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Genotoxic Effect of *Narcuss tazetta* Extracts on *Vicia faba* Seeds and in Some Organs on Mice

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ABSTRACT: In present study evaluated the genotoxic and cytotoxic effects of the crude aqueous extracts of *Narcuss tazetta* on the *Vicia faba* L. seeds after storage for one, three and six months. In the first experiment, the *Narcuss tazetta* extracts caused losing in the mitotic index accompanied with considerable percentage of chromosomal aberrations. These abnormalities include stickiness, disturbed chromosomes, bridges, lagging chromosomes and micro-nuclei. The percentage of inhibition of germination reached to 26.37 % after storage for six months but reached to 63.33 % after storage for one month. The value 7.44% lowest mitotic index was recorded after treated with narcuss extracts storaged for one month. The mitotic inhibition in six months were lower than one and three months. The degree of limit mitotic inhibition reached to 23.14 %, 14.36% and 13.45% after treated with narcuss extract and storage for one ,three and six months. In the second experiment, the cytogenetic effects of extracts *Narcuss tazetta* residues in *Vicia faba* seeds after stored for six months was evaluated in Siwiss mice.The studies included chromosomal aberrations analysis in bone marrow , spleen and spermatocyte cells. The results demonstrated that the cytogenetic effects induced in different mice tissues by extracts *Narcuss tazetta* residues and increased with increasing the feeding periods. Feeding mice with stored seeds of *Vicia faba* for six months had significant effect for the induction of chromosomal aberrations . The percentage of chromosome aberrations reached 10.4±0.84, 11.4±0.77 and 10.2±0.70 ($P<0.01$) in bone marrow, spleen and spermocytes cells respectively after feeding mice for three months. the abnormalities in spermatocytes was significant increase in univalent formation only,predominantly of the sex chromosomes.The obtained results indicate that extracts *Narcuss tazetta* residues in stored *Vicia faba* seeds have potential geneotoxic effect in mice under the conditions tested. These results indicated that the narcuss extracts had a genotoxic effects on germination, mitotic division in plant and bone marrow, spleen and spermatocytes cells in mice.

KEYWORDS: *Vicia faba* , *Narcuss tazetta* extract , chromosome aberration , genotoxicity.

I.INTRODUCTION

The use of plant materials or plant extracts for the protection of crops and stored seeds or grains from insect pest. Some plants contain a toxic components for pathogens are called botanical pesticides. The residues of some natural plant products not have harmful residues and the other caused damage plant or harm to human and animals. These natural products as phenoils, terpenoids and alkaloids don't know how long takes for disintegrate or disappear from nature[1,2].

The secondary compounds of plants have wide range of biological activities. Unlike compounds synthesized in laboratories, secondary compounds from plants are virtually guaranteed to have biological activity and that activity functions highly in protecting the producing plant from a pathogen, herbivore or competitor [3].

The Amaryllidaceae family are well known for the presence of an exclusive group of alkaloids with a wide range of biological activities. Within this group, the genus Narcissus has been extensively used in traditional medicine to treat a variety of health problems.

Chu and Bun [4] represents the first report of purification of a glutamine-rich antifungal peptide (nartazin) from family Amarylliaceace. The peptide, designated as nartazin, was purified from the bulbs of the Chinese daffodil *Narcissus tazetta* var. Peptide (nartazin) is antifungal.

Many reports reveal that extracts of plants origin are genotoxicity to measure their harmful effects on chromosomes during the mitotic and meiotic divisions that may lead to aberration chromosomes. Root tips of *Vicia faba* and mice were used as an experimental material.

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II. MATEREALS AND METHODS

1-Plant experiment

Plant material : the *Vicia faba* (2n=14) seeds var. Giza 3 were used for all the experiments and were obtained from the Crop Research Institute, Agricultural Research Centre, Giza, Egypt. Also, bulbs of Narcissus sp. (2n= 20), var. *Narcissus tazetta.*

Treatment materials:

Preparation of aqueous narcissus extract: About 500g of narcissus bulbs were homogenized in distilled water using blender at high speed for several minutes complete to 1L and let for 48 h in room temperature then filtered and keep the filtrate in refrigerator till it used.

Many *Vicia faba* seeds, weighting 1 Kg, mixed with cured narcissus extracts. The seeds aerated for 24 h at room temperature in order to dry and then stored for one, three and six months under normal conditions (at $20^0C\pm 2^0C$). Non-treated seeds used as control.

Seed germination**:** At the end of the storage periods, the seeds washed and were soaked in tap water for 24 h, then germinated in rolls of filter paper moistened with tap water. Three replicates were selected (15 seeds/replicate) for each treatment and the control. The percentage of seed-germination was estimated when the seedling were three days old.

$$\text{Relative Germination Ratio} = \frac{\text{germination ratio of test plant}}{\text{germination ratio of control}} X100$$

Percentage inhibition on germination of treatment to control were calculated, using the following inhibited establishment of formula as suggested by Sundra and Pote [5,6,7] .

I =100 – (E2 100/E1)

I = % inhibition,

E1 = Response of control plant,

E2= Response of treatment plant

Mitotic studies**:** At the end of the storage period, the seeds washed and soaked in tap water for 24 h, then germinated in rolls of filter paper moistened with tap water. The roots were cut off when reached, 1.5-3.0 cm in length, fixed in acetic acid-ethyl alcohol (1:3) V/V then hydrolysis in 1N HCL and stained using Feulgen squash technique. Three replicates were selected for each treatment and control , three roots were examined/replicate. All experiments were conducted at room temperature
(22±1ºC). The mitotic index and the mitotic inhibition were estimated as follows:

mitotic index = No.of dividing cells/ No.of dividing cells+ No.of interphase cells.X100

$$\text{The mitotic inhibition} = \frac{\text{(mitotic index in control-mitotic index in treated)}}{\text{mitotic index in control.}} X\ 100$$

Chromosome abnormalities were scored in the pro-meta-and ana-telophase stages.

2-Animals experiment:

Male white Swiss mice aged 9-12 weeks were used. The animals were obtained from a closed ,random bred colony at the Egyptian Organization for Biology and Vaccine Production. In all experiments , animals were maintained under controlled conditions of temperature and humidity and were sacrificed after feeding with stored *Vicia faba* seeds .

At the end of the storage period, the seeds were well washed with water-acetone mixture (3:1) at room temperature to remove the extract residues and were air dried.

Bone –marrow and spleen preparations were made according to the technique of [8]. and testes according to the technique of [9] . About 100 well. spread diakinesis –metaphase cells were analyzed per animal for abnormalities in five mice per group.

Statistical evaluation: All cytological data were reported as mean values and Standard Error (S.E.) of the mean. SPSS computer software was used to estimate the t-test for significance at $p\leq 0.05$ and $p\leq 0.01$ level.

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III. EXPERIMENT RESULTS AND DISCUSSION

1-Plant experiment:

Seed Germination**:** The results summaries the effect of narcuss aqueous extracts on the percentage of seeds germination of *Vicia faba* after treatment with narcuss extracts and the seeds storage for one, three and six months were lower than control.

Table (1) clear the percentage of germination revealed a significance decrease and reached to less half with treatment after storage for one month compared to control. Also, it improved after long time of storage. The percentage of germination improved after storage for 6 months and reached to 73.13%. This mean that, the percentage of inhibition of germination reached to 26.37 % after storage for six months but reached to 63.33 % after storage for one month (Table 1). This finding was agreed with that previously studies by [10,11,12] .

Table 1:Relative germination ratio ,Percentage inhibition of germination, Mitotic index, Percentage of limit mitotic inhibition, Percentage of mitotic phases and Percentage of abnormal phases in *Vicia faba* root-tip meristems after seeds treatment with *Narcuss tazetta* extracts and storage for one ,three and six months.

Time of Storage (month)	Relative %of germination rate	% of inhibition germination	% MI± S.E.	% of limited mitotic inhibition	Mitotic phases					
					Prophase		Metaphase		Ana-telophase	
					%	%abn.	%	%abn.	%	%abn.
control	98.13	1.o7	9.68±0.31	00.00	40.10	0.25	26.33	0.52	33.57	1.03
One	36.67	63.33	7.44±0.50**	23.14	38.45	2.70	39.67	61.06	21.88	42.99
control	100	00.00	10.00±0.68	00.00	41.70	0.00	25.34	0.98	32.96	1.03
Three	66.33	33.67	8.29±0.21**	14.36	39.00	1.86	36.33	56.53	24.67	36.91
control	99.00	1.00	9.87±0.67	00.00	41.68	0.00	25.78	1.06	32.54	0.86
six	73.13	26.37	8.33±0.05*	13.95	37.89	1.45	32.87	38.33	29.24	21.38

*: Significant at 0.05 Level(t-test). **: Significant at 0.01 Level(t-test).

Mitotic Studies: Tables (1 and 2) represent mitotic activities and chromosomal aberrations induced after *Vicia faba* seeds treated with narcuss extract and storage . The mitotic index after treated with narcuss extract and storage for one month was recorded that 7.44%.

Highly statistically significant reduction of mitotic index values resulted at all storage periods (Table 1). The degree of limit mitotic inhibition reached to 23.14 %, 14.36% and 13.45%with narcuss extract and all storage period. Percentage of limit mitotic inhibition slightly improved and reached to 13.45 %. This means that long periods of storage were improved the mitotic index.

From the results obtained the cytogenetic analysis indicated that mode of action for narcuss extracts toxicity involved disturbance of mitotic processes and induction of cell division aberrations. In the present study, the treatment of faba seeds induced a decrease in mitotic index (Table 1). The reduction in mitotic index suggests that, the cells undergoing mitosis are toxically(cytotoxic and genotoxic) affected by these treatments at end of time storage, the levels of toxicity which appearance various chromosome related to abnormalities increase toxically affected, after the cells exposed to narcissus extracts.

The narcuss extracts effected on percentage of different mitotic phases and abnormalities of phases, the narcuss extracte have slightly decrease effect on ana-telophase stages (Table 1). Low abnormalities were observed in the prophase stage.

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The extracted from fresh bulbs of many varieties of daffodils two compounds have been extracted. Although their structure are related to many Amaryllidaceae alkaloids, the compounds show nitrogen is amidic in character. The first substance, named narcislasine , which a strong anti mitotic activity and has been assigned structure VIII or its mirror image[13].

The percentage of abnormalities was higher in *Vicia faba* root-tip meristems after seeds-storage for all period of storage. The highest percentage of chromosomal abnormalities was 24.89 ± 0.18** ($p<0.01$) after had treated with narcuss extracts and storage for one month. Prolongation of the storage period to six months had lowering effect on the percentage of abnormalities in the root-tip cells of *Vicia faba* (Table 2).

Table 2: Percentage of abnormal mitosis and the types of mitotic abnormalities in *Vicia faba* root- tip meristems after – treatment with extracts *Narcuss tazetta* and storage for one, three and six months.

Time of Storage (month)	treatments	% of abn. ± S.E.	Type of abnormalities relative to total abnormality				
			Stick.	Distr.	Brid.	Lagg.	Micro.n.
one	Cont.	0.89 ± 0.85					
		24.89 ± 0.18**	76.00	7.13	7.43	5.24	5.20
three	Cont.	0.58 ± 0.76					
		11.78 ± 0.19**	51.32	17.86	15.38	10.30	5.14
six	Cont.	0.48 ± 0.23					
		8.39 ± 0.57**	40.00	23.87	19.13	15.00	5.00

**: Significant at 0.01 Level(t-test).

Most types of chromosome aberrations observed in high percentage were stickiness, disturbance, chromosome bridges in anaphase and telophase, lagging chromosome and micronuclei appearing in interphase cells (Table 2). Treatments with narcissus extracts caused high chromosome frequency after storage for one month caused high chromosome aberration frequency compared to the control. The highly percentage of chromosome aberration reached to 24.89±0.18**(P<00.1)compared to control 0.89±0.85 after treated seeds and storage for one month .

These observations above may be due to the nucleotoxic action of the extracts or disturbance of formation of spindle fibers during cell division which cause to chromosomal aberrations.

Stickiness of chromosomes were the most aberration of this treatments (fig.1.a) .The percentage of stickiness reached to 76% after treated seeds and storage for one month. Percentage of disturbed reached to 23% after treatment and storage for six months.

Stickiness might be due to the effect of pollutants and chemical compounds on the physical-chemical properties of DNA, protein or both, the formation of complexes with phosphate group in DNA, DNA condensation or formation of inter and intra chromatid cross links[14,15] .

Disturbance during metaphase and anaphase (fig.1.d), the plant extract as a toxic agent on formation of the mitotic spindle. Chromosomes bridge (fig.1.c) during anaphase and telophase raises when the chromosomes fail to separate because of chromosomes stickiness. Chromosome fragment is an indication of chromosome break, and can be a consequence of anaphase/telophase bridges.

Micronuclei (MN) (fig.1.e)often result from the a centric fragments or lagging chromosomes that fail to incorporate into daughter nuclei during telophase of the mitotic cells and also, it can cause cellular death due to the deletion of primary genes. These abnormalities have also been reported for several extracts and chemicals already investigated as also observed by [16,17,15] .

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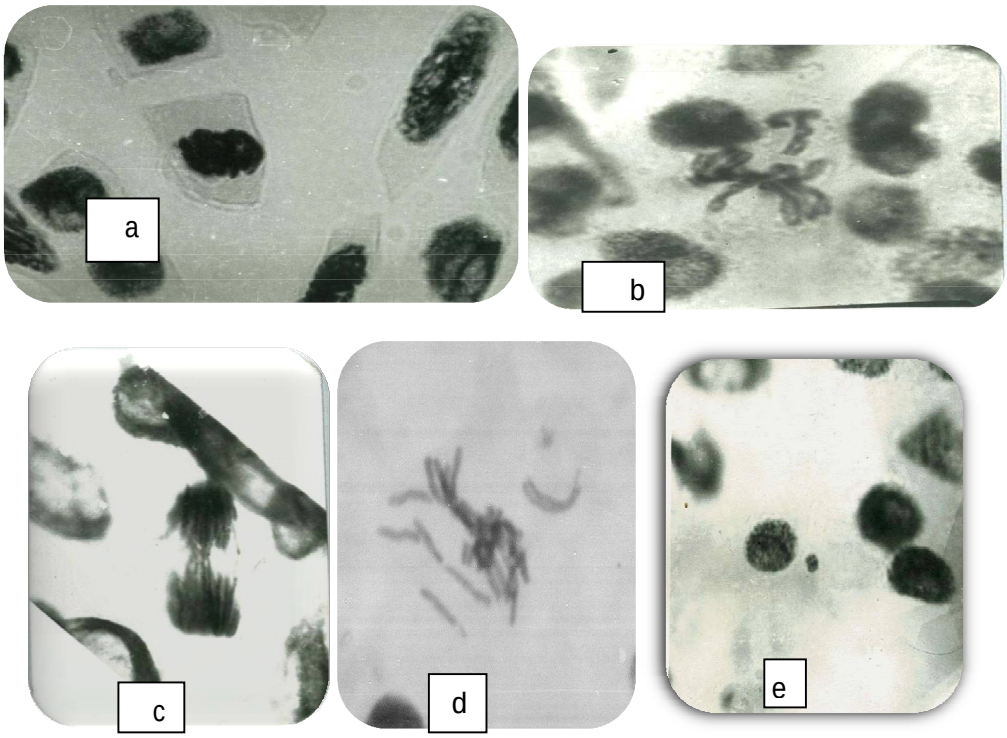

Fig.1(a-e): Chromosomal abnormalities induced in root- tip meristems cells of *Vicia faba* after seeds treatment with *Narcuss tazetta* extracts and storage. (a): Sticky metaphase after storage for one month (b) lagging metaphase after storage for six months, (c):Two Sticky anaphase bridge ,(d): Disturbed anaphase after storage for three months (e): interphase with micro-nuclei after storage for one months.

2-Animal experiment

The cytogenetic studies conducted on mice feed with storage seeds of *Vicia faba* treated with extracts *Narcuss tazetta* revealed the induction of chromosome aberrations in bone marrow, spleen and spermatocytes cells.

Table (3) represents chromosomal aberrations induced in bone marrow cells after feeding mice for one ,two, and three months with storage seeds of *Vicia faba* for 6 months. A significant increase in the percentage of chromosomal aberrations was observed in mice , it reached its maximum of 10.4± 0.84 (P<0.01) after feeding mice for three months.

Table 3: Number and percentage (%) of metaphase with different types of chromosome aberrations in mice bone-marrow cells after feeding mice for one, two and three months with extracts *Narcuss tazetta* and storage for six months(26weeks).

Time of feeding (month)	Mice No.	Metaph. No.	Metaphases with:						
			Chromat. & chromos.gap	Frag.and / or break	Del.	Del.+ frag.&/or break	Frag and/or break+ gap	Poly-ploidy	Mean%±SE
Control	5	500	6	5	4	-	1	-	3.6± 0.39

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One	5	500	16	13	7	2	3	1	8.4±0.63**
Two	5	500	19	14	7	2	3	1	9.2±0.90**
Three	5	500	21	16	6	4	3	2	10.4±0.84**

**: Significant at 0.01 Level(t-test).

Table (4) represent chromosomal aberrations induced in spleen cells after feeding mice. The percentage of chromosomal aberrations increased by increasing the time of feeding. It reached at 8.6±0.67, 10.4±0.83 and 11.4±o.77(P<0.01) after one , two and three months.
The chromosome aberrations observed in both bone marrow and spleen cells were fragments ,breaks, deletions, gaps and few tetraploid metaphases.

Table 4: Number and percentage (%) of metaphase with different types of chromosome aberrations in mice splenocytes after feeding mice for one, two and three months with extracts *Narcuss tazetta* and storage for six months(26weeks).

Time of feeding (month)	Mice No.	Metaph. No.	Metaphases with:						
			Chromat. & chromos. gap	Frag.and/ or break	Del.	Del.+ frag.&/or break	Frag and/or break+ gap	Poly-ploidy	Mean%±SE
Control	5	500	9	7	4	-	1	-	4.2± 0.32
One	5	500	17	15	7	2	2	-	8.6±0.67**
Two	5	500	20	18	7	3	3	1	10.4±0.83**
Three	5	500	23	19	6	3	4	2	11.4±0.77**

**: Significant at 0.01 Level(t-test).

Narcuss tazetta extracts residues in the seeds induced chromosome abnormalities in mice spermatocytes (Table 5). Prolongation of the feeding time affected a significant percentage of abnormalities. The percentage of abnormalities was 10.2±0.70(P<0.01) in mice fed for three months with storage seeds treated with *Narcuss tazetta* extracts after stored for six months. Separation of X-Y and outosomal univalent's were observed (Fig.2) . However, X-Y univalent's dominated . Fragments and numerical chromosomal aberrations were observed at a low frequency (Table 5,Fig.2).

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Table 5: Number and percentage (%) of diakinesis –metaphase1 cells with different types of chromosome aberrations in mice spermatocyte cells after feeding mice for one, two and three months with extracts *Narcuss tazetta* and storage for six months(26weeks).

Time of feeding (month)	Mice No.	Metaph. No.	Metaphases with:					
			X-Y Univ.	Autosom. Univ.	X-Y univ.+ A.U.	Frag. and/or break	Tri. ploidy	Mean%±SE
Control	5	500	11	5	-	-	-	3.2± 0.83
One	5	500	15	13	5	1	-	6.6±0.51**
Two	5	500	18	15	6	2	1	8.4±0.63**
Three	5	500	20	17	10	2	2	10.2±0.70**

**: Significant at 0.01 Level(t-test).

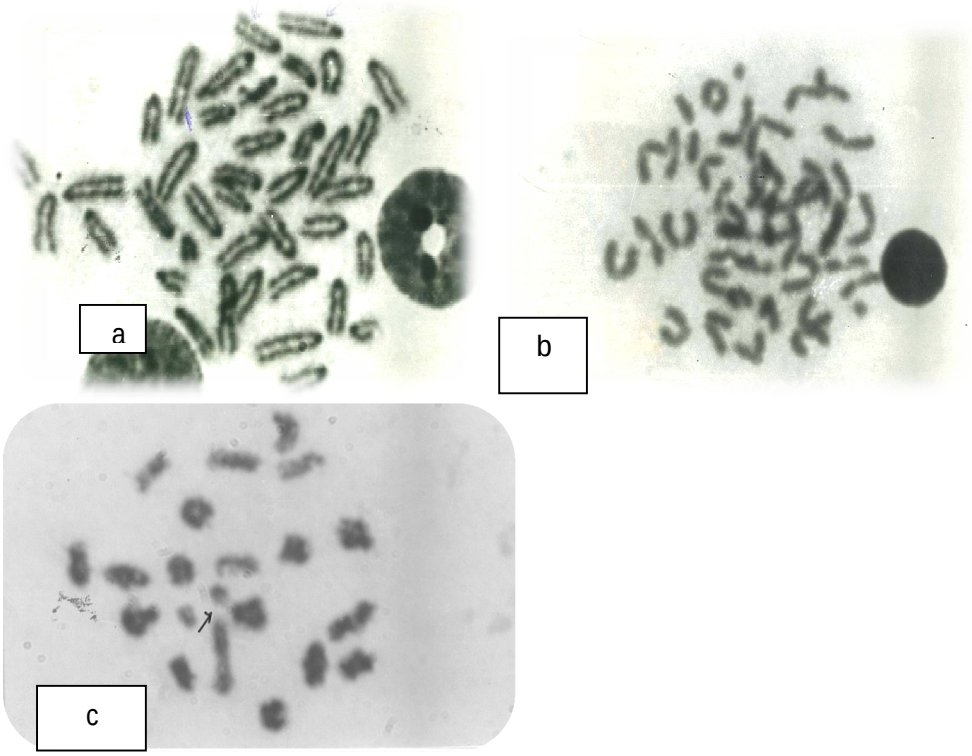

Fig.2(a-c):A metaphase with chromosome and chromatid gaps form mice bone- marrow(a). and metaphase with ring and fragment from mice splenocyte (b) after feeding mice for three months with *Vicia faba* seeds stored for Six months. A metaphase with outosomal univalent and fragment from mice spermatocytes (c) after feeding for one month with *Vicia faba* seeds treated with crud extracts of *Narcuss tazetta* and stored for six months.

The results demonstrated that the percentage of the induced chromosome aberrations in the cells analyzed from three tissues was significant in mice fed with storage seeds treated with *Narcuss tazetta* exctracts. prolongation of the time

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of feeding effected a significant increase of chromosome aberrations. This indicate that the residues of extracts *Narcuss tazetta* increased by increasing the time of feeding . The maximum percentage of chromosome aberrations was observed in mice fed with the storage seeds for three weeks. The present results are in agreement with the findings of Donya[18] who reported that , feeding mice for one,two and three months with *Vicia faba* seeds treated with DDVP (Dichlorovinyl-dimethylphosphate) or "Dursban" and stored for different periods of time (13,26 and 39weeks) mostly affected a significant ($P<0.05$) increase in the percentage of chromosome abnormalities in splenocytes and spermatocytes cells. Amer *et al*.,[19,20] also reported that the period of storage of seeds is an important factor which affected the percentage of the induced chromosomal aberrations in different tissues of the mouse after treatment with the insecticide sevin and DDVP.

It is worth mentioning in this respect that feeding mice with malathion (received in the diet) induced a significant increase in chromosomal aberrations in bone – marrow cells. Its percentage increased by increasing the dose and the time of feeding [21].

Farghaly [22] mentioned that, the Malathion and Pirimiphose methyle residues in stored wheat induced a cytogenetic effects in somatic and germ cells of mouse. According to Anderegg and Madison [23] stored wheat grains treated with ^{14}C –malathion showed considerable binding into grains after a storage period of one year. The percentage of unextractable residues was found to increase with increase of storage time.

The principal type of chromosome aberrations induced in bone-marrow and spleen cells in mouse fed the stored grain includes fragments, breaks, chromatid deletions, chromosome and chromatid gap and few numerical metaphases. Chromatid- gaps represented the dominant type of aberrations scored in mouse bone-marrow and splenocyte cells [24].

The significant increase of abnormalities in spermatocytes of mice fed with the stored seeds with *Narcuss tazetta* exctracts was seen. The separation of chromosome forming univalents was most common type of aberrations. It was reported that malathion increased that frequencies of chromosomal univalency in mouse sepermatocytes after single and multiple dermal treatments [22].

The X and Y univalent's were more after separated than autosomes, a phenomenon observed also by Ibrahim(2003)[25]. Herbicide Glyphosate induced chromosomal aberrations *in Vitro* and *in Vivo* in different tissues in mice[26]. Farghaly *et al*., [27] mentioned that Mancopper induced a cytogenetic effects in somatic and germ cells of the mouse.

V.CONCLUSION

Our results indicate that narcuss crude extract was inhibitor to germination. The experiments showed that reduction in the effect of narcuss extract lowered according to period of storage. After storage for six months, the percentage of germination improved. Significantly higher frequencies of cells with mitotic aberrations indicated the action of narcuss extract to involve chromatin organization and mitotic spindles, leading to the induction of several abnormalities.

These results indicated that the narcuss extracts had a toxic effect on germination, mitotic division in plant and bone marrow, spleen and spermatocytes cells in mice.

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