Cytogenetic and Molecular Estimation on the Effects of Energy Drink "Bison" in *Vicia faba* Plant

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ABSTRACT: The cytogenetic effects of four concentration for energy drink "bison" i.e: 50%, 80%, 90% and 100 % were evaluated on *Vicia faba* plants. Whereas, faba bean plants at the flowering stage were spraying with obvious bision concentrations and meiotic division behavior was studied after (24, 48) h. and 15 days from spraying. SDS protein electrophrosis was estimated in treated *Vicia faba* after spraying with 15 days. RAPD-PCR reaction was conduct in M_2 faba bean plants which treated with the highest bison concentration (100%). All bison treatments caused highly significant of total meiotic abnormalities, which it increased as the time of treatment prolonged in both bison concentrations 50% and 80%. While, this trait decreased after 15 days from spraying at both 90% and 100% bison. On the other hand, this parameter increased as bison concentration increased from 50% to 90% at the 48 h. period. Meiotic abnormalities in the second division were lower than those recorded in the first division in the almost treatments of three bison concentrations(80%; 90% and 100%), these results indicate that a recovery in this age. The most abnormalities were shown in metaphase and anaphase in both two meiotic divisions. On the other hands, stickiness and disturbed were the most dominant of abnormalities. In addition laggards, bridges and micronuclei occurred but with very low percentages in some treatments. All bison treatments caused increasing of four protein bands intensity with molecular weight: 140, 85, 55 and 35 KDa. The highest bison concentration (100%) showed a polymorphic genetic bands by using RAPD-PCR product comparing with control. Results concluded that energy drink "bison" had mutagenic effect on *Vicia faba* plant.
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Key words: Cytogenotoxic effects, meiotic division behavior, SDS protein electrophrosis, RAPD-RCR reaction

1-INTRODUCTION

Energy drinks are soft drinks advertised as being specifically designed to provide energy and it consumption has exploded over the past three decades. Generally they include a combination of methylxanthines including, caffeine, B-vitamins and herbal ingredients (such as: guarana; taurine, ginseng, inositol, carnitine). Many of energy drinks have high caffeine content and are not suitable for: children, pregnant woman, people allergic to caffeine, people with heart disease or high blood pressure, diabetes, disrupt adolescent sleep patterns, exacerbate psychiatric disease and increase the risk of subsequent addiction, (Nielsen, and Popkin, 2004; Bigard, 2010; Payam et al., 2012; Wolk et al., 2012).Caffeine stimulated the central nervous system, cardiac muscle, increased urinary output and relaxation of smooth muscles, (Yukawa et al., 2004; Dash and Gummadi, 2008). Chromosomal aberrations induction and alteration of genetic material are the sensitive and important tests for evaluating genetic hazards of environmental mutagens, and /or carcinogens, because there is a clear association between chromosomal aberrations and certain types of cancer.

Caffeine induced abnormal sperm cells and induction micronucleated polychromatic erythrocytes in mice, (Sylvia and Adekunle, 2010). On other hand, the cytogenetic effects induced by two caffeine concentrations (0.1%, 0.5%) in root meristematic cells of plants belonging to two species of economic importance *Phaseolus vulgaris* and *Raphanus sativus* were studied. Mitotic index, frequency and type of ana-telophase chromosome aberrations, as well as the frequency and categories of metaphase abnormalities were comparatively analyzed for the two species. The results showed that caffeine has genotoxic potential; it induces important alterations at the level on genetic material. The maximum tested concentration (0.5% caffeine) provided the most complex pattern of ana-telophase aberrations, especially in radish. *R. sativus* genotypes presented a higher sensibility to the caffeine action, (Elena et al., 2011). Meanwhile, two food additives: boric acid and sunset yellow caused chromosomal aberrations in *Trigonella foenum-Graecum* and metaphase aberrations were more prominent than anaphase aberrations, (Kumar and Srivastava (2011) two food preservatives: sodium meta-bisulphate and sodium benzoate decreased mitotic index and increased chromosomal abnormalities in *Allium cepa* root tips, (Onyemaobi et al., 2012). On the other side, Altered protein bands

and chromosomal abnormalities observed in mice-bone marrow cells and human-cell culture after treated with two antiepileptic drugs (fenaton and diazebam, 2001). Meanwhile, *Rhazya stricta* leaf aqueous extract (used as folkloric medicine for inflammatory conditions) caused mitotic index depression, induced chromosomal abnormalities, decreased total protein content and caused polymorphic DNA bands in *Allium cepa* root tip meristems(Baeshin et al., 2009). The purpose of this study was to determine the genetic effect of energy drink (bison) in *Vicia faba* plant using cytological and molecular genetics assays.

2. MATERIALS AND METHODS

2.1.MATERIALS:

2.1.1-Biological Material:

Vicia faba plants Variety Giza 40 kindly produced from Crop Research Institute, Agricultural Research Center, Giza, Egypt.

2.1.2-Tested Energy Drink:

Energy drink "Bison" produced by Abuljadayel Beverages INC Jeddah, Saudi Arabia. P.O. Box. 3865.

Amount per serving: Nutrition Facts Serving Size (100 ml)

Total carbohydrate	13. g
Sugar	13. g
Caffeine	24. mg
Taurin	0.3. mg
Vitemin C	25. mg
Niacin	6. mg
Panthotanic acid	2.5. mg
Vitamin B_6	0.6. mg
Follic Acid	0.053. mg

Colories: 51 Koal / 212.5 KJ.

2.2.METHODS:

2.2.1-Meiotic Analysis:

Vicia faba plants (Var. Giza 40) at the flowering stage were sprayed with four bison concentrations (50, 80, 90 and 100%). Control plants were sprayed with distilled water. Ten flower buds from ten different plants were gathered after (24, 48) hours and 15 days from spraying. For meiotic studies the appropriate flower buds were collected and fixed in carnoy's solution (ethyl alcohol absolute and glacial acetic acid with ratio 3:1) for 24h., and then transferred to 70% ethyl alcohol and kept in refrigerator. The cytological analysis were carried out by using 2% aceto carmine stain as described by Darlington and La Cour (1979). The data recorded for different treatments were statistically analyzed using t-test for determining significantly of differences between these treatments.

2.2.2-Molecular Analysis

a-SDS-PAGE protein Analysis:-

SDS- protein were performed on vertical slab (20 cm x 20 cm x 0.2 cm) using the gel electrophoresis apparatus (Manfactured by LABCONCO) according to Laemmli (1970). The fresh leaves were taken from *Vicia faba* plants after 15 days from spraying with the four bison concentrations and the distilled water (control), these leaves were decoated and milled to fine powder. SDS-proteins were extracted over night using OX Tris-Hcl buffer of pH 6.8. Centrifugation was performed at 10000 rpm for 10 min. Then 40µl supernatant of SDS proteins were loaded in SDS-slab gel of 15% acrylamide containing 10% SDS. Gel was run at a current of 15 m. A. for 1 h followed by 25 m. A. for 4-5 h. Molecular weights of different bands were calibrated using the wide range protein marker ranged from 25-230 KDa according to Matta *et al*. (1981). According to the electrophortic results, the treated *Vicia faba* plants with the highest bison concentration (100%) were selected to grow for seedling stage and then this treatment beside the control was planted to obtain the M_2 generation to conduct the

2.2.3.RAPD-PCR analysis.

-RAPD-PCR Analysis:-

**DNA extraction:*

Isolation of DNA from leaves in M_2 treated *Vicia faba* plants with the highest bison concentration (100%), the Protocol for DNA isolation from leaves was taken according to Doyle and Doyle, (1990)

**Polymerase Chain Reaction (PCR):*

PCR reaction was conducted using Perkin Elmer (Germany) thermocycler. RAPD was carried out using ten random 10-*mer* primers (Operon Tech. Inc., USA) with the following sequences ($5^{'}\rightarrow3^{'}$) for RAPD analysis:

Table 1. RAPD primers and their sequences

Primers	Name	Sequence 5^{-}- 3^{-}
Produce variations	B 12	CCTTGACGCA
	C 11	AAAGCTGCGG
	C 16	CACACTCCAG
	G 17	ACGACCGACA
Unable to produce variation	A 3	AGTGAGCCAC
	A 20	GTTGCGATCC
	A 18	AGGTGACCGT
	B16	TTTGCCCGCA
	E18	GGACTGCAGA
	G18	GGCTCATGTG

The reaction conditions were optimized and were mixtures consisted of the following: {dNTPs (2.5 mM) 2.0 µl; Mg Cl_2 (25 mM) 1.5 µl; 10 x buffer 2.5 µl; primer (2.5 µM) 2.0 µl; Template DNA (50 ng/ µl) 20 µl; Taq (5 U/ µl) 0.3 µl and ddH_2O-14.7 µl}.The reaction mixtures were overlaid with a drop

of light mineral oil per sample. Amplification was carried out in the thermocycler programmed for 40 cycles as follows:{94°C/4 min(1 cycle); 94°C/1 min, 37°C/1 min, 75°C/2 min (38cycles); 72°C/12min(1cycle), 4°C(infinitive)}.

**Agarose gel electrophoresis:*

Agarose (1.2%) was used for resolving the RAPD-PCR products.

λ Phage DNA digested with *Bst EII* was used as a standard DNA (15 fragments). Molecular sizes in K bp of the resulted fragments of the standard DNA ranged from 2.64 to 0.16.The run was performed for one hour at 100 V in Pharmacia submarine (20 cm X 20 cm). Bands were detected on UV-transilluminator and photographed by a Polaroid camera. Results were documented with Gel Doc 2000 (Bio RAD).

3.RESULTES AND DISCUSSION

3.1-Meiotic Analysis:

Percentages of abnormal PMC_s in the 1st & 2nd meiotic divisions and total mean meiotic abnormalities after spraying of *Vicia faba* plants with different concentrations of energy drink "bison" for (24, 48)h. and 15 days are shown in table(1). All bison treatments caused highly significant increased in total meiotic abnormalities%, which it increased as the time of treatments pro -longed at both 50% and 80% bison concentrations. These results are in agreement with the results of many researchers, (Ozturk, 2008; Atef, et al., 2011). While, this trait was decreased at the 15 days period in both 90% and 100% bison treatments as a result of recovery in this period (Table 1).

Table (1): Numbers and Percentages of abnormal PMCs in the 1st & 2nd meiotic divisions and total mean of meiotic abnormalities after spraying of *Vicia faba* plants with different concentrations of energy drink "bison" for (24, 48) hours and 15 days

Con. %	Time	Abnormal%in PMC_s meiotic division			Abnormal%in 1st meiotic division			Abnormal%in 2nd meiotic division		
		Divid. cells No.	Abnormal cells No.	Abn.% PMC_s ± S.E.	Divid. cells No.	Abnormal cells No.	Abn. %	Divid. cells No.	Abnormal cells No.	Abn. %
Control		510	18	3.44 ±1.07	276	6	2.17	234	12	5.13
50	24h.	312	52	17 ±1.98	204	24	11.76	108	28	25.93
	48h.	358	102	28.07 ±2.28	212	50	23.58	146	52	35.62
	15days	256	100	37.71 ± 2.10	138	50	36.23	118	50	42.37
80	24h.	598	304	50.83 ± 0.14	266	98	36.84	332	206	62.05
	48h.	755	399	52.93 ± 1.61	396	236	59.60	359	163	45.40
	15days	536	293	54.23 ± 2.10	292	168	57.53	244	125	51.23
90	24h.	662	266	40.08 ± 0.63	288	110	38.19	374	156	41.71
	48h.	762	414	54.34 ±0.08	352	208	59.09	410	206	50.24
	15days	654	314	47.71 ± 1.85	388	178	45.88	266	136	51.13
100	24h.	486	220	43.67 ± 2.20	228	112	49.12	258	108	41.86
	48h.	532	229	43.08 ± 1.50	266	138	51.88	266	91	34.21
	15days	544	211	38.83 ± 2.50	226	80	35.40	318	131	41.19

PMC_s: Pollen mother cells. ** highly significant ($p < 0.01$)

These results are in agreement with the results of many researchers, (Srivastava, and Singh, 2009; Fisun and Goc Rasgele, 2009) On the other hand, the meiotic abnormalities in the second division were lower than those recorded in the first division in the almost treatments of three bison concentrations (80%; 90% and 100%), these results indicate that a recovery in this age, (Srivastava, and Singh, 2009; Fisun and Goc Rasgele, 2009; Srivastava, and Singh, 2009; Fisun and Goc Rasgele, 2009). Results in Table (2) exhibit that the most meiotic abnormalities were present in metaphase and anaphase in both the first and second meiotic division, (Ozturk, 2008; Srivastava, and Singh, 2009; Fisun and Goc Rasgele, 2009) Srivastava, andSingh, 2009; Fisun and Goc Rasgele, 2009;Atef, et al., 2011). Bison significantly increased the percentage of abnormal cells at all treatments compared with control. It has been shown by many investigators that several other chemicals induce chromosomal abnormalities in different plants, (Ozturk 2008; Srivastava, and Singh, 2009; Fisun and Goc Rasgele, 2009; Atef, et al., 2011). Stickiness and disturbed were the most abnormalities which observed in both the two meiotic division. Chromosome stickiness % in first meiotic division ranged from 7.69% to 21.65% and it was increased as period duration increased in all bison concentrations except for 15 days (100% bison), which it was decreased. On the other hand, stickiness% in the second meiotic division ranged from 1.92% to 20.74% and it was lower than those recorded in first meiotic division in all treatments except for the period 24h. (80% and 90% bison).

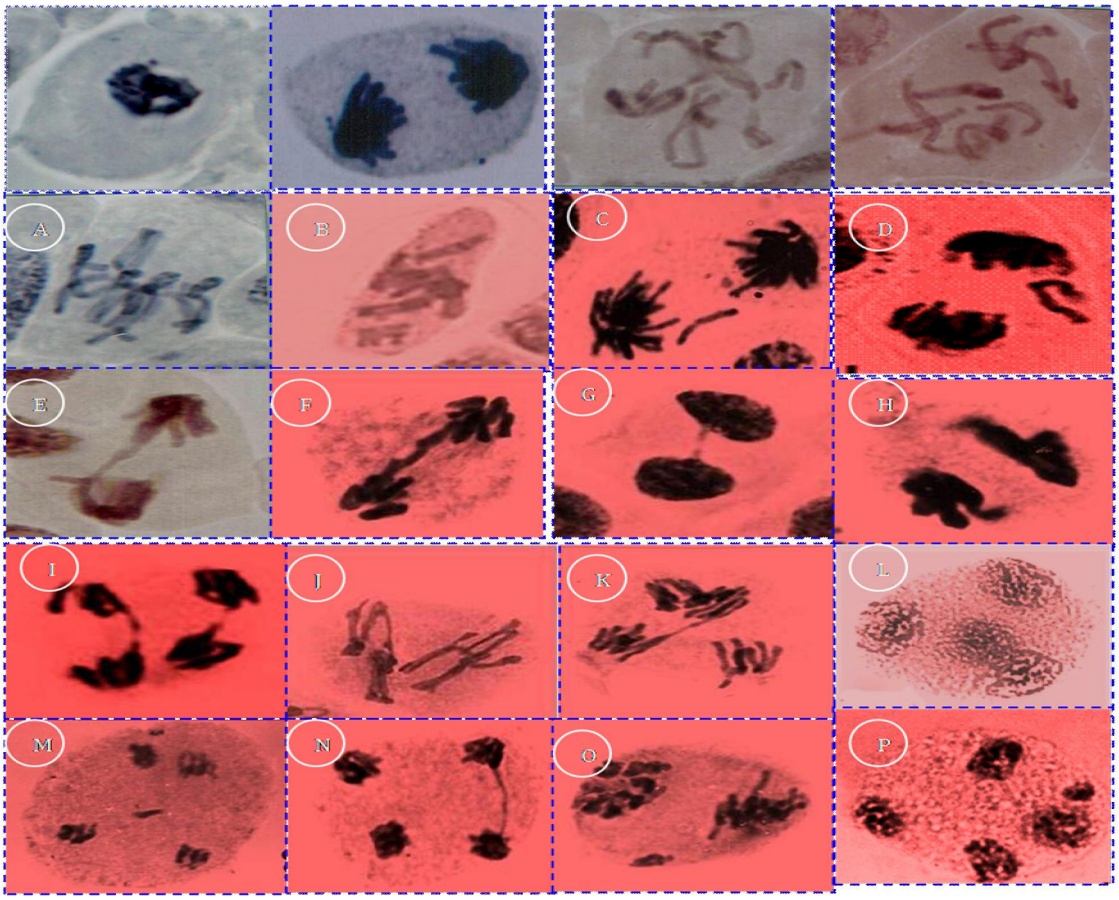
Table (2): Abnormal meiotic phases% in *Vicia faba* plants after spraying with different concent -rations of energy drink "bison" for (24, 48) hours and 15 days

Con. %	Time	First meiotic division				Second meiotic division				
		abn.meta - phase	abn.ana -phase	abn.telo - phase	Total abnormal - ities%	abn.pro - phase	abn.meta - phase	abn.ana - phase	abn.telo -phase	Total abnormal - ities%
Control		1.45	0.72	-	2.17	-	5.13	-	-	5.13
50	24h.	6.86	3.92	0.98	11.76	1.85	11.11	11.11	1.85	25.93
	48h.	15.09	6.60	1.89	23.58	4.11	8.22	15.07	8.22	35.62
	15days	15.94	17.39	2.90	36.23	5.08	22.03	15.25	-	42.37
80	24h.	12.78	20.30	3.76	36.84	4.82	22.89	25.30	9.04	62.05
	48h.	32.32	23.23	4.04	59.60	3.90	20.06	15.04	6.41	45.40
	15days	24.66	26.71	6.16	57.53	7.39	13.52	15.57	14.75	51.23
90	24h.	15.97	19.44	2.78	38.19	4.28	20.32	13.37	3.74	41.71
	48h.	28.41	25.57	5.11	59.09	3.90	25.37	16.10	4.88	50.24
	15days	17.53	22.68	5.67	45.88	4.51	23.31	16.28	6.77	51.13
100	24h.	29.82	16.67	2.63	49.12	6.20	18.60	16.28	0.78	41.86
	48h.	23.31	21.05	7.52	51.88	7.52	10.90	9.02	6.77	34.21
	15days	15.93	13.27	6.19	35.40	7.55	10.69	14.10	8.81	41.19

Also, this trait was decreased by the increasing of period duration at second division in all bison concentrations except for 50% bison, Table(3). Stickiness appeared in metaphase and anaphase in the two meiotic divisions (Fig.1: A, B, F, G, H, I, J, L& M). Our results are in agreement with the results of many researchers (Ozturk 2008 ; Srivastava, andSingh, 2009; Fisun and Goc Rasgele, 2009;Atef, et al., 2011) who suggested that the chromosomes stickiness may result from breakage and exchange between chromatin fibers on the surface of adjoining chromosomes. The second type of abnormalities is the disturbed chromosomes, which ranged from 0.37% - 9.01% and 1.88% - 11.73% in the first and the second meiotic divisions, respectively, after bison treatments (Table 3). This abnormality was shown in metaphase, anaphase in the two meiotic divisions and telophase only in the second division (Fig.1: C, D, N, O& P). Disturbed chromosomes may be due to disturbance of spindle apparatus which allows the chromo somes to spread irregularly over the cell, Srivastava, andSingh, 2009; Fisun and Goc Rasgele, 2009).In addition to the previous common abnormal - ities, it was observed more on meiotic division including: laggards, bridges and micronuclei which they occurred with low percentages in some bison treatments. Laggards chromosomes was observed in some bison treatments with low percentages ranging between 0.79%-2.62% and 0.31%-1.31% in the first and the second meiotic divisions, respectively. Whereas, laggards were present in metaphase in the first meiotic divisions and could be attributed to the failure of spindle apparatus to organize and function in a normal way, (Abdelsalam et al., 1993). These laggards may be distributed randomly to either poles at anaphase or telophase in the first and the second divisions (Fig.1: E, F, G, H &Q), and they may give micronuclei which were observed at prophase II and telophase II in all treatments for 80%;90% and 100% bison concentrations, Table(3) (Fig.1: S& T). While bridges were shown at few bison treatments concentrations with range 0.31%-1.47% in all meiotic divisions (Table 3). bridges were observed in anaphase and telophase in the first and the second divisions (Fig.1: I, J, K, M, O& R). Bridges could be due to the breakage and reunion (Fisun and Goc Rasgele, 2009)or due to the general stickiness of chromosomes, (Abdelsalam et al., 1993) Finally, the induction of these chromosomal abnormalities were pointed to the cytotoxic effects potentiality of the applied concentrations of energy drink"bison" patterns of water soluble proteins for *Vicia faba* leaves after sprayed with four bison concentrations. All bison treatments caused increasing of four protein bands intensity with molecular weight:140, 85, 55 and 35 KDa. Alteration in bands intensity could be attributed to change in the structure or performance of genes and thus they produce of changes in the gene expression of the regulatory genes used in the regulatory system of structural genes, (Ozturk, 2008;Sylvia, and Adekunle, , 2010). The increase in band(s) intensity could be attributed to gene(s) duplication which might result from cytological abnormalities induced by clomid drug. The presence of laggards chromosomes and bridges supported this conclusion which agreed with many researches, (Gangulyet al., 2010; Sefa et al., 2012).

Table (3): Percentages of different meiotic abnormalities in the 1st and 2nd meiotic division after spraying *Vicia faba* plants with different concentrations of energy drink "bison" for (24, 48) hours and 15 days

Con.%	Time	First meiotic division				Second meiotic division				
		Stickiness	Disturbed	Laggard	Bridges	Stickiness	Disturbed	Laggard	Bridges	Micro -nuclei
Control		-	1.18	-	-	0.39	1.96	-	-	-
50	24h.	7.69	-	-	-	1.92	7.05	-	-	-
	48h.	13.97	-	-	-	2.79	11.73	-	-	-
	15days	19.53	-	-	-	9.38	10.16	-	-	-
80	24h.	13.71	2.68	-	-	20.74	11.04	0.67	-	2.01
	48h.	21.46	9.01	0.79	-	13.64	6.09	-	1.06	0.79
	15days	21.65	7, 09	2.62	-	9.89	7.09	0.75	0.37	5.22
90	24h.	12.08	4.53	-	-	17.22	4.53	-	0.60	1.21
	48h.	17.85	6.30	2.36	0.79	16.54	8.13	1.31	0.26	0.79
	15days	18.04	5.81	2.45	0.92	7.65	9.17	0.31	0.61	3.06
100	24h.	14.40	8.23	-	0.41	14.40	7.00	-	-	0.82
	48h.	19.92	3.38	1.50	1.13	12.22	1.88	-	-	3.01
	15days	11.76	0.37	1.10	1.47	11.59	6.99	0.37	0.74	4.41



Figure(1): Different meiotic abnormalities produced after (24,48)h. and 15 days from spraying *Vicia faba* plants with four concentrations of energy drink" bison": A: stickiness at M_1; B: stickiness at A_1; C: disturbed at M_1; D: disturbed at A_1; E: laggard at M_1; F: laggard, stickiness at M_1; G&H: laggard, stickiness at A_1; I: stickiness, bridge at A_1; J: bridge at A_1; K: bridge at T_1; L: stickiness at M_2; M: stickiness, bridge at A_2; N: disturbed at M_2, O: disturbed, bridge at A_2; P: disturbed at T_2; Q: laggard at T_2; R: bridge at T_2, S: micronuclei at P_2; T: micronuclei at T_2 . M_1,A_1,T_1: First(meta,ana, telo)phase; M_2-A_2,T_2: Second (meta,ana,telo)phase.

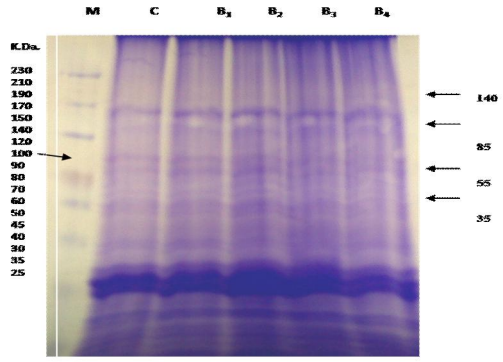
B-RAPD-PCR Analysis:

RAPD profiles of genomic DNA from M_2 *Vicia faba* plants treated with highest bison concentration (100%) by using 10 primers (A03, A18, A20, B12, B16, C11, C16, E18, G17 and G18). RAPD-PCR reaction by using four primers (B12,

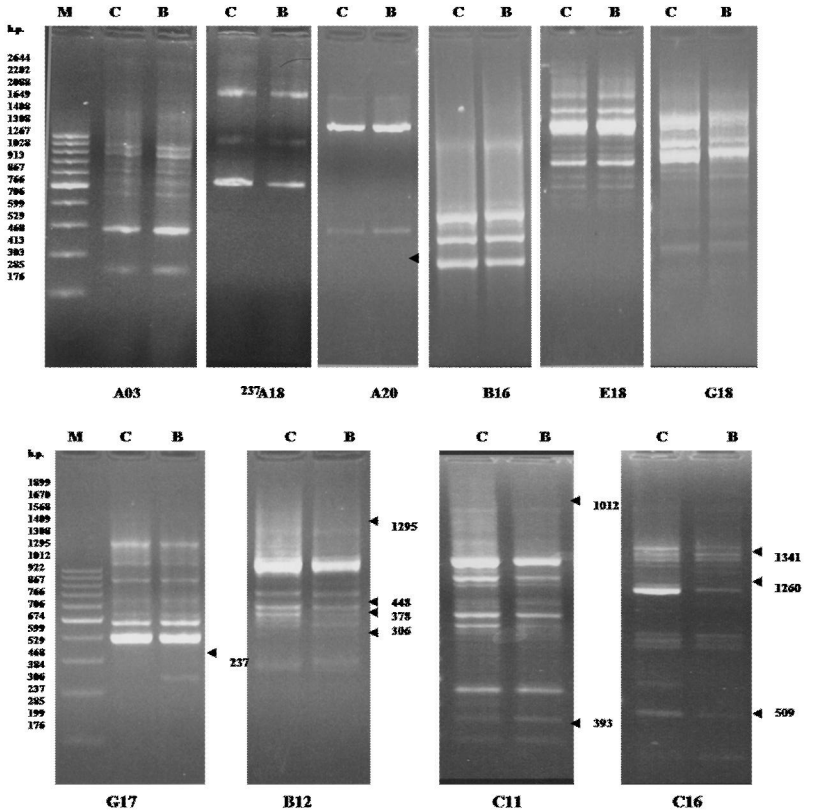
C11, C16 and G17) only revealed variation on DNA bands but the other primers don't exhibited any variation in treated *Vicia faba* plants. Bison treatments altered of 10 DNA bands compared with control, whereas 8 DNA bands disappearance and 2 new bands appearance. The polymorphic bands of the four primers were scored as present (1) and absent (0) as indicated in Table (4).

Bison treatment (100%) induced two new polymorphic bands with molecular sizes of: 237 bp (OP-G17) and 394 bp(OP-C11). On the other hand, this bison treatment caused disappearance of eight polymorphic bands with molecular sizes of:1295, 448, 378, 306 bp (OP-B12); 1012 bp (OP-C11) and 1341, 1260, 509 bp (OP-C16) as compared with the control (Table 4, Fig. 3).

This observation gives good evidence to the ability of energy drink "bison" (100%) to induce insertion or mutations as a result of deletion compromises at least few nucleotides as revealed by the appearance or disappearance of many bands as compared with the control[12]. 150 mg/100 ml clomid treatment may act as intercalation agent or generates free radicals which interact with DNA to account for the observed variation. These are in agreement with many researches[24-29]. From cytological and molecular results, it could be concluded that clomid drug have a cytogenotoxic effects and should be recommend to take this drug under control.



Figure(2): SDS–PAGE banding patterns of water soluble proteins in *Vicia faba* leaves after 15 days from spraying plants with different concentrations of energy drink "Bison".
{ M: mrker ; C: control; B_1– B_4 : Bison concentrations (50%, 80%, 90%, 100%)}



Figure(3): RAPD profiles of genomic DNA from M_2 *Vicia faba* plants treated with 100% energy drink bison by using ten primers.

{ M: marker ; C: control; Cl: clomid drug ;(P_1→ P_{10}) : primers }.

Table (4): RAPD profile alterations in DNA bands as detected with 4-primers in M_2 treated *Vicia faba* plants with 100% energy drink "bison" in compared with the respective control

Primer Code	Sequences 5′ →3′	Size of polym.bands (bp)	Control	Bison treatment
OP - G17	ACGACCGACA	237	0	1
OP - B12	CCTTGACGCA	1295	1	0
		448	1	0
		378	1	0
		306	1	0
OP - C11	AAAGCTGCGG	1012	1	0
		39	0	1
OP - C16	CACACTCCAG	1341	1	0
		1260	1	0
		509	1	0

4-REERENCEFS

[1] Nielsen, S. J. and Popkin, B. M.(2004): Changes in beverage intake between 1977 and 2001 Am. *J. Prev. Med.*, 27:205-210.

[2] Bigard, A. X. (2010): Risks of energy drinks in youths. *Arch. Pediatr.*, 17:1625-1631.

[3] Payam, P. A ; Najmeh, M. M. ;Hassan, J. H. ;Elham, S. S.; Pegah, J. I. ;Sayed, M. A. and Kamran, B. L. (2012): consumption's pattern and knowledge of athletes about energy drink in south of Iran(Shiraz). *Pakistan J. of Nutrition*, 11(8):670-675.

[4] Wolk, B. J. ;Ganetsky, M. and Babu, K. M. (2012):Toxicity of energy drinks. *Current Opinion in Pediatrics*, 24(2):243-251.

[5] Yukawa, G. S. ; Mune, M. O.; Tone, X. M. and Iwahashi, H. L. (2004): Effects of coffee consumption on oxidative susceptibility of low density lipoproteins and serum lipid levels in human. *Biochemical*, 69: 70-74.

[6]Dash, S. S. and Gummadi, S. N. (2008): Inhibitory effect of caffeine on growth of various bacterial strains. *Res. Microbiol.*, 3: 457-464.

[7] Sylvia, U. and Adekunle, A. B. (2010): DNA Damage induced in the germ and bone marrow cells of mice by caffeine. *Res. J. of Biological Sci.*, 5(8): 536-541.

[8] Elena, T. T.; Magdalena, Z. A. and Zenoovia, O. L. (2011): Caffeine induced genotoxic effects in *Phaseolus vulgaris* L. and *Raphanus sativus* L. *Botanica Serbica*, 35(1): 49-54.

[9] Kumar, G. and Srivastava, N. (2011): Genotoxic effects of two commonly used food additives boric acid and sunset yellow in root meristems of *Trigonella foenum-Graecum. Iran. J. Environ. Health Sci.Eng.*, 8 (4): 361-366.

[10] Onyemaobi, O. I. ; Williams, G. O. and Adekoya, K. O. (2012): Cytogentic effects of two food preservatives, sodium meta-bisulphate and sodium benzoate on the root tips of *Allium cepa Linn*. *Lfe. J. of Sci*., 14 (1): 155-165.

[11] El-Souady, A. E.; Badawy, F. M. and Mohamed, M. A. (2001): Cytogenetic effects of antiepileptic drugs on mice bone marrow cells and human a meiotic cell cultures. *Egypt. J.Genet.Cytol.,* 29:115-129.

[12] Baeshin, N. A.; Sabir, J. S. M. and Qari, S, H. (2009): Cytogenetic and molecular evaluations of genetic effects of leaf extract of *Rhaza stricta* (Decne) on *Allium cepa* root tip meristems. *Egypt. J. Genet. Cytol.* (38):73-83.

[13] Darling, C. D. and La Cour, E. F. (1976): The handling of chromosomes. *Sixth edition, George Allan and UnwinLid., London.*

[14] Laemmli, U. K. (1970): Cleavage of structural proteins during the assemble of the head of bacteriophage T4. *Nature*, 227-680.

[15] Matta, N. M.; Gatehouse, J. A. and Boulter, A. E. (1981): The structure of leguminous of *Vicia faba L.*Areapraisal. *J.EXP.Bot*.32 126):183-197.

[16] Doyle, J. J. and Doyle, J. L. (1990): Isolation of DNA from fresh tissue. *Focus,* 12:13-15.

[17] Atef, A. A. H; Abd EL-Hamid, N. R.; Abd EL-Hady, E. A. and AL-Ansary, A. M. (2011): Cyto-genetic effect of insecticide Tellition and Fungicide Dithane M 45 on meiotic cells and seed storage proteins of *Vicia faba. J. of American Science*, 7(1)P.P:19-25.

[18] Ozturk, I. (2008): Effect of fungicide on meiosis of tomato (*Lycopersicon esculantum mill*). *Bangldesh J.Bot.*37(2):121-125.

[19] Srivastava, A. K. andSingh, A. K. (2009): Effects of insecticide profenophos on, early growth, meiotic behavior and chlorophyll mutation of barley. *Acta.Physiol. Plant*.31:537-544.

[20] Fisun, K. and Goc Rasgele, P. (2009): Genotoxic effects of raxil on root tips and anthers of Allium cepa L.*Caryologia*.62(1):1-9.

[21] Abdelsalam, A. Z. E.; Hassan, H. Z.; ElDomyati, A. M.; Eweda, M. A.; Bahieldin, A. and Ibrahim, S. A.(1993): Comparative mutagenic effect of some compounds using different eukaryotic systems. *Egypt. J. Genet. Cytol.*22(1):129-153.

[22] Sefa, P. l.; Atila, Cr. A. and Bahattin, Ai. K. (2012): The toxic and environmental evaluation of pyrolytic liquids by Allium cepa test. *Chemistry and Ecology,* 28 (1): 65-73

[23] Ganguly, S.; Bhattacharya, S.; Mandi, S. and Tarafdar, J. (2010): Biological detection and analysis of toxicity of organophate and zadirctin-base, insecticides in Lathyrus sativus L. *Ecotoxicology*, 19: 85-95.

[24] Rong, Z. Y. A and Yin, H. W. (2004): A method for genotoxicity detection using random amplified polymorphism DNA with Danio rerio. *Ecotoxicol. Environ. Saf*. 58:96-103.

[25] Guzin, K. M.; Serdal, S. and Irem, U. (2010): Assessmen of genotoxic effects of boron on wheat (Triticum aestivum L.) and bean(Phaseolus vulgaris L.) by using RAPD Analysis. *Bull. Environ.Contam.Toxicol* 84, 759-764.

[26] Korpe, A. D. and Aras, S. R. (2010): Evaluation of copper-induced stress on eggplant (Solanum melongena L.)seedlings at the molecular and population levels by use of various biomarkers. *Mutation Research,* 550, (1), 45-55.

[27] Darinka, G. A.; Tatjana, K.; Saša, M.; Biljana, K.; Emilija, K.; Katerina, B. and Trajče S. (2012): Assessment of the genotoxicity of heavy metals in Phaseolus vulgaris L. as a model plant system by Random Amplified Polymorphic DNA (RAPD) analysis. *J. of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering.* V(3):336-373.

[28] Sedat, B. and Ozkan, A. (2012): Application of random amplified polymorphic DNA (RAPD) to detect genotoxic effect of trifluralin on maize (Zea mays). *Drug and Chemical Toxicology*, 13(2):90-99.

[29] Ozkan, A.L.; Filiz, A. E.; Serap, S. S.;Sedat, B.O.and Guleray, A.R.(2013):Assessment of genotoxic effects of 2, 4-dichorophenoxyacetic acid on maize by using RAPD analysis. *Industrial Crops and Products*, 42:552-557.

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