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Effect of the Energy Drink "Bison" on Mitosis And Nucleic Acids Content In *Vicia faba*

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Abstract: The present investigation had been conducted to study the mutagenic effects of four concentrations of energy drink "Bison" (50, 80, 90, and 100%) with three exposure times (6, 12, and 24hours)on root meristems of *Vicia faba* plant. Mitotic index was reduced in all treatments except for two treatments: 90% Bison with two exposure times 6 and 12 h. On the other hand, total abnormalities were highly increased in all treatments. The most common abnormalities were: Stickiness, disturbed, C-metaphase, star shape and lagging chromosomes. In addition, bridges, multipolar, breaks, (micro-multi) nuclii and dinucleated occurred with very low frequencies in some treatments. These results-indicated that Bison had Turbagenic effects. Unbalanced mitotic stages percentages occurred as a result of all treatments compaired with control. Different Bison treatments caused altering of DNA and RNA contents in *Vicia faba* root tips. The inhibitory effects of most Bison treatments were found to depend on exposure time dose. Hundred percentage Bison treatment for 24h caused the highest total abnormalities (90.32%) and the lowest content of DNA and RNA (4.63 and 1.98 mg\g) respectively compared with respective control 2.12%,9.73 and 3.26 mg\g respectively.These findings indicate the mutagenic potentiality of the energy drink "Bison".

Keywords: energy drink, mitosis, nucleic auids, metaphase mitotic indet, Bison, mutagenic potentiality.

Introduction

Evaluating the toxicity of environmental pollutants by plant materials was further expressed by many workshops [1- 3]. As a cytogenetic material, *Vicia faba* has the advantage of having six pairs of relatively large chromosomes that are excellent for assessing chromosomal aberrations[4]. Energy drinks are soft drinks advertised as being specifically designed to provide energy. Generally they include a combination of methylxanthines, caffeine, β-vitamins and herbal ingredients (such as: guarana; taurine, ginseng, inositol, carnitine). Many of energy drinks are not sutiable for children, pregnant women, people has allergic to caffeine, those of heart diseases or high blood pressure and diabetes. The mutagenic potential of energy drinks has not yet been examined, the present investigation was carried out to evaluate the effect of the energy drink "Bison" on mitosis and nucleic acid contents in *Vicia faba* .

Materials and Methods

Seeds of *Vicia faba* (V.Giza 40) were treated with energy drink "Bison" produced by Abuljadayel Beverages INC Jeddah , Saudi Arabia. P.O.Box. 3865.

The list of Bison contents are presented in Table 1. Four Bison concentrations corresponding to 50, 80, 90 and 100% were used for 6, 12 and 24 h. Water control was maintained for assessment of spontaneous aberrations. After exposure, root tips of 2-3 cm length were excised, fixed in Carnoy solution (3:1 ethanol – acetic acid) and stored in 70% alcohol. Aceto – Carmine squash preparations were made and examined cytologically.

Five preparations from each treatment were examined to determine the Mitotic index, mitotic stages percentage, total abnormalities percentage and abnormalities mitotic phases percentage.

On the other hand, DNA and RNA contents from all *Vicia faba* root tips treatments were isolated by a modified method based on Shibko *et al.*[5] RNA was estimated by visible spectrophotometry using the orcinol reaction as described by Ashweel[6], while DNA was estimated by the diphenylamine (DPA) colour reaction described by Burton[7].

Table 1. Nutrition Facts per 100 ml Bison.

Amount per serving:	
Total carbohydrates	13.0 g
Sugars	13.0 g
Caffeine	24.0 mg
Taurin	0.3 mg
Vitamin C	25.0 mg
Niacin	6. 0 mg
Panthotonic acid	2.5 mg
Vitamin B6	0.6 mg
Follic Acid	0.053 mg
Calories : 51 kcal / 212.5 KJ.	

Results and Discussion

1-Mitotic Phases Frequencies

The diferent concentrations of Bison showed a marked effect on the mitotic phases frequency depending on the concentration and exposure time. Prophase %; Metaphase %; and (Ana-Telo) % phases ranged from (9.14 and 34.38%); (35.15 and 71.24%) and (19.62 and 40.26%) respectively, as compared to 22.00; 36.85 and 41.14% , respectively,of the control treatment (Table 2).

2-Mitotic Activity

Mitotic index was reduced at all Bison treatments, except for 90% "Bison" at two exposure times (6 and 12h) which have slightly increased (2.55% after 6h). Further depression in the mitotic activity was induced after 50% "Bison" after 12h exposure time 12h. (2.83%), while it was 4.70% in the respective control treatment (Table 2).

The inhibition of mitotic activity could be due to the effect of "Bison" on the process of DNA synthesis, which is the essential requirement for the progress of the mitotic activity. The reduction of mitotic activity as a result to DNA synthesis was observed by Kononwicz[8]; Dolezel *et al*.[9]; EL-Fiki *et al*.[10]. Chen *et al*.[11]; Polit *et al*.[12]; Kononwicz, Usciati *et al*.[13]; Ryblaczek and Maszwski[14] in many plants such as *Vicai faba*; *Allium sativum; Allium cepa*; *Pinus silvestris*; and *Cicer arietinum* after chemicals treatment by caffeine; 2,4dichlorophenoxy acetic acid; hydroxyl urea; indol acetic acid; indol butyric acid; benzyl amino purine; gibbrellic acid; kinetin; naphthalene acetic acid was investigated.

3-Abnormalities

The various "Bison" treatments induced a wide range of abnormalities. Total abnormalities percentage were increased gradually depending on increasing of the concentration and the prolonged period of treatment. Highest total abnormalities % was induced at longest exposure time with highest concentration (100% Bison with 24h.) up to 90.32%, However, it was 2.15 % in the respective control treatment (Table 2).

Abnormality % of the mitotic phases Pro- Meta- Ana and Telo were increased by increasing the exposure time in each Bison concentrate except for abnormal prophase in 80% "Bison". The highest abnormalities % of (Pro-Meta-Ana and Telo) phases % were recorded at 100% "Bison" after 24h of treatment (Table 3).

All mitotic treatments showed that metaphase stage was the most affected one where the maximum percentage of abnormalities were scored (Table 3). These results showed that the "Bison" may affect also chromosomal regular movement toward the formation of the metaphase plate, *i.e.* they may affect chromosome and kinetochore physical and /or chemical nature.

These results were similar to those obtained by Chen *et al*.[11]; Polit *et al*. [12]; Usciate *et al*.[13]; William and Elizabeth[15]; Ryblaczek and Maszwski [14] after treatments of *Vicia faba* ,*Cicer arietinm* and *Zea mays* plants with caffeine; benzulamino prine; indol acetic acid; hydroxy urea.

The greatest types of abnormalities induced after Bison treatments Were: stickiness (40.00-90.48%), disturbed (1.48-42.86%), C-metaphase (3.82- 12.54 %), lagging chromosome (0.60-7.91%) and star phase (0.33-11.43%), Fig. 1 (a-j). The other kinds of abnormalities, *i.e.* Breaks, bridges, multipolar, (micro-multi) nuclei and dinucleated were found in low frequencies at the same treatments as shown in Table 4 and Fig. 1 (g, i-n).

Stickness was observed in Meta and Ana phases (Fig.1 a, b). Stickness was found to cover the whole chromosomes complement leading to the appearance of chromatin masses where the general appearance of the chromosomes were lost. Chen *et al*.[11]; Polit *et al*.[12]; Usciate *et al*.[13]and Rybazek and Maszwski[14] attributed such stickiness

to the process of depolymerization of DNA, thus the chromosome surface becomes sticky.

Similar results were obtained after treatment of some plants with many chemicals such as caffeine, benzyl aminopurine; indol acetic acid and hydroxy urea[11-15]. On the other hand, disturbed chromosomes were shown in Pro-Meta- and Ana- phases (Fig 1:d- g and j) as a partial action on "Bison" treatments on the spindle formation. Therefore some chromosomes lost their ability to attach with the spindle fiber. Whereas, complete action of "Bison" treatments on the spindle formation resulted in C-metaphase in where the chromosomes lost their ability to continue to anaphase and arrested at metaphase (Fig1, c). Lagging chromosomes were observed in Meta- Ana-and Telo- phases (Fig.1, f, g and i). Occurrence of Laggards at metaphase may result from hinderance of the prometaphase movement of the chromosomes accompanied by adhesion of the centromeres to the adjacent inner surface of the plasma membrane. The Laggards observed at metaphase failed to move properly toward poles and consequently , they appeared at following stages. These results are in agreement with that reported by some workers[11-17] .

4-DNA and RNA Contents

DNA and RNA contents were slightly increased or decreased after Bison treatments except at 100% Bison for 12 and 24 h of treatment, which caused marked decreasing of DNA and also after 6h for RNA contents (Table 5).

Many chemicals such as nicotine; food flavor ; food colorant; water extracts of some umbelliferous plants, and caffeine caused alteration of DNA and RNA contents[11,16,18-20].

Acording to these results, we can conclude that Bison has anti-mitotic agents which caused imbalance in mitotic phases frequencies, reduction of mitotic index in most treatments, alteration of DNA and RNA contents, marked levels of abnormalities. The inhibition effects of Bison treatments were found to be exposure time dependent rather than exposure dose dependent.

On the other hand, "Bison" has highly potent turbogenic effects (spindle poison) whereas, the greatest types of abnormalities were stickiness, disturbed, C-metaphase and lagging chromosome. These

effects may be due to some components in Bison such as :- caffeine, taurin, niacin and panthotonic acid.

Table 2. Mitotic phases percentage,mitotic index and total percentage of abnormalities in *Vicia faba* root tips treated with energy drink ((BISON)).

Treatments		No. of examined cells	No. of dividing cells	Mitotic index%	Prophase		Metaphase		(Ana-Telo) phase		Total of Abnormalities
Dose (%)	Time(h)				No.	%	No.	%	No.	%	
control		11890	559	4.70	123	22.00	206	36.85	230	41.14	**2.15**
50%	6	7926	350	4.42	116	33.14	152	43.43	82	23.43	**10.00**
	12	8201	232	2.83	59	25.43	114	49.14	59	25.43	**46.12**
	24	9065	313	3.45	36	11.50	151	48.24	126	40.26	**64.86**
80%	6	8567	404	4.72	125	30.94	142	35.15	137	33.91	**42.57**
	12	8622	347	4.02	65	18.73	164	47.26	118	34.01	**66.57**
	24	9514	305	3.21	38	12.46	161	52.79	106	34.75	**82.95**
90%	6	9296	448	4.82	154	34.38	170	37.95	124	27.67	**46.21**
	12	10050	566	5.63	132	23.32	256	45.23	178	31.45	**61.48**
	24	10577	324	3.06	47	14.51	192	59.26	85	26.23	**80.86**
100%	6	9638	355	3.68	87	24.51	169	47.61	99	27.89	**34.65**
	12	9472	400	4.22	74	18.50	207	51.75	119	29.75	**75.75**
	24	9895	372	3.76	34	9.14	265	71.24	73	19.62	**90.32**

Table 3. Abnormal mitotic phases percentages in *Vicia faba* root tips treated with energy drink "Bison".

Treatments		Prophase		Metaphase		(Ana-Telo) phase	
Dose%	Time (h)	NO.	%	No.	%	No.	%
control		1	0.81	6	2.91	5	2.17
%50	6	5	4.31	24	15.79	6	7.32
	12	7	11.86	73	64.04	27	45.76
	24	7	19.44	113	74.83	83	65.87
%80	6	39	31.20	83	58.45	50	36.50
	12	34	52.31	131	79.88	66	55.93
	24	14	36.84	150	93.17	89	83.96
%90	6	29	18.83	123	72.35	55	44.35
	12	39	29.55	203	79.30	106	59.55
	24	20	42.55	171	89.06	71	83.53
%100	6	18	20.69	65	38.46	40	40.40
	12	39	52.70	180	86.96	84	70.59
	24	19	55.88	256	96.60	61	83.56

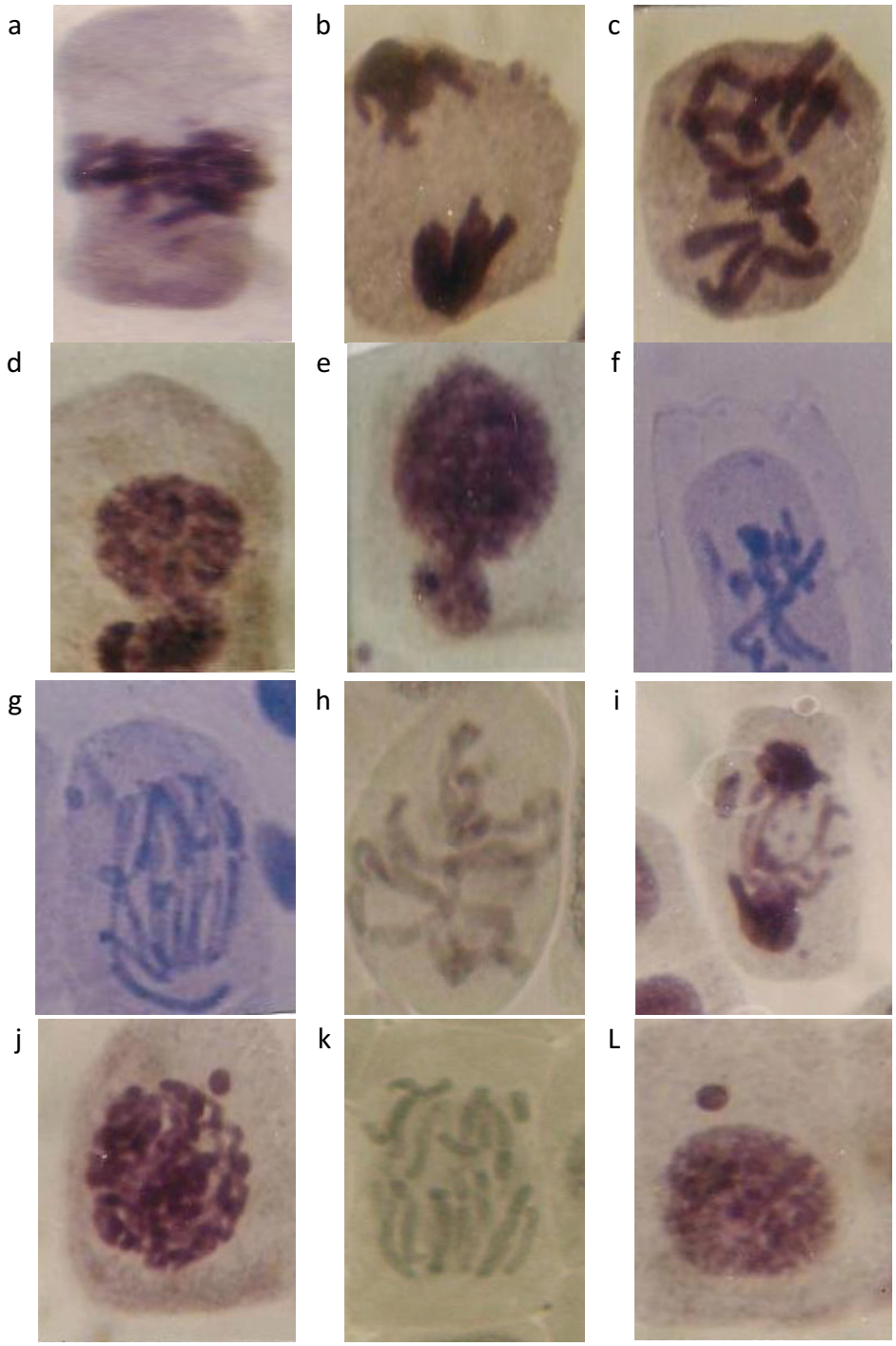
Table 4. Percentage of different types of abnormalities in Vicia faba root tips treated with energy drink " Bison".

Treatments		Stickiness	C- M	Disturbed	Lagging ch.	Star shape	Breaks ch.	Bridges	Multipolor	(Micro-Multi) Nucli	Dinucleated
Dose (%)	Time (h)										
Cntrol		0.18	0.18	1.79	—	—	—	—	—	—	—
%50	6	40.00	5.71	42.86	—	11.43	—	—	—	—	0.03
	12	52.34	4.67	35.51	0.93	5.61	—	0.93	—	—	0.09
	24	64.53	4.43	24.64	2.47	1.46	—	2.47	—	0.01	0.10
%80	6	75.58	8.15	9.30	4.65	1.16	—	0.58	0.58	—	0.15
	12	59.74	12.12	13.00	7.79	1.73	—	3.90	1.73	0.02	0.11
	24	67.19	7.51	9.49	7.91	1.98	0.40	3.16	2.37	0.01	0.20
%90	6	68.60	4.35	20.29	—	4.35	—	—	2.42	0.03	0.46
	12	67.82	6.03	14.66	6.61	1.43	0.29	2.59	0.57	0.01	0.20
	24	84.35	3.82	4.58	3.44	1.53	—	1.91	0.38	0.02	0.27
%100	6	66.67	4.88	25.20	1.63	1.63	—	—	—	—	0.02
	12	72.28	12.54	10.56	2.64	0.33	—	0.99	0.66	0.02	0.14
	24	90.48	5.07	1.48	0.60	0.89	—	1.48	—	0.01	0.11

Table 5. Nucleic acids content (DNA and RNA) in *Vicia faba* root tips treated with energy drink " Bison".

Treatments		DNA		RNA	
Dose%	Time(h)	Value*	Difference	Value*	Difference
Control		9.73	100.00	3.26	100.00
%50	6	12.56	129.09	3.42	104.91
	12	10.75	110.48	2.92	89.57
	24	9.73	100.00	3.60	110.43
%80	6	10.24	105.24	3.26	100.00
	12	9.78	100.51	2.34	71.78
	24	12.56	129.09	2.36	72.39
%90	6	9.41	96.71	3.38	103.68
	12	9.27	95.27	3.33	102.15
	24	9.18	94.35	3.24	99.39
%100	6	9.96	102.36	2.16	66.26
	12	6.12	62.90	2.04	62.58
	24	4.63	47.58	1.98	60.74

Results are expressed as mg/ g fresh weight.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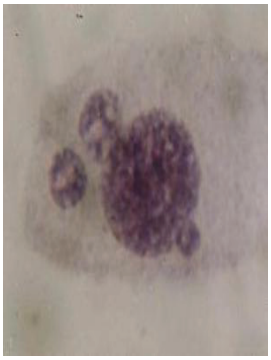

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Fig. 1. a,b: Stickinss at (meta and ana) phase; c: C-meta phase; d,e: disturbed prophase; f: disturbed with lagging at metaphase; g: disturbed with lagging and bridge at anaphase ; h: star metaphase; i: telophase bridge with lagging and break ; j: disturbed anaphase with break; k: micronuclii at prophase; l: micronuclii at interphase ; m : murltinuclii at intenphase; n: dinucleated .

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تأثير مشروب الطاقة "بايسن" على الانقسام الميتوزي و محتوى الأحماض النووية فى نبات الفول

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المستخلص: تم في هذا البحث دراسة التأثير الطفرى لأربع تركيزات لمشروب الطاقة "بايسن" ٥٠، و٨٠، ٩٠، و١٠٠٪ لثلاث فترات تعريض ٦ و١٢ و٢٤ ساعة على القمم النامية لجذور نبات الفول وتم التوصل لما يلي: انخفاض كفاءة الانقسام الميتوزي في كل المعاملات ماعدا معاملتين فقط (٩٠٪ بايسن عند فترتي التعريض ٦ و١٢ ساعة). ارتفاع النسبة الكلية للشذوذات في كل المعاملات وأهمها اللزوجة – التشتت– الاستوائي المستريح – الشكل النجمي والكرموسومات المتلكأة؛ بالإضافة إلى شذوذات أخرى وجدت في بعض المعاملات بنسب ضئيلة مثل: الكباري، عديدة الأقطاب، الكسور، الأنوية الصغيرة وثنائية النواة. وتلك النتائج تثبت سمية البايسن لجهاز مغزل الخلية. عدم اتزان نسب الأطوار الميتوزية في المعاملات مقارنة بالعينة الضابطة. كما أدت المعاملات المختلفة لتغير قيم محتوى الحمضين النووين الـ DNA والـ RNA. التأثير المثبط لأغلب معاملات البايسن يعتمد على فترات التعريض عن الجرعة المعطاة. أدت المعاملة بـ ١٠٠٪ بايسن عند فترة تعريض ٢٤ ساعة لأعلى نسبة شذوذات ٩٠,٣٢٪ وأقل قيم لمحتوى الـ DNA والـ RNA (٤,٦٣ و ١,٩٨ ملليجم/جم) مقارنة بالعينة الضابطة ٢,٢٢٪ و(٩,٧٣ و ٣,٢٦ ملليجم/جم) على التوالي.