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Cytogenetic Effect of Di- Butyl Phthalate on Adult Male Albino Rats and The Possible Protective Role of Ginger and Selenium Against This Effect

Ekram Saleh Mahmoud*[1], Rawya Mohamed Salah El-Din[1], Entesar El-Sayed El-Badry[1], Afaf Abd El- Raouf Shaaban[1] and Amira Abdel Raouf Mohammed[2]

[1]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine for girls, Al-Azhar University, Cairo, Egypt

[2]Cell Biology Department, National Research Centre in Dokki, Giza, Egypt

***E-mail: ekramsmahmoud@gmail.com**

Abstract

Di-butyl Phthalate (DBP) is used as a plasticizer, and it widely used in many consumer products. The male reproductive system is the main target organ of the toxicity of DBP. Ginger has antioxidant and androgenic properties and has a protective role against male reproductive dysfunction. Selenium (Se) is an antioxidant that play a critical role in cell biological processes and DNA repair. The present work aimed to evaluate cytogenetic effect of DBP on adult male albino rats and the possible protective role of ginger and selenium against this effect. One hundred and ten adult male albino rats were divided randomly into eleven groups, control, ginger, Se, DBP (100mg/kg B.W. and 500mg/kg B.W.) alone, with ginger, with Se and with both. All groups were received the treatment by oral gavage daily for 30 days and then samples of testis, epididymis and both femora were dissected and subjected for cytogenetic analysis. In all DBP treated groups, there were significant increase in comet tail length, frequencies of Micronuclei Polychromatic Erythrocytes (MNPCEs) and total count of sperm with abnormal morphology and significant decrease in the total sperm count and viability as compared to the control group in a dose dependent manner. Significant improvement occurred on co-administration of ginger alone, Se alone or both in comet tail length, frequencies of MNPCEs, the total count of sperm with abnormal morphology and total sperm count and viability.

Keywords: Di-butyl phthalate, ginger, selenium, cytogenetic, comet assay, sperm.

1. Introduction

Phthalates or phthalic acid esters are organic substances [1] used to increase transparency and durability of plastic materials [2]. Among the most important phthalate is di-butyl phthalate (di-n-butyl phthalate; DBP). Its molecular formula is C16H22O4 [3&4]. Di-butyl phthalate is used as plasticizer i.e., substance added to plastic to increase its flexibility, stability, resistance to fracture and waterproof properties [3]. Di-butyl phthalate is widely used in many consumer products such as papers, paints, printing inks, adhesives, glass fibers [5], food packaging materials

food containers, and toys. It also used in cosmetics, nail varnish, furniture and dye solvents [6]. Also, it is used in medications and medical devices such as tubing, external feeding bags, peritoneal dialysis bags, infusion tubing, oxygen masks, blood bags and catheters [7]. Because DBP does not bind chemically to polymer carbon chain during the manufacture of plastic and plastic products; therefore, it can easily leach to enter the environment and causing harm to the living organisms [2].

People are exposed to the harmful effects of DBP through contaminated food and water, medications and medical devices containing it, inhalation of volatile DBP presents in air and dust and dermal contact with plastics, cosmetics, or other personal care products containing it [8].

Oxidative stress is considered the main possible mechanism of toxicity of DBP [4,9,10]. Also, it is considered as a peroxisome proliferator, bound to the peroxisome proliferation-activating receptors (PPARs) and activates them resulting in apoptosis of cells [11]. Additionally, DBP acts as an antiandrogen, reduces testosterone hormone level and inhibits its synthesis in Leydig cells [12]. The male reproductive system is considered the main target organ of the toxicity of DBP results in testicular atrophy, decrease in epididymal weight, Leydig cell hyperplasia and deterioration of semen quality [13], impaired spermatogenesis, alteration in activities of steroidogenic enzymes, alteration in testosterone metabolism, decreased level of plasma testosterone and reduced male fertility [8].

Di-butyl phthalate has genotoxic effect. It inhibits the activity of superoxide dismutase (SOD) through binding to the active side of the enzyme responsible for the binding and deactivation of reactive oxygen species (ROS) [9,11].

Di-butyl phthalate causes DNA damage in human mucosal cells and in lymphocytes detected by Comet assay [5]. Also, DBP breaks DNA strand in the liver and testicular germ cells investigated by comet assay [14].

Di-butyl phthalate increases the frequencies of micronuclear polychromatic erythrocytes of bone marrow cells investigated by micronucleus test [15].

There is global trend toward the use of natural substance present in plants as therapeutic antioxidants. The use of natural antioxidants is considered better than synthetic antioxidants in respect of low cost and no harmful effects on the human body [16].

Ginger is used worldwide as a spice and as an alternative medical treatment. Ginger has antioxidant properties as it able to scavenge free radicals and protect cells from lipid oxidation. In many studies, ginger has androgenic properties and has a protective role against male reproductive dysfunction. The antioxidative constituents of ginger improve spermatogenesis, sperm count and viability, fertility, preventing DNA damage, and apoptosis [4]. Ginger also has anti-inflammatory, antimicrobial, and anticancer activities. In addition, ginger has the potential to prevent and manage several diseases, such as neurodegenerative diseases, cardiovascular diseases, obesity, diabetes mellitus, chemotherapy-induced nausea and emesis, and respiratory disorders [17,18].

Selenium (Se) is another antioxidant, which has been found to play a critical role in cell biological processes and DNA repair [9]. Selenium was found to have an effect on cellular redox equilibrium, function as an anti-mutagenic agent, preventing the malignant transformation of normal cells and preventing the activation of oncogenes [19]. Selenium also has an effective role in the production of normal sperm and fertility [9]. So, Se supplementation is protective against a wide variety of chemicals which are associated with DNA damage, mutagenesis, and carcinogenesis [19].

Based on these facts our study was designed to investigate the cytogenetic

effect of DBP on adult male albino rats and the possible protective role of ginger and selenium against this effect.

2. Materials and Methods

2.1 Materials:

I- Experimental Animals: One hundred and ten adult male Albino rats of uniform strain with average body weight ranged from 180 to 200 grams, obtained from Helwan animal breeding house, Egypt.

II- Substances used:

1. Di-Butyl Phthalate (DBP) was purchased from Sigma–Aldrich Company, Cairo, Egypt, as 99% purity in the form of colorless liquid and used as it is

2. Selenium (Se) was purchased from Sigma–Aldrich Company, Cairo, Egypt, as 99.5% purity in the form of black powder and dissolve in distilled water.

3. Ginger was purchased from local market in the form of yellowish powder and dissolved in distilled water.

III- Place of the Study: The cytogenetic studies were done at cell Biology Department of National Research Center in Dokki, Giza, Egypt.

2.2 Methodology:

Preparation of materials:

I- Experimental animals:

- Handling: The handling of animals followed the rules for the experimental research ethics approved by Research Ethics Committee at Faculty of Medicine for Girls Al-Azhar University, Cairo, Egypt. The animals were kept in the Animal House at Faculty of Medicine for Girls, Al-Azhar University. They were housed in clean plastic cages with metal covers; their floors were covered by wood flakes, at room temperature and 12 hours light/dark cycle. The animals were subjected to 7 days period of passive preliminaries in order to adapt themselves to their new environment and to ascertain their physical wellbeing.

- Experimental Design: After one week of acclimatization, animals were divided into eleven groups each group contained ten rats, all groups received the treatment by oral gavage daily for 30 days as follow:

Group I: served as control group, rats were received distilled water.

Group II: served as ginger control group, rats were received ginger in a dose of 400mg/kg B.W [4].

Group III: served as Se control group, rats were received Se in a dose of 1mg/kg B.W [9].

Group IV: rats were received DBP in a dose of 100mg/kg B.W **[**20].

Group V: rats were received DBP in a dose of 100mg/kg B.W. and ginger in a dose of 400mg/kg B.W [4].

Group VI: rats were received DBP in a dose of 100mg/kg B.W. and Se in a dose of 1mg/kg B.W [9], [20].

Group VII: rats were received DBP in a dose of 100mg/kg B.W., ginger in a dose of 400mg/kg B.W. and Se in a dose of 1mg/kg B.W [4],[9] and [20].

Group VIII: rats were received DBP in a dose of 500mg/kg B.W [20].

Group IX: rats were received DBP in a dose of 500mg/kg B.W. and ginger in a dose of 400mg/kg B.W [4],[20].

Group X: rats were received DBP in a dose of 500mg/kg B.W. and Se in a dose of 1mg/kg B.W [9].

Group XI: rats were received DBP in a dose of 500mg/kg B.W., ginger in a dose of 400mg/kg B.W. and Se in a dose of 1mg/kg BW [4], [9] and [20]. Samples of one testis, epididymis and both femora were dissected from each animal cleared of

adhering tissues and were subjected for cytogenetic analysis.

1.The comet assay was performed under alkaline conditions essentially according to the procedure of Singh et al. [21] with modification of Klaude et al. [22] as described by Blasiak et al. [23].

2. Micronucleus polychromatic Erythrocytes (MNPCEs) Assay were prepared according to an improved method adopted by Salamone et al. [24].

3. Sperm affection (morphology, count and viability) were evaluated according to the technique described by Wyrobek et al. [25].

Statistical analysis: Values were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data), so one way analysis of variance ANOVA test was used to compare between groups, followed by post hoc test (LSD) for pairwise comparisons. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with SPSS 20 (Statistical Package for Scientific Studies, SPSS, Inc, Chicago, IL, USA) for Windows.

3. Results

3.1 Comet Assay (The Single Cell Gel Electrophoresis [SCGE]):

Concerning DNA damage evaluated by comet assay in the present study, the results revealed statistically significant increase ($p \leq 0.05$) in DNA damage of spermatocyte as indicated by increase in comet tail length in all DBP treated groups compared to control group in a dose dependent manner. In addition, there was a significant decrease ($p \leq 0.05$) in DNA damage of spermatocyte as indicated by decrease in comet tail length in rats co-treated by DBP with ginger alone, Se alone or both as compared to DBP treated rats but co administration with both ginger and Se produced better effects on the level of comet tail length than co-administration with ginger alone or Se alone (Table 1& Figures1-4).

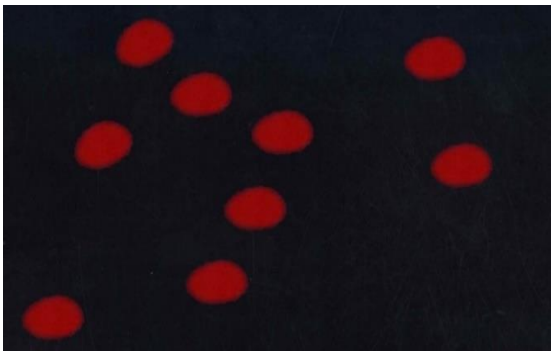

Figure (1): Fluorescent microscope photomicrograph spermatocyte of groups I, II, III, V, VI and VII showing no migration of DNA out of the nucleus into the tail of the comet (Propidium iodide stain).

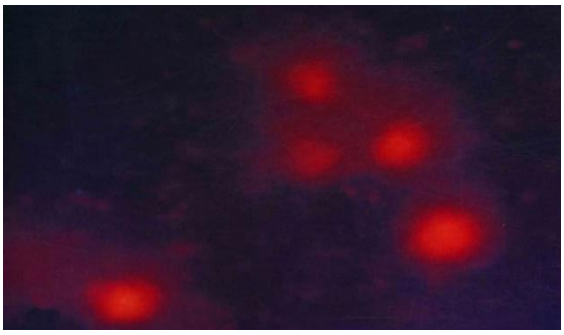

Figure (2): Fluorescent microscope photomicrograph of spermatocyte of group IV showing DNA migration out of the nucleus into the tail of the comet as compared to the control group (Propidium iodide stain).

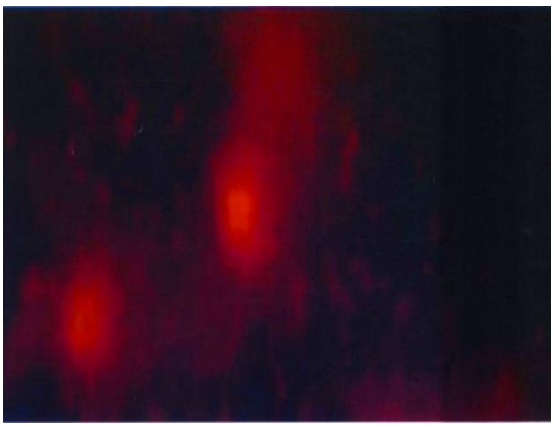

Figure (3): Fluorescent microscope photomicrograph of spermatocyte of group VIII showing greater DNA migration out of the nucleus into the tail of the comet as compared to the control group (Propidium iodide stain).

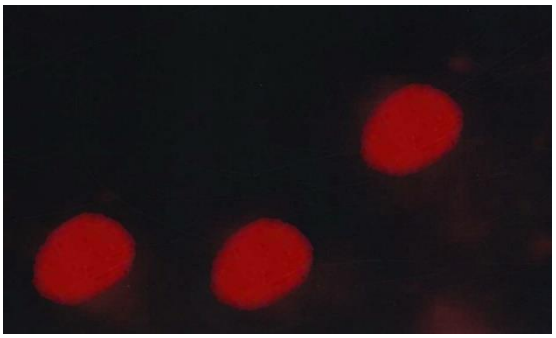

Figure (4): Fluorescent microscope photomicrograph of spermatocyte of groups IX, X and XI showing reduction in the migration of DNA out of the nucleus into the tail of the comet as compared to the group VIII (Propidium iodide stain).

3.2 Micronuclei Polychromatic Erythrocytes (MNPCEs) assay in femur bone marrow cells:

The results of the present study indicated that, there was statistically significant increase ($p \leq 0.05$) in the frequencies of MNPCEs in femur bone marrow cells induced by DBP intoxication as compared to the control group in a dose dependent manner. Concomitant administration of DBP with ginger alone, Se alone or both showed significant improvement ($p<0.05$) in the frequencies of MNPCEs but co-administration with ginger alone or Se alone produced better effects on the frequency of MNPCEs than co-administration with both (Table 2 & Figures 5&6).

Figure (5): A photomicrograph of normal polychromatic erythrocytes in bone marrow of control (I) or ginger (II) or Se (III) groups (Giemsa stain, x 100).

Table (1): Statistical analysis (One Way ANOVA test and post hoc test {LSD}) for the effect of low dose (100mg/kg b.w.) & high dose (500mg/kg b.w.) di-butyl phthalate (DBP) on the changes in the DNA damage by comet assay of spermatocytes of adult male albino rats (n=110; Mean±SD) and the possible protection by ginger and selenium (Se) on the studied groups.

Groups n=10rats/group		Comet tail length(uM) of DNA damage
		Mean ± SD
Group I (Control)		14.31 ± 2.017 g
Group II (Ginger)		13.99 ± 1.201 h
Group III (Se)		14.15 ± 1.597 gh
Group IV (DBP100mg)		22.88 ± 2.871 b 59.88%
Group V(DBP100mg+ginger)		15.40 ± 2.579 e ↑7.61%
Group VI(DBP100mg+Se)		15.44 ± 2.102 e ↑7.89%
Group VII(DBP100mg+ginger+Se)		14.70 ± 3.038 f ↑2.72%
Group VIII(DBP500mg)		31.63 ± 3.406 a ↑121%
Group IX(DBP500mg+ginger)		17.26 ± 1.999 c ↑20.61%
Group X(DBP500mg+Se)		17.52 ± 2.361 c ↑22.43%
Group XI(DBP500mg+ginger+Se)		16.27 ± 2.626 d ↑13.69%
One way ANOVA	F test	2715
	P-Value	<0.001
Post hoc test	LSD	0.285

DBP= Di-butyl phthalate; **Se**= Selenium; **n**= number; **S.D**.= standard deviation; **%**=% change from Control group**;** LSD= least significant difference, means with different superscript letters are significantly different at $p< 0.05$, whereas means with the same superscript letters are non-significant in the same column.

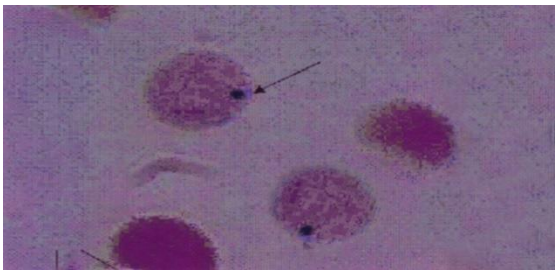

Figure (6): A photomicrograph of normal polychromatic erythrocytes in bone marrow of rat exposed to di-butyl phthalate (DBP) (Giemsa stain, x 100).

3.3 Sperm affection (morphology, count and viability):

3.3.1 Sperm morphology (total count of sperm with abnormal morphology)

Concerning sperm morphology in the present study, there were statistically significant increase ($p \leq 0.05$) in the total count of sperm with abnormal morphology represented by amorphous head, divided head, coiled tail, and divided tail in all DBP treated groups as compared to the control in a dose dependent manner. Concomitant administration of DBP with ginger alone, Se alone or both showed significant improvement ($p \leq 0.05$) in sperm morphology but co-administration with ginger alone or Se alone produced better effects on the amorphous head, divided head, divided tail and total count of sperm with abnormal morphology than co-administration with both ginger and Se. While in coiled tail, co-administration with ginger alone produced better effects than co-administration with Se alone or both ginger and Se (Table 3 & Figures7-10).

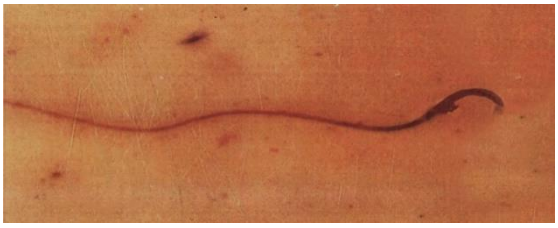

Figure (7): A photomicrograph of normal sperm morphology showing normal head and tail (Eosin stain, x 400).

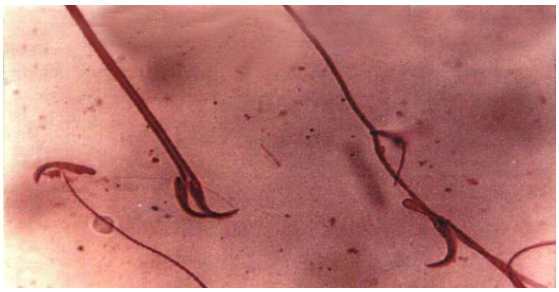

Figure (8): A photomicrograph of sperm head abnormality showing divided head (black arrow) with fused tail (double arrow) in rats exposed to di-butyl phthalate (DBP) (Eosin stain, x 400).

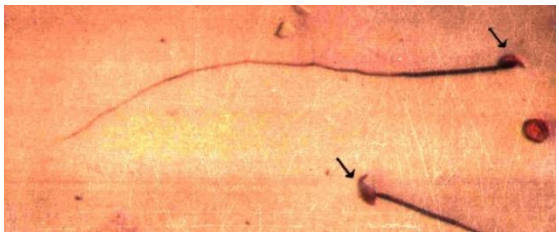

Figure (9): A photomicrograph of sperm head abnormality showing amorphous head in rats exposed to di-butyl phthalate (DBP) (Eosin stain, x 400).

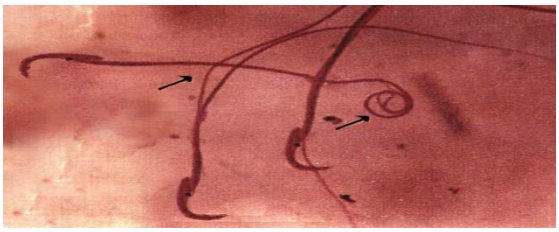

Figure (10): A photomicrograph of sperm tail abnormality showing divided tail and coiled tail in rats exposed to di-butyl phthalate (DBP) (Eosin stain, x 400).

3.3.2 Total Sperm Count and Viability

Concerning total sperm count and viability in the present study, there were statistically significant decrease ($p \leq 0.05$) in the total sperm count and viability in all DBP treated groups compared to control group in a dose dependent manner. Concomitant administration of DBP with ginger alone, Se alone or both showed significant improvement ($p \leq 0.05$) in the total sperm count and viability but co-administration with ginger alone or Se alone produced better effects on the total sperm count and viability than co-administration with both (Table 4).

Table (2): Statistical analysis (One Way ANOVA test and post hoc test {LSD}) for the effect of low dose (100mg/kg b.w.) & high dose (500mg/kg b.w.) di-butyl phthalate (DBP) on the Micronuclei Polychromatic Erythrocytes (MNPCEs) in femur bone marrow cells of adult male albino rats (n=110; Mean±SD) and the possible protection by ginger and selenium (Se) on the studied groups.

Groups n=10rats/group		Frequencies of Micronuclei Polychromatic Erythrocytes (MNPCEs) Mean ± SD
Group I (Control)		12.70 ± 3.12 ^{e}
Group II (Ginger)		10.90 ± 2.601 ^{e}
Group III (Se)		11.90 ± 2.601 ^{e}
Group IV (DBP100mg)		56.60 ± 9.754 ^{b} ↑345.66%
Group V (DBP100mg+ginger)		18.10 ± 2.601 ^{d} ↑42.51%
Group VI(DBP100mg+Se)		19.50 ± 2.173 ^{d} ↑53.54%
Group VII(DBP100mg+ginger+Se)		20.20 ± 2.820 ^{d} ↑59.05%
Group VIII(DBP500mg)		97.20 ± 9.554 ^{a} ↑665.35%
Group IX(DBP500mg+ginger)		26.90 ± 3.314 ^{c} ↑111.81%
Group X(DBP500mg+Se)		30.50 ± 3.535 ^{c} ↑140.15%
GroupXI (DBP500mg+ginger+Se)		31.20 ± 2.780 ^{c} ↑145.66%
One way ANOVA	F test	277.556
	P-value	<0.001
Post hoc test	LSD	4.317

DBP= Di-butyl phthalate; **Se**= Selenium; **n**= number; **S.D**.= standard deviation; **%**=% change from Control group**; LSD=** least significant difference, means with different superscript letters are significantly different at p< 0.05, whereas means with the same superscript letters are non-significant in the same column.

4. Discussion

Di-butyl phthalate is one of the most important phthalate esters [4] and it is widely used in many consumer products [5]. In the present study, we investigated the cytogenetic effect of DBP on adult male albino rats and the possible protective role of ginger and selenium against this effect. Concerning DNA damage evaluated by comet assay in the present study, the results revealed statistically significant increase in DNA damage of spermatocyte as indicated by increase in comet tail length in all DBP treated groups compared to control group in a dose dependent manner. In addition, there was a significant decrease in DNA damage of spermatocyte as indicated by decrease in comet tail length in rats co-treated by DBP with ginger alone, Se alone or both as compared to DBP treated rats but co-administration with both ginger and Se produced better effects on the level of comet tail length than co-administration with ginger alone or Se alone. The findings of the present study could be explained by Alam & Hoque [9] who said that the genotoxic effect of DBP and its metabolite, mono-butyl phthalate, attributed to their ability to inhibit the activity of SOD and increase ROS formation and oxidative stress. The results of the present study were in agreement with those of Jing et al. [15] who reported that mice exposed to DBP (350mg/kg) by gavage once a day for five days showed statistically significant increase in the percent of tail DNA as indicated by increase in comet tail length compared with control group. In the present study, co-administration of ginger showed significant improvement ($p<0.05$) in comet assay.

Table (3): Statistical analysis (One Way ANOVA test and post hoc test {LSD}) for the effect of low dose (100mg/kg b.w.) & high dose (500mg/kg b.w.) di-butyl phthalate (DBP) on the sperm morphology of adult male albino rats (n=110; Mean±SD) and the possible protection by ginger and selenium (Se) on the studied groups.

Groups n=10rats/group		Amorphous head	Divided Head	Coiled Tail	Divided Tail	Total Count of Sperm with Abnormal Morphology
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group I (Control)		1.30±0.89 ^{e}	0.90±0.83 ^{g}	0.70±0.40 ^{g}	0.60±0.44 ^{g}	3.50±1.715 ^{f}
Group II (Ginger)		0.90±0.83 ^{e}	0.60±0.44 ^{g}	0.50±0.5 ^{g}	0.30±0 ^{g}	2.30±1.251 ^{f}
Group III (Se)		1.10±0.83 ^{e}	0.70±0.40 ^{g}	0.60±0 ^{g}	0.500±0 ^{g}	2.90±1.197 ^{f}
Group IV(DBP100mg)		8.70±2.21 ^{b} ↑569.23%	7.60±1.50 ^{b} ↑744.4%	8.40±1.50 ^{b} ↑1100%	8.20±1.68 ^{b} ↑1266.6%	32.90±2.98^{b} ↑840%
GroupV (DBP100mg+ginger)		4.10±1.28 ^{d} ↑215.38%	2.70±1.05 ^{f} ↑200%	2.80±0.63 ^{f} ↑300%	2.90±0.99 ^{ef} ↑383.3%	12.50±2.38^{e} ↑257.1%
GroupVI (DBP100mg+Se)		4.70±1.33 ^{cd} ↑261.53%	3.60±0.69 ^{ef} ↑300%	3.30±0.67 ^{ef} ↑371.4%	2.50±0.52 ^{f} ↑316.6%	14.10±2.33^{e} ↑302.8%
GroupVII(DBP100mg+ginger+Se)		5.00±1.49 ^{cd} ↑284.61%	4.30±1.15 ^{cde} ↑400%	4.20±1.03 ^{de} ↑500%	3.30±0.67 ^{ef} ↑450%	16.80±2.5^{d} ↑380%
Group VIII (DBP500mg)		14.00±1.94 ^{a} ↑976.92%	11.20±1.87 ^{a} ↑1144.4%	11.20±1.54^{a} ↑1500%	11.40±2.36 ^{a} ↑1800%	47.80±2.78^{a} ↑1265.7%
GroupIX (DBP500mg+ ginger)		4.50±1.43 ^{cd} ↑246.15%	3.80±1.31 ^{de} ↑322.2%	4.40±1.50 ^{cd} ↑528.5%	3.80±1.03 ^{de} ↑533.3%	16.50±2.93^{d} ↑371.4%
Group X (DBP500mg+ Se)		5.30±1.63 ^{cd} ↑307.69%	4.80±1.22 ^{cd} ↑433.3%	5.20±1.54 ^{c} ↑642.8%	4.40±0.96 ^{cd} ↑633.3%	19.70±2.39^{c} ↑462.8%
GroupXI (DBP500mg+ ginger+Se)		5.60±1.42 ^{c} ↑330.76%	4.90±1.37 ^{c} ↑444.4%	5.00±1.15 ^{cd} ↑614.2%	5.00±1.15 ^{c} ↑733.3%	20.50±2.45^{c} ↑485.7%
One way ANOVA	F test	62.202	69.795	87.602	87.196	335.978
	P-Value	<0.001	<0.001	<0.001	<0.001	<0.001
Post hoc test	LSD	1.341	1.063	0.994	1.019	2.080

DBP= Di-butyl phthalate; **Se**= Selenium; **n**= number; **S.D**.= standard deviation; **%**=% change from Control group**; LSD=** least significant difference, means with different superscript letters are significantly different at p< 0.05, whereas means with the same superscript letters are non-significant in the same column.

The findings of the present study could be explained by Abd El-Monem & Elwakeel [18] who reported that ginger revealed marked antioxidant activity and exhibited DNA-protective effects as antioxidation plays vital role in chelating metal ions and scavenging hydroxyl and hydrogen peroxide radicals. also ginger extract protects against oxidative stress by enhancing superoxide dismutase and catalase activity, increasing reduced glutathione content and decreasing malondialdehyde levels.

The results of the present study were in agreement with those of Abd El-Monem & Elwakeel [18] who reported that, administration of 250 mg/kg b.w. of ginger extract by gavage for 14consecutive days to rats before exposure to gamma-rays significantly reduced cellular DNA damage of bone marrow cells, as demonstrated by decreased comet tail length compared with irradiation alone group.

In the present study, co-administration of Se showed significant improvement ($p<0.05$) in comet assay.

The results of the present study agreed with those of Hassan et al. [26] who found that treatment by Se (0.5 mg/kg intraperitoneal for 5 consecutive days) with Hexavalent chromium (Cr VI) showed significant decrease in DNA damage as indicated by decrease comet tail length compared to Cr VI toxic group.

Table (4): Statistical analysis (One Way ANOVA test and post hoc test {LSD}) for the effect of low dose (100mg/kg b.w.) & high dose (500mg/kg b.w.) di-butyl phthalate on the total sperm count and viability of adult male albino rats (n=110; Mean±SD) and the possible protection by ginger and selenium (Se) on the studied groups.

Group No= 10 rats /group		Total Sperm Count Millions/gm testes	Sperm Viability (%)
		Mean ± SD	Mean ± SD
Group I (Control)		33215000±1497600 [b]	71.72±0.013 [a]
Group II (Ginger)		34925000± 1280462 [a]	73.42±0.015 [a]
Group III (Se)		34450000±1223610 [a]	72.99± 0.016 [a]
Group IV (DBP100mg)		22300000± 1619328 [d] ↓32.86%	41.61± 0.019 [d] ↓41.98%
Group V (DBP100mg+ginger)		28000000± 1312335 [c] ↓15.7%	53.77± 0.029 [b] ↓25%
Group VI (DBP100mg+Se)		27775000± 1145341 [c] ↓16.4%	53.49± 0.027 [b] ↓25.4%
Group VII (DBP100mg + ginger+Se)		27725000± 975035.6 [c] ↓16.5%	52.33± 0.023 [b] ↓27%
Group VIII(DBP500mg)		18850000± 1599479 [e] ↓43.24%	28.79± 0.028 [e] ↓59.85%
Group IX (DBP500mg+ginger)		23200000± 1159502 [d] ↓30.15%	46.94± 0.030 [c] ↓34.55%
Group X (DBP500mg+Se)		22775000± 1023678 [d] ↓31.43%	46.14± 0.028 [c] ↓35.66%
Group XI (DBP500mg+ginger+Se)		22650000± 936898 [d] ↓31.8%	45.98± 0.029 [c] ↓35.88%
One way ANOVA	F test	184.415	336.313
	P value	<0.001	<0.001
Post hoc test	LSD	1129155.034	2.172

DBP= Di-butyl phthalate; **Se**= Selenium; **n**= number; **S.D**.= standard deviation; **%**=% change from control group**; LSD=** least significant difference, means with different superscript letters are significantly different at p< 0.05, whereas means with the same superscript letters are non-significant in the same column.

In the present study, co- administration with both ginger and Se produced better effects on the level of comet tail length than co-administration with ginger alone or Se alone. Concerning Micronuclei Polychromatic Erythrocytes in the present study, there was statistically significant increase ($p<0.05$) in the frequencies of MNPCEs in femur bone marrow cells induced by DBP intoxication as compared to the control group in a dose dependent manner. Concomitant administration of DBP with ginger alone, Se alone or both showed significant improvement ($p<0.05$) in the frequencies of MNPCEs but co-administration with ginger alone or Se alone produced better effects on the frequency of MNPCEs than co-administration with both.

The results of the present study were in agreement with those of Jing et al. [15] who reported that mice exposed to DBP (350mg/kg) by gavage once a day for five days showed statistically significant increase in the micronuclear rate of bone marrow cells compared with control group.

In the present study, co-administration of ginger showed significant improvement ($p < 0.05$) in MNPCEs.

The results of the present study were in agreement with those of Abd El-Monem & Elwakeel [18] who reported that, administration of 250 mg/kg b.w. of ginger extract by gavage for 14consecutive days to rats before exposure to gamma-rays significantly ($P<0.01$) reduced the mean percentage of MNPCEs compared with irradiation alone.

In the present study, co-administration of Se showed significant improvement ($p < 0.05$) in MNPCEs.

The results of the present study agreed with those of Mohammed & Abd-Elwahab [16] who reported that co- administration of

selenium by oral gavage (0.5 mg/kg b.w.) daily for 6 weeks caused significant decrease in the frequencies of MNPCEs in bone marrow cells compared to toxic group in the rats treated by Hexavalent chromium (Cr VI).

In the present study, co-administration of ginger alone or Se alone produced better effects on the frequency of MNPCEs than co-administration with both.

Concerning sperm morphology in the present study, there were statistically significant increase ($P<0.05$) in the total count of sperm with abnormal morphology represented by amorphous head, divided head, coiled tail, and divided tail in all DBP treated groups as compared to the control in a dose dependent manner. Concomitant administration of DBP with ginger alone, Se alone or both showed significant improvement ($P<0.05$) in sperm morphology but co-administration with ginger alone or Se alone produced better effects on the amorphous head, divided head, divided tail and total count of sperm with abnormal morphology than co-administration with both ginger and Se. While in coiled tail, co-administration with ginger alone produced better effects than co-administration with Se alone or co-administration with both ginger and Se.

The findings of the current study could be explained by Huang et al. [27] and Alam & Hoque [9] who reported that, DBP has adverse effects on the reproductive system of male, including disturbing the secretion of testosterone, influencing the production and morphology of sperm and damaging the seminiferous tubule, which may induce through oxidative stress from their ROS-metabolites. Di-butyl phthalate causes atrophy in the seminiferous tubules, decreases SOD and GSH-Px activities and increase catalase activity, which indicates an oxidative stress state in the testis.

The results of the current study were in agreement with those of Yin et al. [3] who stated that DBP (100, 200 and 500 mg/g b.w.) via oral gavage for 21 days significantly elevated the sperm deformity rate compared to control group in rats.

Similarly, the results of the current study were in agreement with the results of Rihani et al. [13] who reported that DBP (250,500 and 750 mg/kg/day) administrated to rabbits for 4 weeks resulted in significantly increase in the abnormal sperm shapes (no head, banana shape head, excessive hook, pin head, tow head, amorphous head, two tails) compared to the corresponding control group. In the present study, co-administration of ginger showed significant improvement ($p<0.05$) in the sperm morphology. The results of the present study were in agreement with those of Abd El-Monem & Elwakeel [18] who reported that, administration of 250 mg/kg b.w. of ginger extract by gavage for 14consecutive days to rats before exposure to gamma-rays significantly attenuated the sperm abnormalities caused by irradiation, as demonstrated by the lower percentage of abnormal sperm in the group receiving ginger and radiation than in the radiation-only group ($P<0.01$).

In the present study, co-administration of Se showed significant improvement ($p<0.05$) in sperm morphology.

The results of the present study agreed with those of Mistry et al. [28] who reported that improvement in sperm morphology after selenium supplementation in 64 men in Scotland.

In the present study, co-administration with ginger alone or Se alone produced better effects on the amorphous head, divided head, divided tail and total count of sperm with abnormal morphology than co-administration with both ginger and Se. While in coiled tail, co-administration with ginger alone produced better effects than co-administration with Se alone or co-administration with both ginger and Se.

Concerning total sperm count and viability in the present study, there were statistically significant decrease ($p\leq0.05$) in the total sperm count and viability in all DBP treated groups compared to control group in a dose dependent manner. Concomitant

administration of DBP with ginger alone, Se alone or both showed significant improvement in the total sperm count and viability but co-administration with ginger alone or Se alone produced better effects on the total sperm count and viability than co-administration with both.

The results of the present study were in agreement with those of Oda & Waheeb [4] who found that sperm count, and viability exhibited a significant reduction ($P<0.05$) in DBP-treated rabbits. In the present study, co-administration of ginger showed significant improvement ($p<0.05$) in sperm count and viability.

The findings of the present study could be explained by Amr & Hamza [29] and Abd El-Monem & Elwakeel [18] who stated that, ginger supplementation increases testicular blood flow and improves the fertility index by increasing sperm mobility, viability, count and morphology via protective effect.

The results of the present study were in agreement with those of Soleimanzadeh et al. (30) who reported that ginger (500, 1000 and 2000 mg/kg/d) by gavage improved sperm count and viability in formaldehyde treated mice for 35 days.

In the present study, co-administration of Se showed significant improvement ($p<0.05$) in sperm count and viability. The findings of the current study could be explained by Mehdi et al. [31] who stated that, selenium plays an important role in synthesis of sperm, and sperm mobility. Selenium deficiency results in reduction of sperm motility. Ragozzino et al. [32] reported that, in male, selenium is a constituent of selenoproteins as glutathione peroxidase that protects against oxidative damage to spermatozoa throughout the process of sperm maturation. Thus, Se ensures viability of spermatozoa as well as providing protection against ROS. Qazi et al. [33] mentioned that Se has been implicated to play an important role in spermatogenesis. Selenium might have strong implication in the process of spermatogenesis in males. The results of the present study were in agreement with those of Mossa et al. [34] who observed that selenium supplementation (50microgram) once daily at night for 3 months significantly increased sperm count and viability of infertile men comparing with before treatment.

In the present study, co-administration with ginger alone or Se alone produced better effects on the total sperm count and viability than co-administration with both ginger and Se.

5. Conclusion

Considering the results obtained from the present study it can be concluded that, Di-butyl phthalate induced cytogenetic effect by increasing in DNA damage which estimated by comet assay and increase in the frequency of Micronuclei Polychromatic Erythrocytes in bone marrow cells and decrease in the number of sperm count and sperm viability with increase count of abnormal sperm (amorphous head, divided heads, coiled tail and divided tail). concomitant administration of ginger or Se or both improved cytogenetic changes which resulted from DBP toxicity in adult male albino rats.

6. Recommendation

- Avoid food contact with plastics and materials containing DBP. Food should be prepared, served and packaged in a glass container.
- Avoid cosmetics, personal care products, medications and medical devices, toys and childcare articles containing DBP.
- Dietary intake of natural antioxidants as ginger or selenium is recommended. As they can inhibit the oxidation of cellular structures.

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