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MITODEPRESSIVE EFFECT AND DNA IHIBITION INDUCED BY CAMEL URINE IN *A. CEPA* ROOT MERISTEM CELLS.

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ABSTRACT

Camel urine is used by Beduines in the treatment of many diseases, since it contains chemical compounds affect the physiology of human body. Therefore, the effects of camel urine on the process of mitosis and chromosomal behaviour in normal cells (the root meristem cells of *A. cepa*) were studied in direct and recovery experiments. Also, an attempt was made to find out a relationship between the mitotic activity and the DNA content. The results clearly indicated genotoxic activity of camel urine however no clastogenic effect was observed. The observed mitodepressive effect depends on the concentration and exposure time of direct treatment and it may be due to the presence of camel urine cytotoxic constituents such as purine bases, hypoxanthine and creatinine. The non-clastogenic nature probably was attributed to the antioxidant and the antimutagenic compounds (uric acid and creatinine) present in camel urine. The mitostatic action may be due to an accumulation of clumping stickimetaphase cells. Thus, chromosomes lose the ability to undergo anaphase and telophase stages and are completely arrested at metaphase in cells treated with high doses for long exposure time. Beside stickiness, other physiological aberrations were observed which probably attributed to abnormal spindle formation. On the other hand, the mitostatic feature may be due to the reduction in the number of cells entering mitosis and the depression of DNA synthesis at S phase. During recovery periods, the process of mitosis and DNA concentration were recuperate in meristem cells treated with 10% of camel urine. On the contrary, the attempt to revive the meristem cells was not effective for the sublethal dose (20%). It revealed complete genotoxic effect and continuous decrease of DNA content at all recovery times. Further studies are in progress to explain the genotoxic activety of camel urine and its active compounds on the cell cycle phases and gene expression.

INTRODUCTION

Camel urine is used in folklore medicine as treatment for several illnesses. Beduines in the Arab desert mix it with milk and give the mixture to patients suffering from stomach infections, tuberculosis (Gauthier-Pilters and Dagg, 1981) and cancer (Kabarity *et. al.*, 1988). They add milk to the urine only to overcome its strong odour. The urine must be fresh and excreted from young animals.

Using the micronucleus test, Al-Harbi *et.al.*(1996) reported the cytotoxic effect of camel urine. They indicated that it potentiate the cytotoxicity of cyclophosphamide in the bone marrow cells of mice. Also, the same natural product was shown to be cytotoxic in *Candida albicans*, *Aspergillus niger* and *Fusarium oxysporum* cells (Al-Awadi and Hakal, 1997 and Al-Gadabi and Al-Awadi, 1999).

The chemical composition of camel urine showed the presence of purine bases, hypoxanthine, creatinine, urea, uric acid, sodium, potassium and phosphates (Mura *et. al.*, 1987). Some of these compounds are similar to the

ingredients present in common urine of other animals such as purines, urea, creatinine and hypoxanthine. The difference in composition was due to low level of hepatic guanase and hypoxanthine guanine phosphoribosyl transferase (HPRT) resulting in an increased level of purine bases and hypoxanthine (Mura *et. al.*, 1986).

Purines, urea, creatinine and hypoxanthine exhibit cytotoxic and antitumour activity in different animals, human tumours and cell lines (Gallent *et. al.*, 1993; Lillie *et. al.*, 1993 and Miller *et. al.*, 1993).

Most of the purine analogues and derivatives were shown to be cytotoxic in *E.coli* (Basu *et. al.*, 1993); *Saccharomyces cerevisiae* (Shcherbakova and Pavlov, 1993); mouse lymphoma cells (Cole *et.al.*, 1991); *Salmonella typhimurium* and Chinese hamster lung cells in culture (Matsuda *et. al.*, 1991); V79 cells and human lymphocytes (Bonatti *et. al.*, 1986). Hypoxanthine was reported to be clastogenic in human lymphocytes (Emerit *et. al.*, 1985) and was shown to potentiate the mutagenicity induced by monofunctional alkylating agents in Chinese hamster cells (Peterson and Peterson, 1979).

However, the complex nature of natural products has the potential to inhibit the clastogenicity and/or the mutagenicity effects. Uric acid and creatinine present in camel urine are known to be potent antioxidants (Glazer, 1988). Al-Bekairi *et.al.*(1991 b) reported that uric acid act as inhibitor of clastogenicity and biochemical changes induced by cyclophosphamide in mice. Human uric extracts also showed to be antimutagenic in *Salmonella typhimurium* (Malaveilla *et. al.*, 1992).

The complex natural products supress also secondary malignancies. The active peptides and major protein fraction of human urine were found to be cytotoxic against human Leukemic cells, Osteosarcoma and Hela cells (Burzynki *et. al.*, 1976 and Sloane *et. al.*, 1986). In addition, camel urine was reported to inhibit the formation of C-Tumors induced by cholchicine in *Allium cepa* root tips (Kabarity *et. al.*, 1988).

In view of the cytotoxic,mutagenic, antioxidative and antimutagenic nature of ingredients present in camel urine and its folklore use in illnesses and cancer therapy, the present study was done to investigate the cytological effect of camel urine during direct treatments and recovery durations upon the process of mitosis and the behaviour of chromosomes in normal cells (*A. cepa* root meristem cells). Furthermore, an attempt was made to find out a relationship between mitotic activity and DNA content.

MATERIALS AND METHODS

Urine samples:

Samples of fresh urine were obtained aseptically from the urinary bladder of camels (*Camelus dromedarius*) slaughtered in the Government Slaughter House, Riyadh, Saudi Arabia. These camels live in remote areas in the deserts and depend mostly on xerophytic vegetation as food. Preliminary tests on variability of urine from different individual camels of different sexes revealed a lack of differences in their mitodepressive action. In the present

study urine of female camels was used. Different concentrations (2.5, 5, 10 and 20%) of freshly obtained camel urine were prepared by dilution with distilled water (Al-Harbi *et. al.*, 1996).

Plant samples preparation:

Roots of *A. cepa* bulbs were obtained by placing on beakers containing tap water. When the roots were about 1.0mm long, the bulbs were placed on beakers containing the different concentrations of the urine. The exposure times of the treatments for each concentration were 4, 8, 12 and 24 hours. Five bulbs were used for each concentration and exposure time as well as for the control grown in tap water (Williams and Omoh, 1996). In order to determine whether treated roots would recover from the effects of the urine, bulbs with roots which had been treated for 8 hours with 10% and 20% of the urine were transferred to tap water for 24, 48 and 72 hours before fixation (Lopez-Saenz *et. al.*, 1996).

Cytological procedure:

After treatment, the root tips were cut off, washed in distilled water and fixed overnight in a mixture of ethanol: acetic acid (3:1) at 4°c (Wilson and Morrison, 1971). Subsequently, the roots were hydrolyzed in 1N HCl for 50 minutes at 22°c and stained in Schiff's reagent. Feulgen-squash technique of Darlington and La-Cour (1976) was used. Schiff's reagent was stored in dark at 4°c until use.

For cytological determination, at least two slides from each bulb were prepared and five microscope fields (40X objective) were scored for each slide noting the total number of all cells, cells in mitosis and those affected for each type of anomaly. The sum of the 50 fields gave the total number of examined cells for each treatment.

Determination of DNA content:

After each treatment, one g. of the root tips were cut off, washed in distilled water and stored at –20°c until analysis. The CTAB method of Dellaporta *et.al.*(1983) for preparation of plant DNA was followed. The purity of DNA was checked by an UV- spectrophotometer using the following coefficient (Fellenberg, 1974): $A_{260}/A_{280} = 0.5\text{-}0.6$, where A: is the absorbance at a given wavelength. A dilution of the DNA- containing solution was measured by Shimadzu UV- spectrophotometer at 260nm (OD of 1 at 260 nm ≅ 50ug/ml of DNA).

Statistical analysis:

Spsspstt computer software was used to estimate the mean (x), standard error and t-test for significance. The levels of significance in t-tests were $p \leq 0.05$ and $p \leq 0.01$(Williams and Omoh, 1996).

RESULTS AND DISCUSSION

Direct treatment:

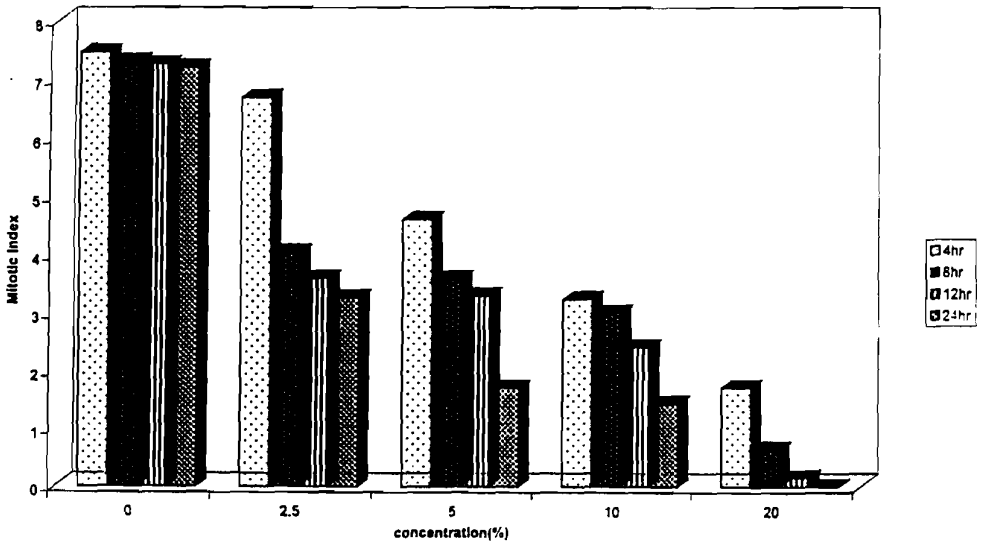
Mitotic index:

In the present study, camel urine showed inhibitory effect on the rate of cell division in *A. cepa* root tips. As shown in Table (1) and Figure (1), there was significant decrease of mitotic indices below control values after direct treatment with different concentrations and durations of exposure to camel urine. This finding cleared that the mitodepressive effect depends on the dose and the time of treatment. After 24hr.of treatment by 20% camel urine, such mitodepression was completed and there was not any cell in the meristems undergoing mitosis. These results were in agreement with those obtained by AL-Harbi *et.al.*(1996); AL-Awadi and Hakal (1997) and AL-Gadabi and AL-Awadi (1999) in which they indicated the cytotoxicity of camel urine in different types of normal cells.Also, the present data could be explain the inhibitory action of camel urine on C-Tumor cells induced by cholchicine in *A. cepa* root tips(Kabarity *et. al.*, 1988). The mitodepressive effect in normal cells was previously reported for several medicinal plant extracts such as *Ricinus communis* (George and Geethamma, 1990); *Tylophora indica* L. (Saggoo *et.al.*, 1991); Coumarin and its derivatives (Podbielkowska *et. al.*, 1994) and *Allium sativum* L. (Riad, 2002).

Table (1): Mitotic indices and percentage of mitotic phases in the root meristem cell of *A. cepa* after direct treatment by different concentrations of camel urine at different times of exposure.

Concentration (%)	Duration of Treatment (hr)	Mitotic Index ±SE	Mitotic Phases Percentage of dividing cells± SE		
			Prophase	Metaphase	Anaphase + Telophase
0	4	7.47±0.12	35.43±0.38	35.06±0.04	29.51±0.34
	8	7.31±0.12	34.5±0.44	35.48±0.21	30.02±0.23
	12	7.27±0.14	33.2±0.29	35.66±0.19	31.14±0.17
	24	7.21±0.05	33.33±0.18	37.17±0.1	29.5±0.26
2.5	4	**6.7±0.07	**31.01±0.49	**39.15±0.08	29.84±0.6
	8	**4.06±0.02	**18.6±0.12	**59.55±0.07	**21.85±0.06
	12	**3.61±0.11	**10.62±0.12	**69.93±0.04	**19.45±0.08
	24	**3.28±0.05	**8.37±0.1	**79.76±0.29	**11.87±0.32
5	4	**4.62±0.04	**20.58±0.24	**56.58±0.19	**22.84±0.26
	8	**3.61±0.08	**16.64±0.11	**61.46±0.02	**21.9±0.09
	12	**3.31±0.05	**9.66±0.08	**71.61±0.1	**18.73±0.13
	24	**1.71±0.1	**4.53±0.13	**87.44±0.1	**8.03±0.04
10	4	**3.25±0.04	**13.52±0.16	**66.39±0.1	**20.09±0.2
	8	**3.02±0.04	**7.38±0.08	**79.66±0.11	**12.97±0.04
	12	**2.42±0.1	**3.16±0.04	**91.52±0.08	**5.32±0.07
	24	**1.45±0.07	**0±0	**100±0	**0±0
20	4	**1.72±0.06	**8.52±0.14	**78.34±0.14	**13.14±0.25
	8	**0.65±0.04	**5.52±0.18	**84.3±0.11	**10.18±0.17
	12	**0.16±0.02	**0±0	**100±0	**0±0
	24	**0±0	No dividing cells		

**Significant at 0.01 level.

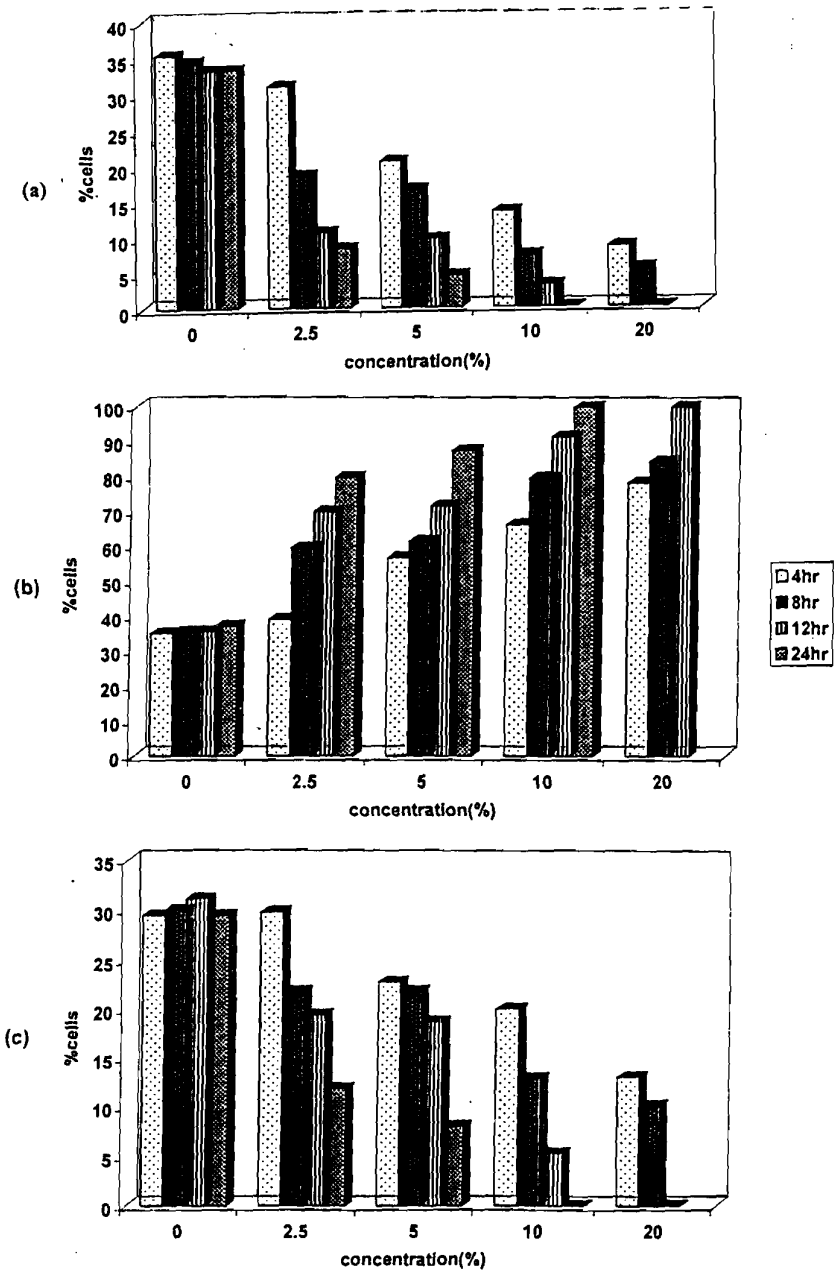


Figure(1): Mitotic indices in the root meristem cells of *A.cepa* following direct treatment by different concentrations of camel urine at different times of exposure.

The essential compositions of camel urine such as purine bases, urea, creatinine and hypoxanthine (Mura *et al*, 1986 and 1987) were reported as cytotoxic and antitumor compounds (Gallent *et. al.*, 1993; Lillie *et. al.*, 1993 and Miller *et. al.*, 1993). Therefore, these compounds may cause the mitodepressive and the lethal effects observed during the direct treatment.

Frequency of mitotic phases:

The harmful effects of camel urine on the rate of cell division induced an imbalance in the frequencies of mitotic phases. Roots treated with different doses of camel urine at different exposure times showed fluctuation in the frequency of various mitotic phases (Table 1). There was significant decrease in the number of prophase, anaphase and telophase stages (Figure 2-a and c) and significant increase in the number of metaphase cells (Figure 2-b) as compared with control samples. These changes in the frequencies increased as the concentration and the duration of the treatment increase until there was complete metaphase arrest at 10% dose for 24hr.and 20% concentration for 12hr. No dividing cells were observed in the root tips after 24hr.of 20% dose. Therefore, there were not any available values to calculate the frequency of mitotic phases beyond these conditions.The metaphase arrest feature was also observed by Banerjee (1992); Carballo *et.al.* (1992); Podbielkowska *et.al.*(1994) and Riad (2002)when they studied the cytological effects of some plant extracts.



Figure(2): Frequency of mitotic phases in the root tips of *A. cepa* after direct treatment by different concentrations of camel urine at different times of exposure, (a) prophase, (b) metaphase and (c) anaphase and telophase.

Cytological aberration:

Camel urine induced a variety of aberrants (Figure 3). The data recorded in Table (2) and Figure (4-a) illustrate that the total abnormalities were increased gradually and significantly versus control values and depended on the dose and the time of exposure. Stickiness of chromosomes at metaphase was the most common aberration (Figure 4-b) reaching a frequency of 100% at 10% concentration for 24hr and 20% dose for 12hr. As a result of the high frequency of stickiness, the rate of total aberration increased to the same frequency at the same doses and times of exposure to camel urine. Also, as a result of the absence of cell division at 20% dose for 24hr, the frequencies of aberrations have not been calculated.

In fact, due to the effect of camel urine containing compounds, there was highly accumulation of cells in metaphase stage is related to stickiness which covers the whole chromosome complement leading to clumping stickimetaphase. Thus, the chromosomes lose the ability to undergo anaphase and telophase stages and they were arrested at metaphase. These results coincide with those obtained by George and Geethamma (1990); Sahi and Singh (1996) and Riad (2002) as a result of some natural compound treatments. Darlington and Mcleish (1951) and La-Cour and Rutishauser (1954) attributed stickiness to the process of depolymerization of DNA, while Saggoo *et.al.*(1991) proposed that it may be due to alteration in the surface nucleoprotein configuration or improper folding of chromosome fibre.

During the present study, other physiological types of aberrations were observed such as C-metaphase, tetraploid, bridges, multipolar and multinucleated cells.Therefore, the reduction of mitotic activity could be also due to the effect of camel urine ingredients on the spindle formation. Similar aberrants have been reported to be induced by a number of physical and chemical reagents (Kabarity and Malallah, 1980; Saggoo *et.al.*, 1991 and Riad, 2002). The formation of chromatin bridges at anaphase probably due to stickiness of chromosomes. Sticky bridges have been discribed by many workers using plant extracts of *Achillea fragrantissima* (Shehab *et.al.*, 1978); *Ipomea carnea* (Alam *et.al.*,1987); *Cymbopogon proximus* (Adam and Farah, 1989); *Tylophora indica* (Saggoo *et.al.*, 1991) and *Allium sativum* L. (Riad, 2002).

The data obtained in the present investigation indicated the absence of any chromosomal breaks. The non-clastogenic nature appeared to be due to the effect of antioxidative and antimutagenic components of camel urine such as creatinine and uric acid (Glazer, 1988 and Al-Bekairi *et.al.*, 1991b).

DNA content:

In this investigation the results indicated that the mitodepressive effect may be due to the strong reduction in the number of cells entering mitosis. Such phenomenon may be due to lengethening of cell cycle time (Adam and rashad, 1984) or/and inhibition of DNA and protein synthesis in S and G_2 periods of interphase stage (Shehata, 1993). It is of intrest to note that camel urine containing compounds reduced gradually and significantly the amount of DNA in *A.cepa* root meristem cells during the direct treatment (Table 3 and Figure 5a). These results were in agreement with those obtained by Al-Harbi

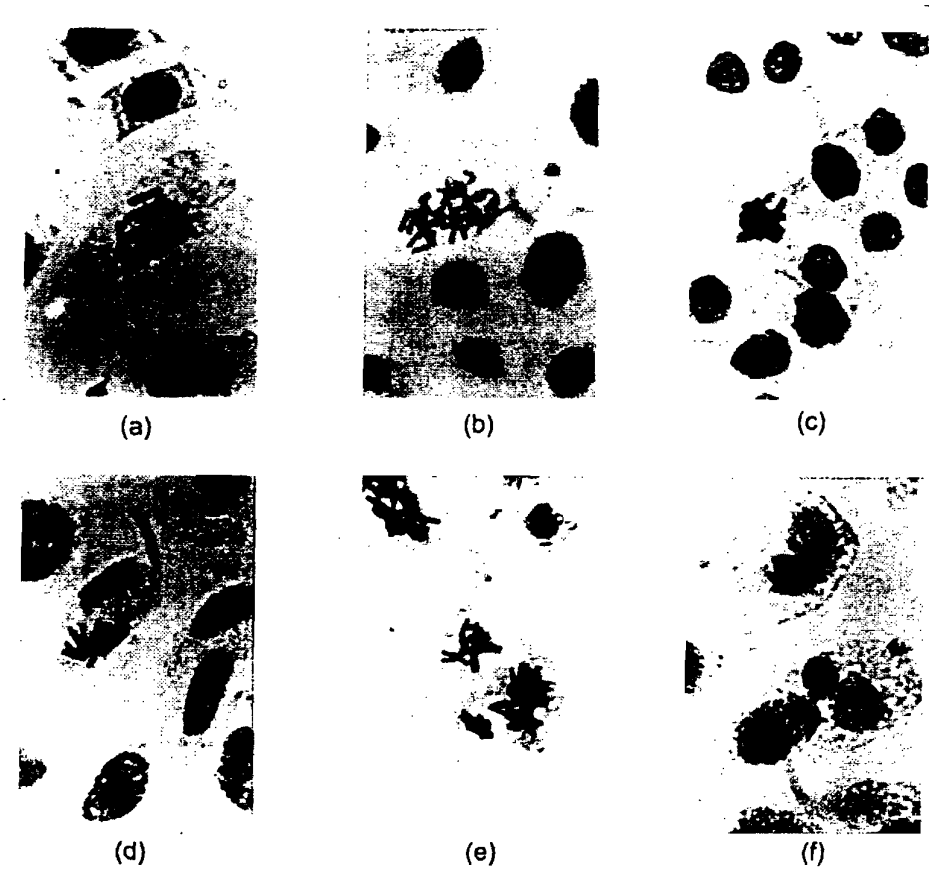
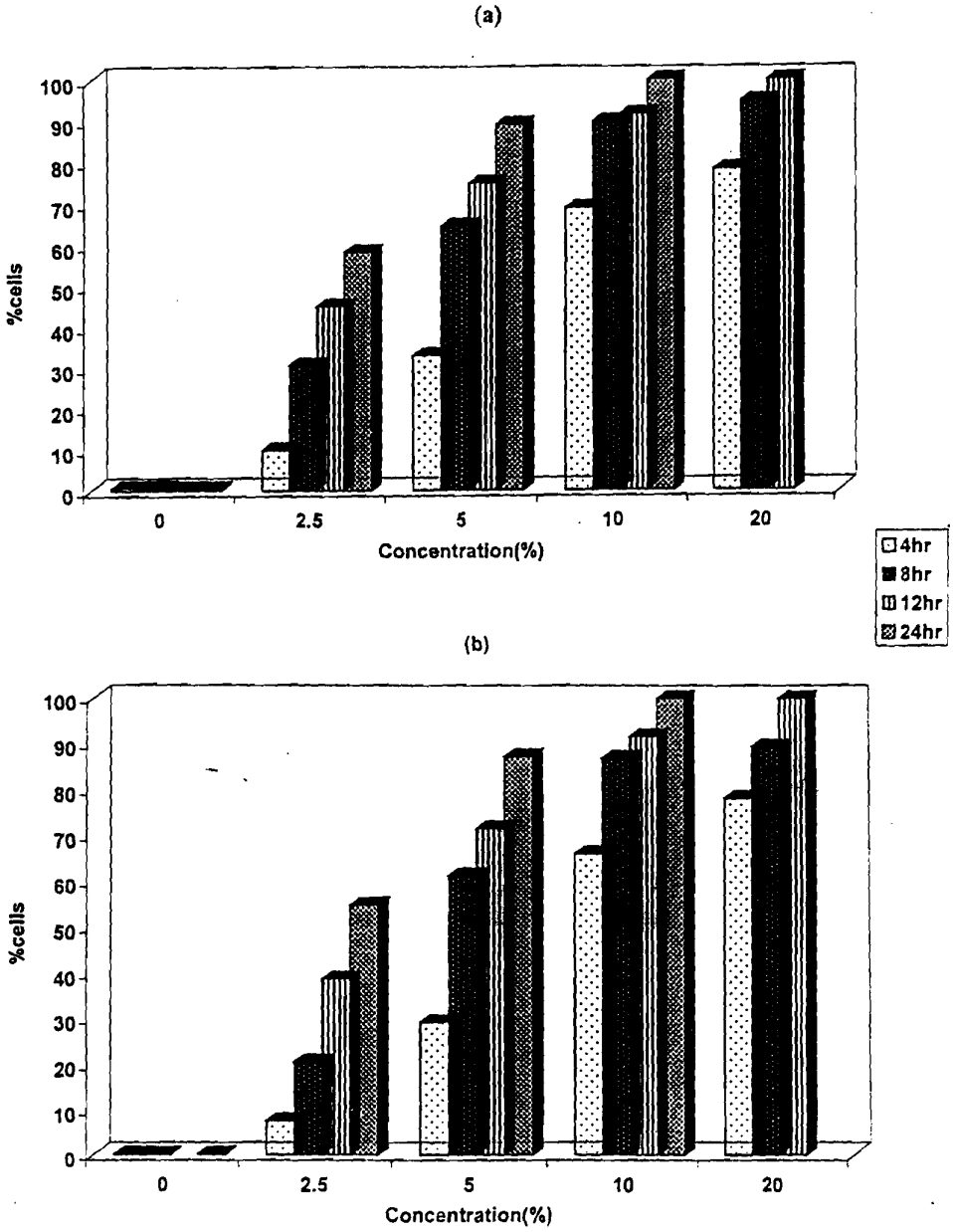
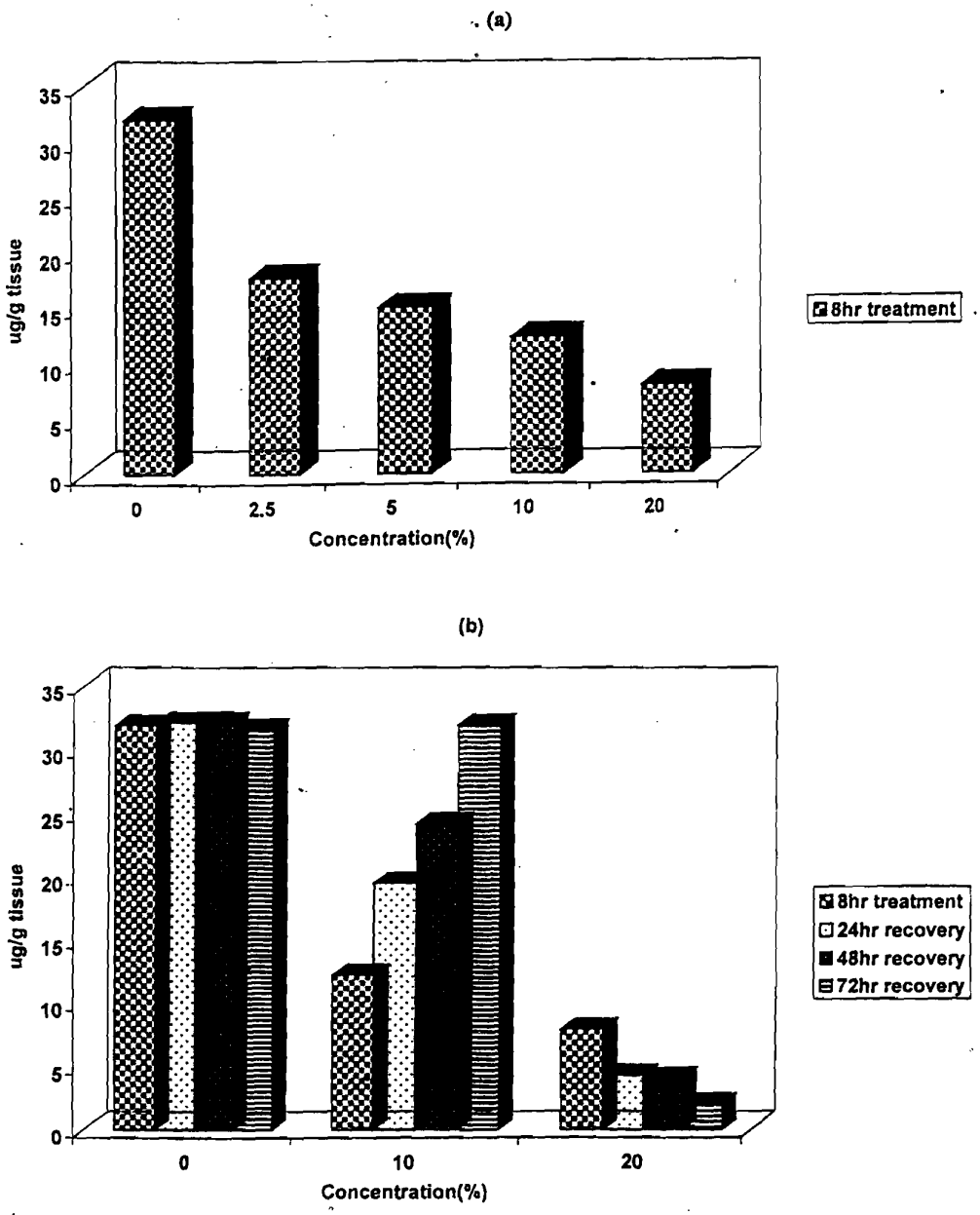


Figure (3): photomicrographs showing some different aberrations induced in *Allium cepa* root meristem cells after direct treatments by camel urine: (a) c-metaphase, (b) tetraploid cell, (c) clumping stickimetaphase, (d) bridge, (e) multipolar cell and (f) multinucleated cell.



Figure(4): (a) total percentage of aberrations, (b) percentage of stickiness induced in the root tip cells of *A.cepa* after direct treatment by different concentrations of camel urine at different times of exposure.



Figure(5): DNA content in the root tip cells of *A.Cepa*, (a) after direct treatment by different concentrations of camel urine, (b) after 8hr treatment by high doses and recovery periods.

et.al.(1996) which showed that camel urine enhanced the reduction of liver DNA concentration during cyclophosphamide and camel urine treatments in mice. Also, the depression of DNA concentrations was previously reported in *A.cepa* root meristem cells following the direct treatments by different medicinal plant water extracts such as: *Artemisia herba alba*; *Capsicum annuum*; *Anthemis nobilis* and *Carum carvi* (Shehata, 1993). Thus, at least the inhibitory effect on the DNA synthesis and the reduction of the number of cells in prophase probably caused the mitostatic feature in the present study.

Table (2): Percentage of induced aberrations in the root meristem cells of *A. capa* after direct treatment by different concentrations of camel urine at different times of exposure.

Duration of Treatment (hr)	Concentration (%)	Percentage of aberrations						Total±SE
		C	Tp	S	L	B	Mp	
4	0	0.21	-	0.21	0.21	-	-	0.63±0.01
	2.5	0.55	-	7.7	-	1.1	0.55	**9.91±0.05
	5	0.92	-	29.36	-	1.83	0.92	**33.03±1.24
	10	-	-	66.22	-	-	2.7	**68.92±0.26
	20	-	-	78.26	-	-	-	**78.26±0.12
8	0	0.23	0.23	0.23	-	-	-	0.69±0.03
	2.5	0.99	-	20.79	-	5.94	2.97	**30.69±0.29
	5	-	1.04	61.46	-	2.08	-	**64.58±0.3
	10	-	1.45	86.96	-	1.45	-	**89.86±0.1
	20	-	5.26	89.47	-	-	-	**94.73±0.2
12	0	0.21	-	-	0.21	-	-	0.42±0.02
	2.5	-	-	38.94	-	0.88	5.31	**45.13±0.05
	5	-	-	71.74	-	-	3.26	**75.00±0.28
	10	-	-	91.67	-	-	-	**91.67±0.13
	20	-	-	100	-	-	-	**100±0
24	0	0.22	-	0.22	-	-	-	0.44±0.01
	2.5	-	-	55	-	-	33.3	**58.33±0.12
	5	-	-	87.3	-	-	1.87	**89.17±0.08
	10	-	-	100	-	-	-	**100±0
	20	No dividing cells						

** Significant at 0.01 level.

C = C-metaphase. Tp = Tetraploid cells.
S = Stickiness. L = Laggard.
B = Bridge. Mp = Multipolar and micronucleated cells.

Recovery effect:

At the same time of treatment (8hr.), 10% dose of camel urine showed intermediate mitotic activity and 20% concentration exhibit sublethal effect. Therefore, to investigate the stability of camel urine effects, 8hr.treated roots with each dose were let to recover for 24, 48 and 72hr. before fixation.

DNA content:

In comparison with 8hr.treatment, DNA content in the meristem cells treated with 10% of camel urine increased gradually and significantly during recovery periods (Table 3 and Figure 5-b). These values were still significantly below the control level at 24 and 48hr.of recovery times. It was however normalized after 72hr. On the other hand, at all recovery periods, 20% dose exhibited low DNA concentration in the meristem cells to control and 8hr.treatment. Such reduction was significant and increased gradually during recovery durations.

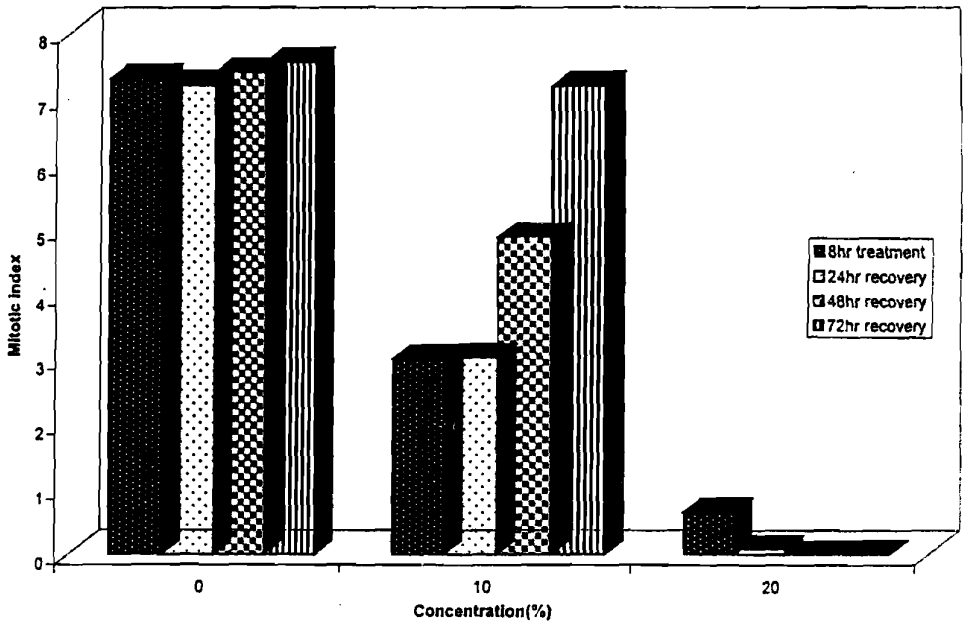
Table (3): DNA concentration in the root meristem cells of *A. cepa* after direct treatment for 8 hr. by different concentrations of camel urine and during recovery periods for higher doses.

Concentration (%)	DNA content (ug/g tissue) ± SE			
	Recovery Period (hr.)			
		24	48	72
0	31.95±0.82	32.2±0.78	32.2±0.84	31.7±0.77
2.5	**17.7±0.52			
5	**15.02±0.44			
10	**12.3±0.32	**19.6±0.49°°	**24.3±0.8°°	32.04±0.9°°
20	**7.95±0.39	**4.32±0.93°	**4.1±0.82°	**2.05±0.11°°

** Significant to control at 0.01 level.
° Significant to 8 hr. treatment at 0.05 level.
°° Significant to 8 hr. treatment at 0.01 level.

Mitotic index:

From Table (4) and Figure (6), it could be noticed that 72hr.of recovery times were enough to normalize cell division pattern in the roots treated with 10% concentration of camel urine. However, at 24 and 48hr. the mitotic indices remained significantly below control values. For the same dose and after 48hr.of recovery durations, the frequency of the cell division increased gradually and significantly above 8hr.treatment value.On the contrary, all recovery times were not effective for the cells treated with 20% concentration. The mitotic indices decreased versus control and 8hr.treatment values and were reduced to zero after 48hr.of recovery durations.

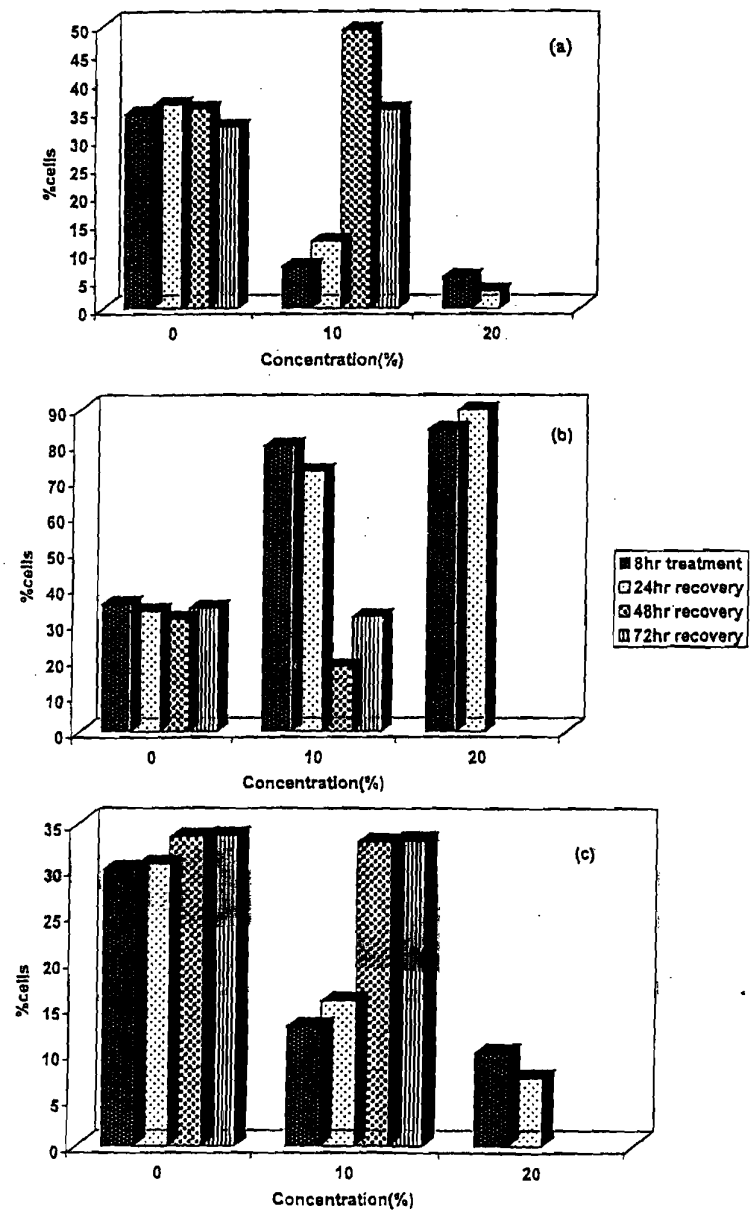


Figure(6): Mitotic indices in the root tip cells of *A. cepa* after 8hr treatment by high doses of camel urine and recovery periods.

Frequency of mitotic phases:

As shown in Table (4) and Figure (7), the recovery periods were not effective for the meristem cells treated with 20% concentration of camel urine. At 24hr., the frequencies of prophase, anaphase and telophase stages continued to decrease significantly below control and 8hr.treatment values (Figure7-a and c). At the same time, there was continuous accumulation of the cells in metaphase (Figure7-b).The value of this stage was significantly higher than that of the control and 8hr.treatment samples. At other recovery periods there was not any cell undergoing mitosis in the root tips allowing the calculation of mitotic phase frequencies.

For 10% concentration, prophase stage percentages increased significantly above 8hr.treatment level during all recovery times (Figure7-a). The frequency of this stage was still significantly below control value at 24hr.period. But, at 48hr.the percentage increased significantly above control value and was normalized to control after 72hr.of recovery durations.The accumulation of cells in metaphase significantly decreased below 8hr.treatment level at all recovery times (Figure7-b). At 24hr.of recovery, the number of metaphase cells were still significantly higher than that of the control sample, and at 48hr.the frequency of the same stage decreased significantly below control value, then it was normalized after 72hr.of recovery periods. The percentage of anaphase and telophase elevated gradually and significantly above 8hr.treatment level (Figure7-c).The number of cells in these stages were significantly below control sample at 24hr.of recovery but after 48hr., their frequencies were completely normalized.



Figure(7): Percentage of mitotic phases in the root tip cells of *A. cepa* following 8hr treatment by high doses of camel urine and recovery periods, (a) prophase, (b) metaphase and (c) anaphase and telophase.

Table (4): Mitotic indices and percentage of mitotic phases in the root meristem cells of *A. cepa* after 8 hours treatment by high doses of camel urine and recovery periods.

Concentration (%)	Time of Recovery(hr)	Mitotic Index ±SE	Mitotic phases Percentage of dividing cells ± SE		
			Prophase	Metaphase	Anaphase + Telophase
0	24	7.21±0.2	36.03±0.08	33.4±0.08	30.57±0.08
	48	7.4±0.03	35.27±0.19	31.22±0.19	33.51±0.01
	72	7.55±0.17	32.12±0.04	34.23±0.06	33.66±0.08
10	24	**3.04±0.04	**11.79±0.22^{oo}	**72.44±0.17^{oo}	**15.76±0.14^{oo}
	48	**4.9±0.07^{oo}	**49.02±0.23^{oo}	**18.01±0.12^{oo}	32.97±0.13^{oo}
	72	7.2±0.06^{oo}	35.09±0.36^{oo}	31.78±0.17^{oo}	33.13±0.5^{oo}
20	24	**0.08±0.01^{oo}	**2.99±0.16^{oo}	**89.63±0.26^{oo}	**7.38±0.15^{oo}
	48	**0±0^{oo}	No dividing cells		
	72	**0±0^{oo}			

** Significant to control at 0.01 level.
^{oo} Significant to 8 hr. treatment at 0.01 level.

Cytological aberrations:

The percentages of the total abnormalities and stickiness gradually decreased and significantly below 8hr.treatment during value different recovery periods of 10% camel urine dose (Table 5 and Figure 8-a and b). After 72hr., the total aberration frequency decreased from 89.86% to 4.08% and the percentage of stickiness decreased from 86.96% to 1.96%. However, these values were still significant to control sample. Beside stickimetaphase, multipolar and tetrapliod cells were observed during recovery periods.

On the contrary, at 24hr.recovery time of 20% treated samples, the frequency of the total aberrations and stickiness increased significantly to 100%. During 48 and 72hr.of recovery durations, there were not any dividing cells.

Thus, recovery experiments illustrated gradual activation of DNA synthesis in meristem cells treated by 10% dose of camel urine. Concequently, the number of cells entering mitosis was increased. On the other hand, the chromosomes lost most of their stickiness and the effect on the spindle formation probably reduced. Therefore, high percentage of arrested metaphase cells continued the mitotic process and finally mitotic indices were normalized at the end of recovery durations. The recuperation of the mitotic activity in *A.cepa* meristem cells was also observed during recovery periods for *Retama spaerocarpa* (Chacon *et.al.*, 1994); parasitic *Viscum cruciatum* (Gomez *et.al.*, 1996) and *Achillea ageratum* (Saenz *et.al.*, 1999) extract effects.

On the other hand, the present data indicated genotoxic effect at all recovery times for root tips treated by 20% concentration of camel urine. It means that the sublethal dose has strong inhibitory action on the genetical, cytological and biochemical activities of normal cells. Similar effect was noted for different medicinal plant extracts (Kabarity and Malallah, 1980; Adam and Rashad, 1984; Saggoo *et.al.*, 1991 and Banerjee, 1992).

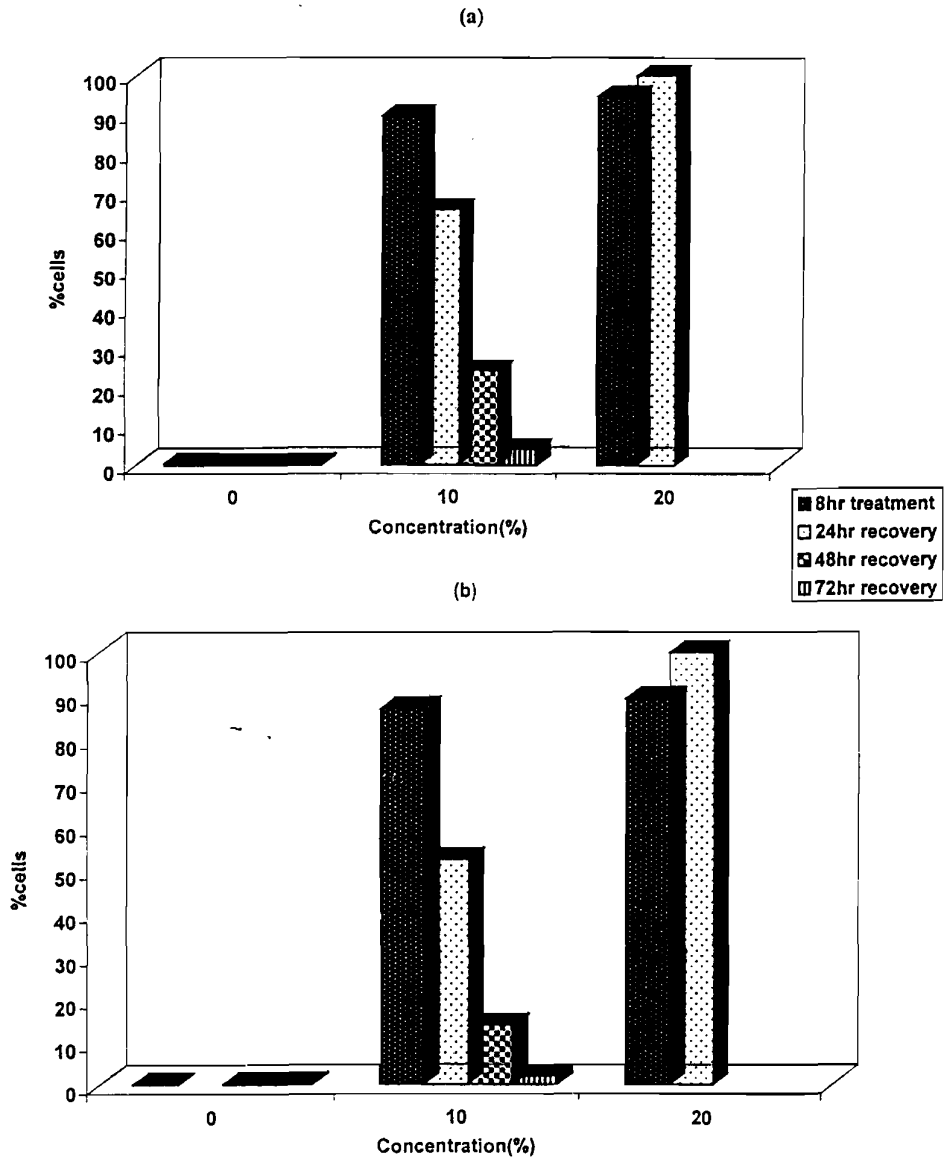


Figure (8): (a) total percentage of aberrations, (b) percentage of stickiness in the root tip cells of *A.cepa* after 8hr treatment by high doses of camel urine and recovery periods.

Therefore, it is very important to estimate the exact doses of camel urine which are not harmful for human normal cells.In addition, the effective components shoud be isolated from such natural product and their genetical, cytological and biochemical actions on normal cells must be study. Then, they could be used as a drug in exact doses for human.

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تثبيط الانقسام الميتوزي و محتوى المادة الوراثية بواسطة بول الإبل في خلايا القمم النامية لجذور البصل

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يستخدم البدو بول الإبل في علاج كثير من الأمراض حيث أنهم يعتقدون أنه يحتوي على مركبات تؤثر فسيولوجياً على جسم الإنسان. لذلك إستهدف هذا البحث دراسة تأثير بول الإبل على السلوك الميتوزي و الكروموسومي في الخلايا الطبيعية (خلايا القمم النامية لجذور البصل) و علاقة ذلك بكمية المادة الوراثية بتلك الخلايا.و قد تم تحقق ذلك من خلال المعاملة المباشرة للجذور بتركيزات مختلفة من بول الإبل و لمدد زمنية مختلفة. وأيضاً للتعرف على مدى ثبات تأثير هذا المركب الطبيعي، تم إنعاش الجذور للمعاملات عالية التركيز لفترات زمنية مختلفة. أظهرت النتائج أن لبول الإبل 'سمية وراثية على الخلايا إلا أنه في نفس الوقت لم 'يحدث أي كسور كروموسومية. فقد تبين التأثير المثبط للانقسام الميتوزي في الخلايا المعاملة ببول الإبل وإتضح أن هذا التأثير يزيد بزيادة التركيز و مدة المعاملة الزمنية. و من المحتمل أن تكون تلك الفاعلية ناتجة عن الطبيعة السمية الخلوية لبعض مكونات بول الإبل و الممثلة في القواعد البيورينية و الهيبوكسانثين و الكرياتينين. كما قد يكون عدم إستحداث بول الإبل لكسور كروموسومية ناتج عن تأثير بعض مركباته المضادة للأكسدة و الطفرات مثل حامض اليوريك و الكرياتينين. إتضح أيضاً من الدراسة أن التأثير المثبط للإنقسام الميتوزي قد يكون ناتج عن تراكم الخلايا في الدور الاستوائي كنتيجة للزوجة الكروموسومات الشديدة و التي أظهرتها ككتلة كروماتينية و أعاقتها من الاستمرار الى الأدوار الميتوزية التالية خاصة في المعاملات عالية التركيز لفترات زمنية طويلة. الى جانب اللزوجة الكروموسومية، شوهدت أيضا أنواع أخرى من الشذوذات الفسيولوجية الناتجة عن التأثير على تكون خيوط المغزل. من جهة أخرى قد 'يعزى التأثير التثبيطي للإنقسام إلى إنخفاض أعداد الخلايا المقبلة على الإنقسام و كذلك لتثبيط بناء المادة الوراثية في المرحلة الخلوية S. تبين من خلال تجارب الإنعاش أن فتراتها كانت كافية وفعالة في إستعادة خلايا الجذور المعاملة بالتركيز ١٠% من بول الإبل للمعدلات الطبيعية للإنقسام و نسب الأدوار الميتوزية و كذلك كمية المادة الوراثية. وعلى العكس من ذلك فقد إستمر إنخفاض معدل الإنقسام و كذلك كمية المادة الوراثية إلى جانب التراكم التام للخلايا في الدور الإستوائي لزيادة اللزوجة الكروموسومية خلال إنعاش خلايا الجذور المعاملة بالتركيز ٢٠% إلى أن بلغت الخلايا التثبيط الميتوزي الكامل. 'يجرى حالياً مزيد من البحث لتفسير تأثير بول الإبل على المراحل المختلفة من الدورة الخلوية وكذلك على التعبير الجيني.