polysomes and endoplasmic reticulum were constantly submitted to exploration during growth under variable conditions (Zaki & Tawab, 2001). Indeed, protein synthesis has a remarkable influence on mitotic process and cell cycle control.

One of the most interesting chemical components is obtained from medicinal plants which comprise biological active constituents. *Rosmarinus officinalis* L. is a medicinal plant used in folk medicine as analgesic, anti-inflammatory and anti-microbial agent (Falerio *et al.,* 1995), owing to the presence of variable active constituents defined as volatile oil, diterpines, tannis, flavonoids and rosmarinic acids (Chevallier, 1996).

The work presented herein focuses on studying the mode of action of the crude extract from *Rosmarinus officinalis* L. on mitotic processes. Structural changes that occur at cellular level and associated with drug administration at various levels are investigated during this study. Moreover, ultrastructure survey on the protein synthesis devices was investigated and compared by untreated counterparts.

MATERIALS AND METHODS

Cytological studies. Bulbs of *Allium cepa* L. var Giga 6 Mohassan were germinated in glass vials using tap water. Water extract of *Rosmarinus officinalis* was prepared by

Fig. 1. Some types of abnormalities in *A. cepa* L. root tip cells after different treatments with *R. officinalis* extract. a) disturbance at metaphase (6 h, 0.5%), b) contracted chromosomes at metaphase (12 h, 0.5%), c) stickiness and disturbed metaphase (6 h, 1%) d) absence of spindle assembly at anaphase (6 h, 1%), c) disturbance at anaphase (3 h, 2%), f) multinucleated cell (6 h, 0.5%).

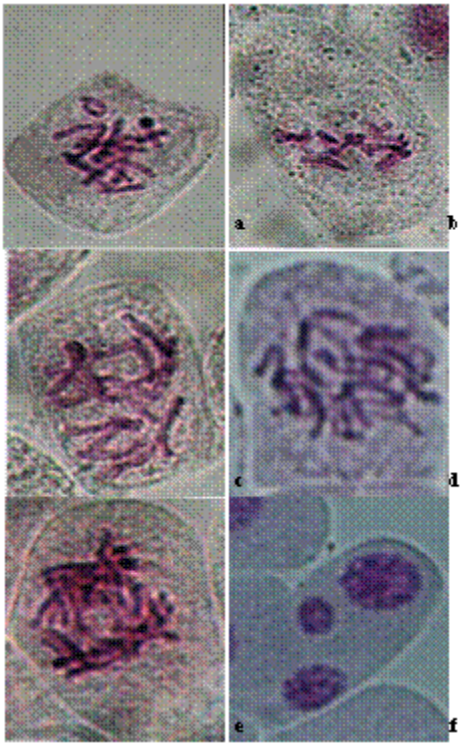


Fig. 2. Electron micrograph of enlarged portion of meristematic cell representing the control roots. This figure shows cell wall (CW), plasma membrane (PM), vacuole (V), mitochondria (M) proplastids (P), Golgy bodies (G), endoplasmic reticulum (ER), ribosomes and polysomes spread in the cytoplasm. (x 14000).

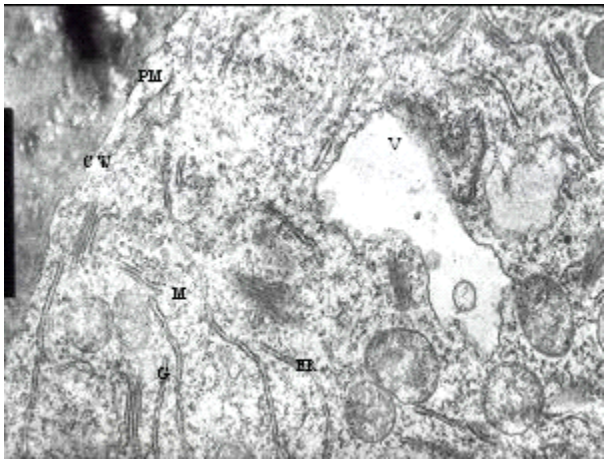


Fig. 3. Electron micrograph of cells with thin cell wall (CW) encloses the plasma membrane (PM). Occasionally, plasmodesmata (PD) are seen connecting the two neighbor cells (arrows). Elongated endoplasmic reticulum (ER), numerous mitochondria (M) and proplastids (P) in affluent cytoplasm with ribosomes when treated with 1% for 6h (x. 11500)

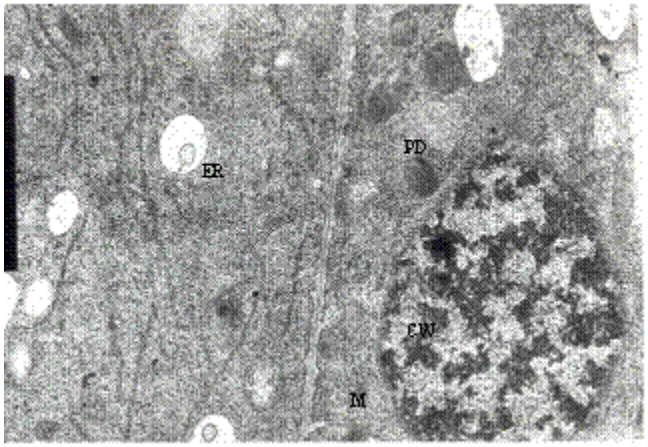
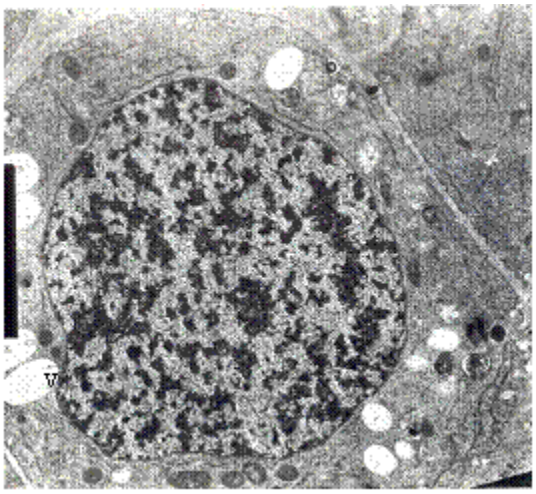


Fig. 4. Electron micrograph of an enlarged portion of cell where nuclear envelope is intact and surrounded by dense cytoplasm rich in ribosomes. The presence of numerous mitochondria (M) with performed structure, and endoplasmic reticulum (ER). (1% of 6 h). (x 6.000)



boiling the whole plant for 1 h, evaporated water was replaced. The roots were treated with 0.5, 1 and 2 g 100 mL^{-1} water extract for 3, 6 and 12 h control roots were simultaneously soaked in tap water. Following treatments, roots were detached, fixed in Carnoy's fixative for 24 h, repeatedly washed in water; then Feulgen squash technique was carried out. Six temporarily slides were prepared for each treatment and the control. At least 1000 cells per slide were examined; percentage of cell division, number and types of abnormalities in the mitotic phases were recorded. Statistical analysis for cell division was made using analysis of variance (ANOVA) in the statistical analytical system (SAS) software.

Electron microscopy. Samples of untreated root tips as well as treated roots were processed for electron microscopy studies. The samples were fixed for four h in 2% glutaraldehyde in 0.05 M phosphate buffer (pH 7.0) at room temperature. Following fixation, roots were washed in several changes of buffer, post-fixed in 1% osmium tetroxide for 4 h at room temperature and dehydrated in graded ethanol water series. Following passage through a graded propylene oxide/ethanol series, the root tips were gradually infiltrated with resin by placing them for 24 h in each of series of resin/propylene oxide mixtures, followed by three changes in 100% Epon substitute. Finally, materials were embedded in freshly prepared resin mixture, and polymerized in oven at 60°C for 48 h. Sections were cut 0.1 µm, mounted on copper grids and stained with uranyl acetate and lead citrate as described by Reynolds (1963). Observations were made with a Phillips Electron microscope operating and accelerating voltage of 75 kv.

Molecular analysis. Concentration of total protein for the treated as well as the untreated root was determined according to method reported by Bradford (1976), where 0.1 g of fresh tissue was powdered in liquid nitrogen, extracted by 1 mL 80% ethyl alcohol, precipitated for 15 min at 4000 rpm at -5°C and dissolved in 1 mL phosphate buffer (pH 7.0). Optical density was read at 595 µm.

Distribution of protein pattern was detected using continuous polyacrylamide gel electrophoresis (SDA-PAGE) of 12.5% and 0.75 mm thick by means of Hoefer (SE 245) dual vertical mini-gel. 0.1 g of each treatment was powdered by liquid nitrogen, extracted with 1 mL of extraction buffer (1.21 g tris HCl, 1 mL 10% SDS, 0.5 mL β-mercaptoethanol & 5 g sucrose completed to 50 mL distilled water- pH 8.0), precipitated by cooling centrifuge at 4000 rpm for 15 min., pellets were dissolved with sample buffer (1.2 mL tris HCl, 2 mL 10% SDS, 1 mL glycerol, 0.5 mL 0.4% bromophenol, 0.5 mL β-mercaptoethanol, 4.8 mL distilled water) following protocol of Gallagher and Smith (1995). The homogenate were boiled in water bath for 90 seconds, loaded on gel and allowed to run at 70 volts, 40 mA. Finally, the gel was stained with comassie blue stain and destained according to LaemmLi (1970) and Wilson (1983). Gel was photographed and scanned for further documentation system.

Estimation of nucleic acids inducing total RNA was applied using Schneider method (1954), where 0.5 g powdered samples were homogenized in 10% perchloric acid and extracted in a gradient pH series of perchloric acid. Estimation of total RNA was carried out using orcinol reaction and the absorbency were measured at 660 µm.

RESULTS AND DISCUSSION

Cytological analysis. A progressive decrease in mitotic index of *Allium cepa* root meristematic cells was obvious after all treatments with *Rosmarinus officinalis* water extract as compared with their respective control (Table I).

This universal highly significant mitotic depression

Table I. Mitotic index and percentage of phases in *Allium cepa* L. root tips treated with *R. officinalis* extract

Treatment	Total No. of cells	Mitotic Index S.D.	Prophase	Metaphase	Anaphase-(telophase)
Control					
3 h	5072	3.5±0.212	36.9	23.8	39.3
6 h	5003	3.9±0.124	39.3	25.8	34.9
12 h	5038	4.2±0.113	42.2	32.1	25.7
0.5 g/100 mL					
3 h	5053	2.42±*0.111	29.9	38.1	25.4
6 h	5032	1.86*±0.138	25.1	29.7	44.0
12 h	5052	0.63*±0.073	31.9	33.0	30.5
1 g/100 mL					
3 h	5048	1.93*±0.093	30.2	35.3	37.8
6 h	5031	1.49*±0.190	31.3	47.2	20.6
12 h	5000	0.0±0.00	0.0	0.0	0.0
2 g/100 mL					
3 h	5044	1.78*±0.096	17.7	57.8	27.9
6 h	5047	1.31*±0.079	30.5	44.0	28.4
12 h	5000	0.0±0.00	0.0	0.0	0.0

was dose and time-dependent and the mean differences

Fig. 5. Electron micrograph of a cell with evident nuclear envelope surrounds the condensed chromatin (ch). Numerous large vacuoles (v) spread in the dense cytoplasm (c) when A. cepa roots were treated with 2% water extract for 6 hrs (x. 8900)

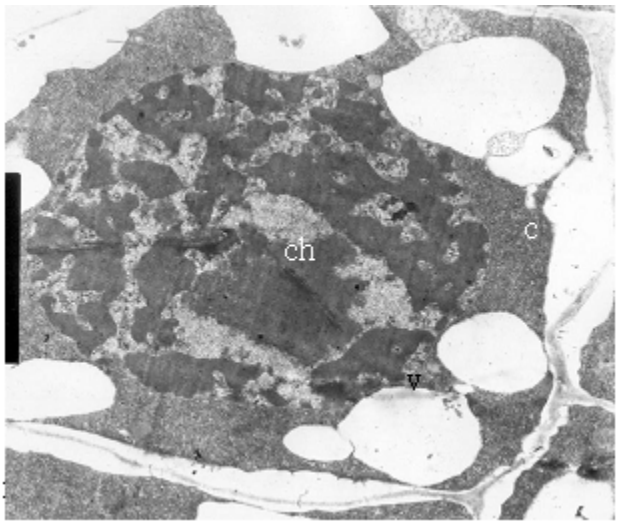
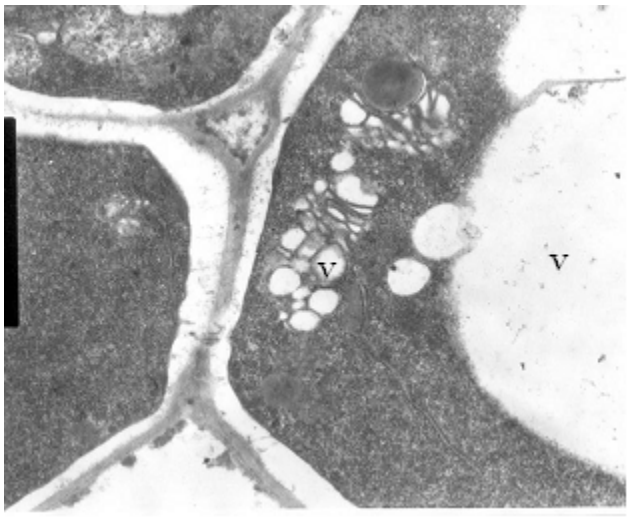


Fig. 6. Electron micrograph of treated cells with 2% water extract for 12 h, elucidates congregation of numerous small vacuoles (v) aside in the dense cytoplasm, voluminous vacuole occupies about 90% of the cell; indistinguishable compartments in the dense cytoplasm. (x 15500)



between concentrations and time duration was significant at 0.05 levels. However, the maximum proportion of mitotic division was 2.42% after 3 h of exposure to the lowest concentration 0.5% *R. officinalis* water extract while the minimum proportion showed drastic decrease to 0.63% after 12 h exposure to the same concentration.

Application of maximum dose 2% water extract exhibits an explicit decrease in mitotic activity whereas; mitotic index was reduced to 1.31% after 6 h exposure. Moreover, mitotic process was completely discontinued after 12 h of exposure to 1 and 2% extract as a result of an inhibitory activity of the drug.

Earlier studies reported complete inhibition in mitotic process after the use of both tackle herbicide, (Mohamed, 1995) and exotoxin (Mohamed & El-Shimy, 1995). Depression in mitotic indices was previously reported by Badr (1988), Ash and Abdou (1990), El-Khodary *et al.* (1990), Haliem (1990), Salam *et al.* (1993) and Abdel Salam *et al.* (1997).

Periodic pattern of cyclins condensation for entry into mitosis and degrading for exit from mitosis (Evans *et al.,* 1983) may be destructed by the drug. This process requires a signal sequence in the cyclin polypeptide chain that targets it for degradation (Murray & Kirschner, 1989). However, the continuous activation of MPF prevents cells to exit from mitosis (Murray *et al.,* 1989). Furthermore, the metaphase to telophase transition is blocked and was proofed by the presented results herein of metaphase arrest.

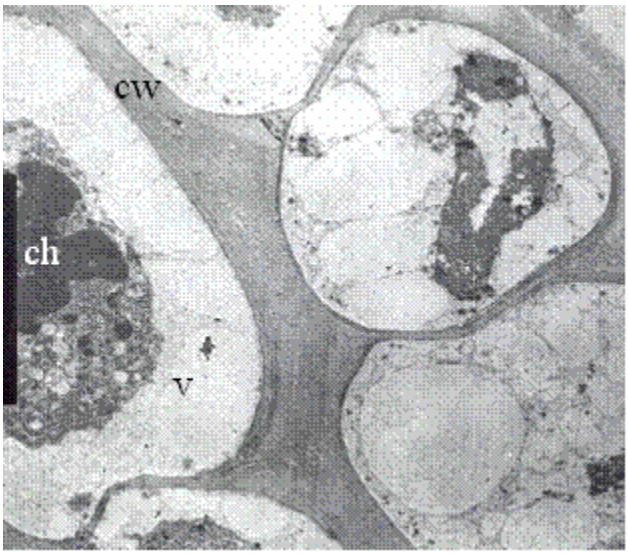
This suggestion is in agreement with that recently provided by Kamath and Jordan (2003), they suggest that epothilone B is similar to paclitaxel for inducing mitotic arrest by suppression and complete stabilization of microtubule dynamics and, were associated with mitotic arrest in metaphase, and the drug was concentration dependent.

Frequency of mitotic phases revealed an obvious accumulation in metaphase stage on response of both prophase and anaphase, after all treatment with the extract. The maximum proportion was 57% after 3 h exposure to 2% water extract.

The only exception was after 6 h exposure to 0.5%, where accumulation in anaphase took place. This accumulation was previously discussed by Farr and Cohen-Fix (1999) as abolishing mitotic cyclin degradation by mutations in the destruction box motive lead to an arrest late in anaphase.

Accumulation of mitosis on metaphase stage was previously reported by El-Bayomi *et al.* (1988), El-Khodary *et al.* (1990), and Barakat and Hassan (1997). However, metaphase arrest was formerly reported after the use of the purified vinca alkaloids vinblastine and vincristine (Inoué, 1981; Tawab, 1983) after the use of colchicine alkaloid. Each molecule of colchicine binds to one tubulin molecule by replacement of methane group and prevents its polymerization (Salmon *et al.,* 1984). Therefore, cells arrest in metaphase when the spindle is disassembled (Inoué, 1981) and the inactivation of MPF that signals the metaphase to anaphase transition is blocked. Thus,

Fig.7. Electron micrograph of cells with enclosed mucleoplasm containes dark clumps condensed chromatin (ch), thick fibrillar cell wall (cw) and large vacuoles (v) due to treatment with 2% water extract for 12 h (x 3900)



disrupting the spindle with the drug would be expected to produce a strong signal that greatly prolongs metaphase, as discussed earlier by Salmon *et al.* (1984). On the other hand, metaphase arrest can be achieved when the drug binds tightly to the microtubules and stabilizes them as did taxol (Debrabander, 1986).

It can be concluded that, metaphase arrest can be direct reflect not only on polymerization but also on depolymerization during mitosis indicating that chemical equilibrium exists through continual exchange subunits as given by Sammak and Borishy (1988).

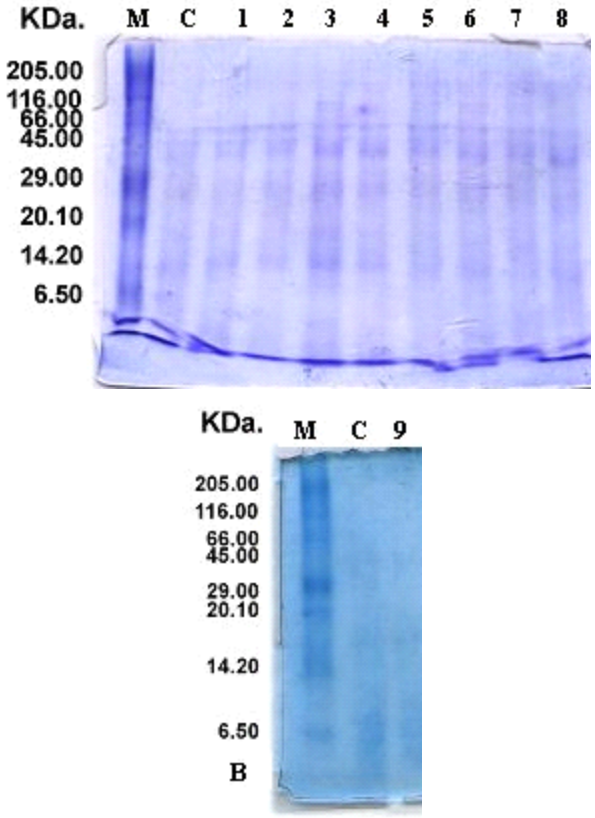
Total percentage of abnormalities was extremely high following treatment with *R. officinalis* water extract as compared by their relative control, and was concentration and time exposure dependent. The maximum proportion was 67.4% after 12 h exposure to 0.5% water extract, while the minimum percentage was 37% after 3 h exposure to the same dose. All data were statistically high significant.

Failure of properly segregate chromosomes at metaphase to anaphase transition may be a consequence of defeats in its regulatory mechanisms resulting in abnormalities (Cahill *et al.,* 1998).

The induction of mitotic abnormalities was common feature after treatment with a variety of compounds as previously recorded by Amer and Farah (1983), Badr and Ibrahim (1987), Habib *et al.* (1988), Adam *et al.* (1990), Salam *et al.* (1993), Guha and Das (1994) and Abdel Salam *et al.* (1997).

Spindle disturbance was the most common abnormality that occurs as a result of treatment with water

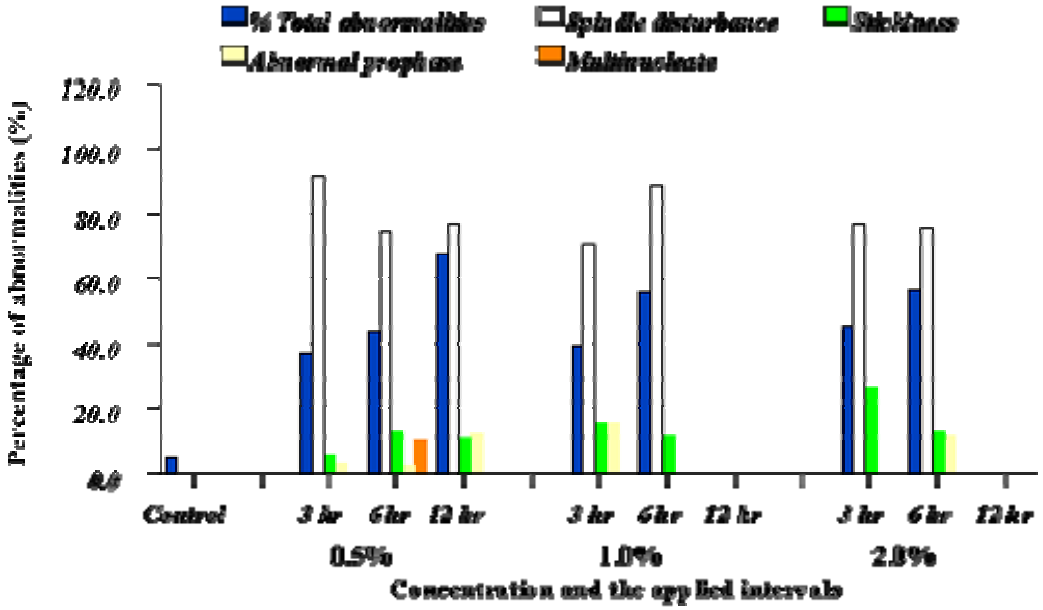
Fig. 8. SDS-PAGE electrophoretic analysis for *A. cepa* roots. Lanes: M marker, C control. l,2,3 for 0.5% *R.* extract. 3,4,6 for 1%. 7,8,9 for 2%



extract at various levels (Fig. 1). The maximum proportion was 91.8% after 3 h of exposure to 0.5% water extract, while the minimum proportion was 70.4% after 3 h of exposure to 1%. Stickiness was induced with maximum proportion 26.4% after 3 h exposure to 2% water extract. This stupendous proportion of spindle disturbance corroborates our exposition in which the drug impedes the continuity of subunits exchange leading to spindle disassembled.

Ultrastructure survey. Electron microscopic observations of meristem prior to and following treatment revealed a series of gradual changes that associated with sub-cellular organelles. Cells treated with two doses of *R. officinalis* water extract were compared by their untreated counterpart. The latter features a thin cell wall, cytoplasm including general matrix, ribosomes lying either free or else aggregate in cluster as polyribosomes, endoplasmic reticulum bears attached ribosomes classified as rough. Mitochondria with elaborated internal structure were present in cytoplasm along with fairy ground substance; vacuoles were spread in the cytoplasm of the cell. Some contained fragmented organelles (Fig. 2). Although numerous dictyosomes were detected in various location in the cytoplasm, but vesicles

Fig. 9. Percentage of total abnormalities induced by *R. officinalis* extract on *A. cepa* roots and types of abnormalities

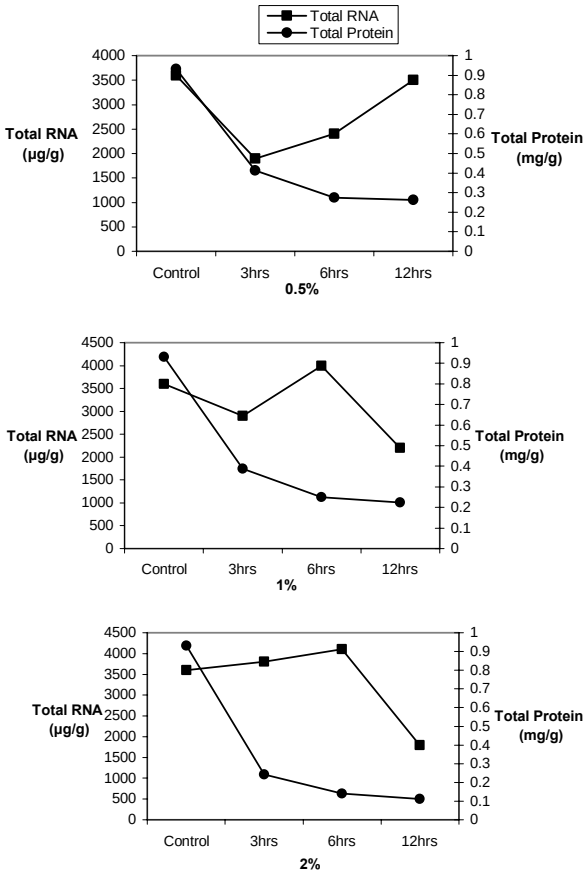


were rare and hardly observed in cytoplasm (Fig. 2). Following 6 h exposure to 1% water extract little structural changes have been observed. However, the vacuole has fragmented into numerous small vacuoles when cell underwent division (Figs. 3 & 4). Similar results were obtained during the induction of salt tolerant microspore of

Table II. Effect of *Rosmarinus officinalis* water extract on the electrophoretic banding pattern of *Allium cepa* L. roots

Types of protein	Mol. wet. (kD)	RF	Control	0.5%			1%			2%		
				3h	6h	12h	3h	6h	12h	3h	6h	12h
	280.9	0.07	-	-	-	-	-	-	+	-	-	-
Myosin, Rabbit muscle	205.0	0.11	+	+	+	+	-	-	-	+	+	-
	158.9	0.15	+	+	+	+	+	+	+	+	+	-
	131.1	0.17	-	+	-	-	+	+	+	+	+	-
β-Galactosidase	116.0	0.2	+	-	+	+	-	+	+	+	-	-
	78.2	0.23	-	-	-	+	+	+	-	+	-	-
Albumin, Bovine serum	66.0	0.25	-	-	+	+	-	-	+	-	-	-
Ovalbumin, Chicken egg	45.0	0.30	+	+	+	+	+	+	+	+	+	-
	38.8	0.33	+	+	+	+	+	+	+	+	+	+
	35.9	0.36	-	-	-	+	+	-	-	-	-	-
	33.9	0.38	+	+	+	+	+	+	+	+	+	-
	32.4	0.40	-	-	-	-	-	+	-	-	+	-
	30.7	0.42	+	-	+	+	+	-	+	+	+	-
Carbonic Anhydrase, Bovine Erthocytes	29.0	0.44	-	+	+	+	+	+	+	+	-	+
	27.6	0.46	+	+	+	+	+	+	+	+	+	-
	23.7	0.51	-	+	-	-	-	-	+	-	+	-
Trypsin Inhibitor, Soybean	20.0	0.59	+	+	+	+	+	+	+	+	-	+
	17.11	0.62	-	-	-	+	+	+	+	+	+	+
	15.9	0.65	-	-	-	-	-	-	-	+	+	-
α-Lactalbumin, Bovine milk	14.2	0.68	+	+	+	+	+	+	+	+	+	+
	11.6	0.71	-	+	-	-	-	-	-	-	-	-
	9.25	0.73	-	-	+	+	+	-	-	-	-	+
Aprotinin, Bovine Milk	6.5	0.78	+	+	-	-	-	+	+	-	+	+
	4.7	0.81	+	+	+	-	+	-	-	+	+	+
	3.5	0.84	+	-	-	+	-	-	-	-	-	-
	2.5	0.87	-	+	-	-	+	-	+	-	-	-
	2.1	0.89	-	-	+	+	+	-	-	-	-	-
Sum	27.0		13	15	15	18	17	14	17	16	15	8

Fig. 10. Portein and Nucleic acid (RNA) contents in *Allium cepa* L. root (mg/g fresh weight) after treatment with *Rosmarinus officinalis*



oilseed rape, Zaki and Tawab (2001).

Cells exposed to long term of treatment (12 h) feature thick cell wall, proplastids lacking their internal structure. Similar results were previously reported by Piqueras *et al.* (1994) and Bressan *et al.* (1990). Moreover, thick cell wall was a common feature after inoculation of tomato roots with different species of fungi as described by Beswetherick and Bishop (1993).

One of the striking observations in the present work is the accumulation of ribosomes, the expansion of endoplasmic reticulum and the presence of numerous mitochondria. Similar results were reported by Vartapetian *et al.* (1985), Tawab *et al.* (2000) and Zaki and Tawab (2001). These organelles are known as a device for protein synthesis and refer to active phase of protein synthesis that in turn reflects activation of genes. Following the processes of transcription and translation, proteins are formed in the cell that is equipped to become potentially competent to the unfavourable condition to survive rather than to proliferate, these regulations are done by extracellular specific signals, Raff (1992).

However, the gradual decrease in protein synthesis (introduced later) although the accumulation of such devices can be explained as an influence of the crude drug which causes a deleterious effect on them by mean of blocking the translation reaction or blocking the initiation of RNA chains by binding to polymerase II and preventing RNA synthesis as previously reported by Lord *et al.* (1991).

On the other hand, the exposure of meristematic cells to 2% *R. officinalis* water extract for 12 h revealed the deposition of thick fibrillar cell wall, congregation of numerous small vacuoles aside the equivocal compartments and alongside the enormous vacuole that occupy about 90% of the cell volume. The appearance of grouped ribosomes either in close contact with the cortical endoplasmic reticulum or contiguous to the tonoplast in the dense cytoplasm, indistinct compartments were observed. Large nucleus appeared with dark clumps condensed chromatin and surrounded by nuclear envelope (Figs. 6 & 7). Similar results were reported by Chauhan and Sundararaman (1990) as a result of isoproturon on *A. cepa* root cells. The dark clumps chromatin was previously described by Vaux *et al.* (1992) as cell programmed for death.

Gene expression in response to mitodepressive agent. A progressive decrease in protein synthesis of all treated *A. cepa* root cells which was directly influenced by all used *R. officinalis* water extract. Quantitative decreases in total soluble protein are obvious as compared by its control. The maximum amount is 0.412 mg/g fresh weight which was 50% less than the untreated cells. The least amount 0.112 mg/g was scored after 12 h of treatment with the maximum dose 2%. Thus the emphatic decline in protein synthesis was concentration and time exposure dependent. The decrease in total soluble protein was previously reported by Tawab *et al.* (2003) using *Brassica napus* cells.

Different virus families inhibit the expression of host genes during their replication (Hardwick & Griffin, 1997). Picornaviruses act directly to inhibit solely host protein synthesis (Knipe, 1996). While poliovirus protein inactivates either enhancer-binding protein or else cyclic responsive element-binding protein: so that inhibits genes expression (Yalamanchili *et al.*, 1997).

The severe decrease in total percentage of protein after all treatment can be explained by that the crude drug affect one of the three steps of protein synthesis machinery on ribosome as to block either the translocation reaction or polyptidyl transferase reaction as reported by Jiménez (1988), or to block the m RNA synthesis by binding to RNA as did α-amanitin as previously reported by Perentesis *et al.* (1992).

Protein banding profiles representing the treated cells are demonstrated in (Table II) and (Fig. 10). Number of bands per lane and their distribution among the treatments varied and exceeded their relative control. The highest number of bands (18) was recorded after 12 h exposure to

the maximum dose, 2%. Fourteen newly elaborated bands with different molecular weights were evident after all treatments. However, all protein bands represented by the untreated root cells are observed constantly in the protein pattern of treated root cells. The molecular weight of the newly elaborated bands altered between high 280 kDa after 12 h of exposure to 1% water extract, and low 2.1 kDa after 6, 12 h and 3 h of exposure to 1 and 2% water extract.

The dominant newly elaborated polypeptides were at low molecular weight (29 & 17.11 kDa). Similar results were previously reported by Moreno *et al.* (1994) in the proliferation of *A. cepa* cells during cold and heat conditions.

The novel protein bands of low molecular weights are believed to be involved in determining the substrate of specificity of cyclosome/anaphase promoting complex as did the two proteins Cdc 20p in degrading the substrate at metaphase to anaphase transition (Fang *et al.,* 1998); and also Cdh 1p for the degradation of mitotic cyclins and other substrates at the exit of mitosis (Zachariae *et al.,* 1998).

Persistence of protein bands of low molecular weights 20 and 27.7 kDa was obvious even after all treatments. Similar proteins are cyclin dependent kinase inhibitory which have been described in *Arabidopsis* by De Veylder *et al.* (2001) and were as such as mammalian p 27^{kip1} but their function remains abstruse in plants.

The rates of cellular RNA synthesis decline during the mitotic phases (Frenster *et al.,* 1974) and also during cell differentiation (Frenster, 1999). A progressive decrease in relatively RNA during early and late prophase, returned back to the basal level (as for control) after exit from mitosis precisely in interphase as previously determined by Nakatsu *et al.* (1974). These findings are in agreement with our data presented herein, especially after the use of 0.5% dose. The temporal increase in RNA after 6 h of applying 1 and 2% (Fig. 10) is due to the significant decrease in mitotic indices and the relatively high number of interphase cells. Recently, this temporary overshoot of such nucleic acid are referred to the re-programming of daughter cells after mitosis (Prasanth *et al.,* 2003), and could reflect active sites which have a role in mitotic re-programming (Frenster & Hovsepian, 2003).

In conclusion, the drug inhibits mitotic process and exhibits mitotic arrest in metaphase and forfeits the chemical equilibrium of the tubulin and microtubules for spindle assembly. The drug inhibits the protein synthesis and binds to one of the three steps during protein synthesis in consequence of the sensitivity of ribosomes to inhibitors. Alteration in ultrastructure of the protein synthesis devices leads to significant inhibition of cell division. The seminormal induction of nucleic acid RNA indicated our suggestion that the drug bind to one of the three steps of protein synthesis and exclude the possibility of acting as α-amanitin. Thus, further investigation would be carried out to identify the accurate mode of action of the purified active constituents of the drug.

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