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Original Research

Cytogenetic and testicular histological alterations induced by sulphur dioxide in dried apricot leather

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Abstract: Sulphur dioxide (SO_2) is used as a preservative in food to prevent its discolouration, and to inhibit the growth of bacteria. Little data is available concerning its *in vivo* hazardous impact.The present study is therefore designed to examine the cyto-genotoxic potential and the testicular histological alterations in adult mice, induced by SO_2 present in the dried apricot leather used to prepare the oriental drink Qamar Al-Deen. Two different forms of drinks were tested; cold and boiled drinks. Animals were placed into 4 groups. The first group received distilled water as a negative control.The second and third groups received orally the drink for 28 days in the form of a cold and a boiled drink, respectively. Animals of the fourth group received cyclophosphamide, they were used as a positive control for cyto-genotoxic tests. The chromosomal aberrations, as well as sperm abnormalities, were significantly elevated in animals that received the two different drink preparations. The mitotic index significantly decreased in comparison with negative and positive controls. Furthermore, histological examination showed different degrees of alterations in the testis. Our results suggest that the presence of SO_2 inside the apricot leather might be responsible for these changes. Thus, these remarkable hazardous effects of SO_2 on male albino mice could be used as a potential guide for the prediction of its human health impact. Furthermore, consumers could be advised to prevent excessive consumption of the drink (Qamar Al-Deen) prepared from dried apricot leather.

Key words: Sulphur dioxide; Dried apricot leather; Genotoxicity; Histology; Mice; Testis.

Introduction

Dried apricot leather is used to prepare a Middle Eastern drink called Qamar Al-Deen. It is a famous drink in several parts of the Arabian world especially during the month of Ramadan. Dried apricot leather is produced by placing apricot and sugar on fire and straining them by a wooden strainer soaked in olive oil, left to dry in direct sunlight and then packaged (1). Sulphur dioxide (SO_2) is used in the processing and preservation of this dried apricot leather.

Sulphur dioxide (E220) is used as a preservative agent in foods and drinks. It prevents discolouration of foods and inhibits the growth of bacteria. The use of SO_2 is associated with potential health hazards. It may induce asthma when eaten or inhaled by sensitive persons, even with high dilutions, whereas, in healthy ones it is not hurmful when being used with the advised concentrations (2). For sensitive people to this preservative or have asthma, the health risks of SO_2 range from mild to severe (3).

Numerous studies have demonstrated that the utilization of SO_2 as a food preservative in apricot sulphurization and several fabricated foods has a mutagenic effect on the cells. Meng et al. (4) revealed that exposure to SO_2 damage the DNA of many organs, which could lead to mutations, cancer and other diseases. Moreover, Uren et al. (5) shown that human lymphocytes treated with different concentrations of SO_2 caused significant increases in the frequency of sister chromatid exchange and micronuclei in the middle and high dose groups in addition to the induction of mitotic delays and decreased mitotic index and replication index. The results have confirmed that SO_2 cause mutagenicity and genetic damage leading to malignancies.

In this regard, *in vitro* toxicological studies were realized on a mouse bone marrow micronucleus test complemented with hematological endpoints (6). Studies on human bronchial epithelial cells showed that the treatment of cultured human bronchial epithelial cells with sulfite or bisulfite caused over expression of mRNA and protein for two protooncogenes c-fos and c-jun and the tumor suppressor gene c-myc at all tested treatments (7). In addition, Bakand et al. (8) reported SO_2 cytotoxicity on A549- human pulmonary type II-like epithelial cell lines.

This frequently used preservative has been stated to generate reproductive toxicity in experimental animals (9). It was also reported that SO_2 caused a decrease in testosterone levels, inappropriate spermatogenesis and variation in epididymal morphometry in rats (10). In addition, it has been involved to have harmful

consequences on human reproduction (11). In fact, although it is widely used by the food and drink industries, its potential toxicity has not been described well. Therefore, the present investigation is done to evaluate the influence of SO_2 present in the drink prepared from dried apricot leather namely (Qamar Al-Deen), regarding histological alterations to the testis and certain genotoxic parameters such as; sperm abnormalities (SpA), mitotic index (MI) and chromosomal aberrations (ChA).

Materials and Methods

Animals and management

Adult male Swiss Albino mice (*Mus musculus*), 6-8 weeks of age (22±2.0 g) were used. Animals were randomly reared in stainless steel cages (4 per cage) and kept under laboratory conditions (25±2ºC, 50-70% humidity and a 12 h light: 12 h dark cycle) for 14 days before beginning the experiments with free access to tap water and standard rat chow *ad labium*.

Chemicals

Cyclophosphamide (CP) white powder was obtained from Asta Medica AG, (Frankfurt am Main, Germany). Colchicine was obtained from Sigma Chemical Company (MO, USA). Giemsa stain was acquired from Merck India Ltd., (Mumbai, India). The testosterone immunoassay kit was provided by Chemux Bioscience Inc. (California, USA).

Preparation of Qamar Al-Din drink

Ten samples (each 500 gm) of dried apricot leather were collected from different local markets in Alexandria, Egypt. The samples were stored at 4°C until the preparation of the drink. The drink was prepared by two methods namely cold soaking and boiled. The cold drink was prepared by soaking 18 gm of dried apricot leather in 100 ml H_2O at room temperature (22-25°C). The soaked were homogenized at 5.000 RPM using a union homogenizer and stored at 4°C until analysis. The boiled drink was prepared using the above method and after homogenization, the drink was heated in a water bath at 90°C for 15 min and stored at 4°C until analysis. The percent total soluble solids (TSS) of the cold or boiled drink were 14% as determined by ATAGO Abbe Refractometer.

Determination of total and free sulphur dioxide

The total sulphur dioxide was determined in cold and boiled drinks using the spectrophotometric method recommended by the Association of Political Analytical Chemists (AOAC, 1984) using a p-Rosaline reagent. The results were calculated as ppm of drink and mgg^{-1} of the dried apricot leather. Free sulphur dioxide was determined using the iodine titrimetric method of (AOAC, 1984) and the results were calculated as mentioned before (12).

Experimental Design

Animals were placed into four groups including 10 mice each to carry out the experimental procedure. These groups were as follows: Group I; (The negative control group) received distilled water; Group II, (The cold drink group) received dried apricot drink prepared by cold extract daily by oral gavage; Group III, (The boiled drink group) received dried apricot drink prepared by boiled extract daily by oral gavage and Group IV, (The positive control) for cyto-genotoxic tests, received 25 mg CP/kg b.wt. After 28 days mice were sacrificed and bone marrow and spermatozoa were obtained for cytogenetic analysis, blood samples were collected for biochemical analysis and the tissue samples from the testis were collected for histological analysis. The experimental design and investigation protocols were conducted with the guidelines of Institutional Animal Care and Use Committee, Alexandria University, Egypt

Chromosomal aberrations assay

Mice were intravenously injected with freshly prepared colchicine solution (4 mg/kg b.wt.) as a metaphase-arresting agent according to Brusick, (13) and then sacrificed after 3 hours. Briefly, the harvest cells into hypotonic solution (0.075 M % KCl) were incubated at 37 °C for 30 min and then centrifuged at 1200 rpm for 10 min. Cells were fixed in Carnoy solution twice, pipetted onto clean glass slides to release chromosomes, slides were dipped into the Giemsa stain 5% for 10 min and then counted 100 cells for each mouse under a microscope using oil immersion at 1000X magnification according to Preston et al. (14) There was a group of animals used as a positive control injected with CP (25 mg/kg b.wt.). All the slides were coded. A total of 100 cell spread metaphases was counted to record the chromosomal aberrations. Mitotic index (MI) was calculated from 1000 cells/animal and expressed as percentages.

Sperm morphology assay

To evaluate the spermatozoa morphologic abnormalities, a drop of sperm suspension was added to 1% Eosin-Nigrosin stain and kept for 5 min then placed on a clean slide and spread gently to make a thin film according to the method of Blom (1983). Morphological abnormalities of spermatozoa at 400x magnification were observed under a microscope and expressed as percentage according to the method of Wyrobek and Bruce (15). For each mouse, 100 spermatozoa were scored.

Testosterone hormone measurements

Blood samples were collected from all mice examined by the retro-orbital venous plexus. Sera were separated and stored at -80ºC until being used, as reported previously by Zaki et al. (16). The serum concentration of testosterone was measured using the commercial testosterone immunoassay kit according to Amballi et al. (17).

Histological assessment

Specimens from the testis were taken for histological assessment. The testis was excised and cut into 2 specimens, one was instantly cut into small pieces (1/2-1 mm^3), fixed in 3% glutaraldehyde solution and processed to obtain ultrathin sections for transmission electron microscope (TEM) examination. The other specimen was fixed in Bouin solution, processed to make 3–5 µm thick paraffin sections, then stained with Haematoxylin and Eosin (H&E) stain for light microscopic assessment. Haematoxylin and Eosin (H&E) stained sections were used to get digital images at magnification (100X)

and (400X), using (Olympus DP20) digital camera which was connected to (Olympus BX41) microscope.

Statistical analysis

All data were expressed as the mean ± SD and analyzed using the SPSS for Windows software package version 18.0 (SPSS, Chicago, IL, USA) was applied. One-way analysis of variance (ANOVA) was done, and differences between groups were determined using the Duncan test. The statistical significance was set at < 0.001

Results

Total and free sulphur dioxide (SO_2) content

Sulphur dioxide concentration was estimated in certain widely consumed dried apricot leather samples in Egyptian markets. The total amounts of sulphur dioxide are ranged from 583.7±1.05 to 1766.0±2.0 ppm while the free amounts of sulphur dioxide are ranged from 102.0±1.7 to 409.0±2.3 ppm in the cold drink but ranged from 83.6±0.9 to 327.6±2.1 ppm in the boiled drink as shown in Tables 1 and 2. Two samples were found at an acceptable level (550 ppm) whereas 8 samples had an unacceptable level.

Chromosomal aberrations and mitotic index

The results obtained for the chromosomal aberrations (CAs) analysis of bone marrow cells are given in Table 3 and Figure 1. These CAs include fragments, rings, breaks, gaps, deletions, centromeric attenuation, centric fusion, stickiness and end-to-end association. It can be observed that the treatment with CP produced the highest level of chromosomal aberrations (90.4 ± 1.15). The aberrations induced by the two different prepared drinks (cold and boiled) increased significantly when

Table 1. SulphurSulphur dioxide content of Qamar Al-din drinks prepared by the cold soaking method.

Sample number	Sulphur dioxide (ppm) in cold drink		
	Total	Free	Combined
1	1766.0±2.0	332.8±2.2	1433.2
2	1095.7±1.55	409.6±1.91	686.1
3	1011.2±1.43	409.6±1.44	601.6
4	1090.6±1.35	307.2±1.83	783.4
5	1241.6±1.6	338.4±2.2	903.2
6	1290.2±2.15	332.0±1.9	958.2
7	583.7*±1.05	102.0*±1.7	481.7
8	588.8*±1.07	102.0*±2.1	486.8
9	1582.1±2.37	409.0±2.3	1173.1
10	1617.9±2.83	307.0±1.91	1310.9

Data are represented as mean ±S.D of three replicates measurements. *Acceptable level of Sulphur dioxide concentration.

Table 2. Sulphur dioxide content of Qamar Al-din drinks prepared by the boiled method.

Sample number	Sulphur dioxide (ppm) in boiled drink		
	Total	Free	Combined
1	1766.0±2.0	249.6±1.8	1516.4
2	1095.7±1.55	327.6±2.1	768.1
3	1011.2±1.43	311.3±1.9	699.9
4	1090.6±1.35	267.3±1.7	823.3
5	1241.6±1.6	243.4±1.6	998.2
6	1290.2±2.15	242.3±1.6	1047.9
7	583.7*±1.05	86.7*±0.9	497.0
8	588.8*±1.07	83.6*±0.9	505.2
9	1582.1±2.37	347.7±1.8	1234.4
10	1617.9±2.83	273.2±2.1	1344.7

Data are represented as mean ±S.D of three replicates measurements. *Acceptable level of Sulphur dioxide concentration.

Table 3. Frequencies of different mitotic aberrations in bone marrow cells induced by the cold and boiled drink preparations.

Groups	Mean of chromosomal aberrations (%)									Total CA mean ± SE	% of mitotic index mean ± SE
	F	R	B	G	D	CF	CA	ETE	S		
Control	-	3.36	1.80	2.25	-	4.80	2.43	2.01	1.95	18.6±0.8**	8.8 ± 0.11
Cyclophosphamide	12.49	6.61	11.32	9.81	7.71	10.35	7.72	9.84	14.55	90.4±1.15**	3.9±0.25**
Cold drink	8.43	5.54	7.33	6.45	8.21	6.33	7.44	7.95	9.92	67.6±1.28**	5.7±0.38**#@
Boiled drink	10.60	5.74	9.98	8.90	9.46	7.50	8.74	7.88	11.92	80.7±1.80**	4.3±0.19**#@

Data are represented as mean ±S.D; n = 5 mice/group. Abbreviations: R: ring, F: fragment, G: gap, B: break, CF: centric fusion, D: deletion, ETE: an end to end association, CA: centromeric attenuation, S: stickiness. **Significantly different from negative control (P< 0.001). # Significantly different from cyclophosphamide group (P< 0.001). @Significantly different from cold drink group (P< 0.001).

Table 4. Frequencies of sperm abnormalities of adult male mice induced by the cold and boiled drink preparations.

Groups	Mean of sperm abnormalities									Total sperm Abnormalities Mean ± SE
	Head abnormalities						Tail abnormalities			
	Banana	Amorphous	Twins	Attached two heads	Detached	Without hook	Detached	Possess two tails	Looped	
Control	-	1.21	-	1.52	1.34	0.97	1.19	-	1.67	7.9 ± 0.1
Cyclophosphamide	1.26	1.45	0.85	3.98	1.04	1.70	3.80	0.20	9.18	23.46±1.66**
Cold drink	2.0	1.80	0.90	2.4	1.85	2.56	1.44	0.35	4.0	17.3±1.70**#
Boiled drink	1.74	0.76	0.94	1.64	2.22	2.18	4.3	0.49	6.50	21.0±1.11**#@

Data are represented as mean ±S.D; n = 5 mice/group.

**Significantly different from negative control (P< 0.001)

Significantly different from cyclophosphamide group (P< 0.001)

@Significantly different from cold drink group (P< 0.001)

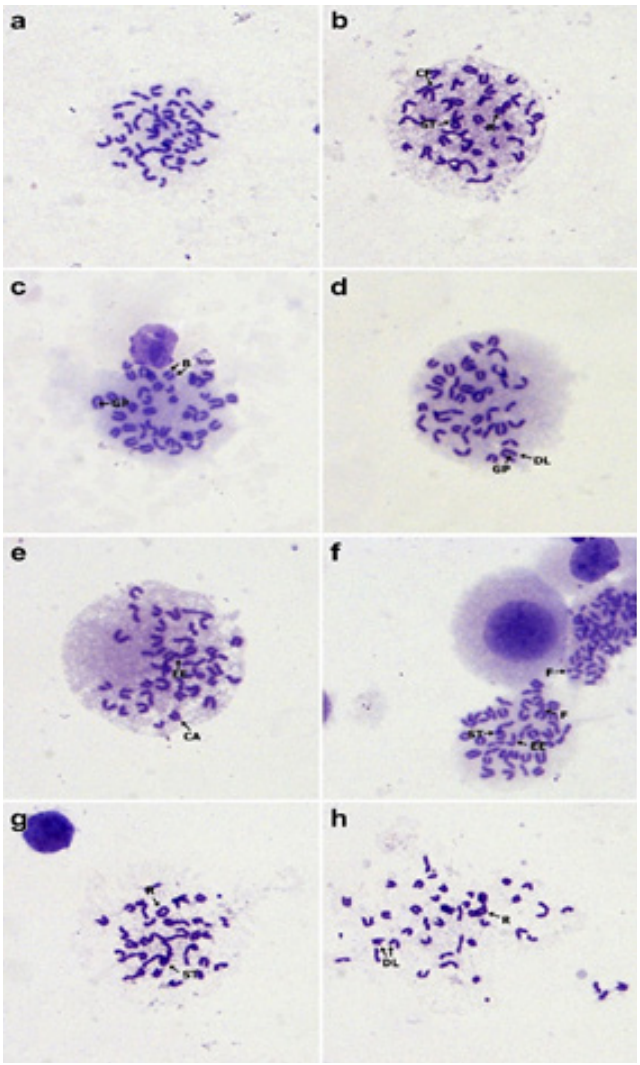

Figure 1. Photomicrograph of metaphase complements in mice bone marrow cells **(a)** normal spread. **(b-h)** Photomicrographs of Qamar Al-Deen drink treated groups, illustrating different types of chromosomal abnormalities. CF; Centric Fusion, ST; Stickiness, R; Ring chromosome, DL; Deletion; GP; Gap, B; Breaks, F; Fragment, EE; End to End association, CA; Chromosome Attenuation. (Giemsa stains 1000x mag.)

compared to the negative control group giving values of (67.6±1.28; 80.7±1.80), respectively. Meanwhile, it is noticed that the values in the boiled drink group are significantly higher than the cold one. The negative control group gave the lowest number of aberrations (18.6 ± 0.8) when compared to the ones received the drinks and CP. Concerning the effect of the drinks on the cell division, it can be observed from Table 3 that there was a significant decrease in the values of the mitotic index in the animals received cold and boiled drinks which were (5.7±0.38; 4.3±0.19), respectively, when comparedto the negative control group (8.80±0.11). The lowest number of dividing cells was observed in mice treated with 25 mg/kg b.wt. of CP for 28 days, where the

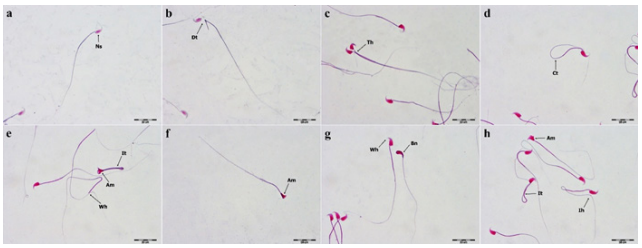

Figure 2. Photomicrograph of mice sperm: **(a)** Ns; normal sperm. **(b-h)** Photomicrographs of Qamar Al-Deen drink treated groups, illustrating different types of sperm abnormalities Dt; detached head, Th; twins head, Ct; curved tail, It; inverted tail, Am; amorphous, Wh; without the head, Bn; banana shape, Ih; inverted head. (Eosin-nigrosin stain 400x mag.).

mitotic indices value was (3.9±0.25).

Sperm abnormality

Sperm abnormality parameters include Banana, amorphous, twins heads, without hook, big head, small heads, detached tail, possess two tails and looped tail (Fig. 2). The data show that the total number of sperm cells found with abnormality in the control group was (7.9±0.1) and the treatment with CP produced the highest number of abnormal sperms (23.46±1.66). Whereas a significant increase in the value of sperm abnormalities was detected in the cold and boiled groups compared with a negative control group, where the recorded values were (17.3±1.70 and 21.0±1.11), respectively. In addition, sperm abnormalities in the boiled drink group are significantly high compared to the cold group (Table 4).

Testosterone analysis

The data demonstrate a significant reduction in the testosterone measurements in the groups that received the cold and the boiled drinks with values (2.60±0.15 and 4.06 ± 0.11), respectively, when compared with the control group received distilled water (4.06±0.11) (Table 5).

Histopathological assessment

Light microscopic findings

Light microscopic examination of testicular sections from the control group (Group I), stained by H&E stain, showed normal testicular tissue. Seminiferous tubules are packed together with an intact basement membrane lined with regular layers of spermatogenic cells with mature spermatozoa in the lumina. In addition, Sertoli cells were noticed in the seminiferous tubules supporting spermatogenic cells. In between the seminiferous tubules, groups of interstitial cells of Leydig were revealed in the interstitial space connective tissue stroma (Fig. 3).

Table 5. Measurement of testosterone activity of male albino mice after oral administration of two types of Qamar el din drink for 28 days.

	Negative Control	Cold drink	Boiled drink
Testosterone (ng/ml)	4.06 ± 0.11*	2.60 ± 0.15*	1.53 ± 0.22*@

Data are represented as mean ±S.D. *Significantly different from negative control group ($P < 0.001$). @Significantly different from cold drink group ($P < 0.001$)

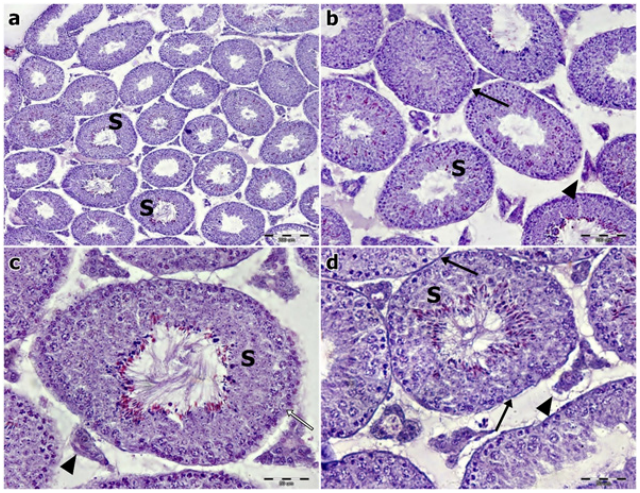

Figure 3. Photomicrographs of testis of the control group (Group I). **(a-d)** Photomicrographs showing, normal testicular tissue. Notice, seminiferous tubules (S) are packed together with an intact basement membrane (thin black arrow). Seminiferous tubules are lined with regular layers of spermatogenic cells in addition to Sertoli cells (thin white arrow). **(b-d)** Notice interstitial cells of Leydig in between the seminiferous tubules (black arrowhead); (H&E stain a; 100x mag. b; 200x mag. c,d; 400x mag.).

Testicular sections from the group received the cold drink (Group II), showed normal testicular tissue organization in many areas with normal seminiferous tubules and spermatogenic cells arrangement. On the other hand, some areas showed disturbed tissue structure, where widening of the interstitial spaces was noticed in these areas and some blood vessels appeared congested with the thickened wall. Some seminiferous tubules showed the irregular distribution of the germinal epithelium, as spaces in between spermatogenic cells were revealed, with a reduction in germ cells layers and the number of spermatozoa in the lumen of seminiferous tubules. In addition, some interstitial cells of Leydig showed dark pyknotic nuclei (Fig. 4).

Interesting, testicular sections from the mice that received the boiled drink (Group III), showed disturbing testicular tissue. Disorganized seminiferous tubules, with the widening of the interstitial space in most areas, where congested dilated blood capillaries with thickened walls were observed. The interstitial cells of Leydig were decreased in number some showed pyknotic nuclei. Some seminiferous tubules showed disturbed basement membrane and shrinkage in germ cells layers. Spermatogenic cells showed spaces between them, moreover, vacuolations in their cytoplasm were revealed. The lumen of many seminiferous tubules showed, decrease or disappearance of spermatozoa, in addition to exfoliated spermatogenic cells (Fig. 5).

Ultrastructure findings

In accordance, electron microscopic examination of testicular sections from the control group (Group I), showed normal ultra-structure of testicular tissue. Seminiferous tubules showed intact basal lamina surrounded by myoid cells, narrow interstitial spaces were noticed between them. The Sertoli cells were revealed with their indented nuclei and tubular cristae, forming a junctional complex with adjacent Sertoli cells. Spermatogonia

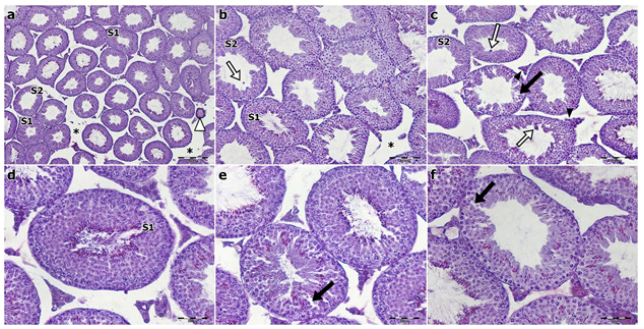

Figure 4. Photomicrographs of testis of (Group II). **(a-f)** Photomicrographs show some areas with normal seminiferous tubules (S1) and spermatogenic cells arrangement. Other seminiferous tubules show spaces in between spermatogenic cells (thick black arrow) and exfoliated germ cells in the lumen (thick white arrow). Notice seminiferous tubules (S2) showing a reduction in germ cells layers with the wide lumen and decreased number of spermatozoa. **(a,b)** Notice congested blood vessels with a thickened wall (white arrowhead) and areas with widening of the interstitial spaces (*). **(c)** Some interstitial cells of Leydig show dark pyknotic nuclei (black arrowhead). (H&E stain a; 100x mag. b,c; 200x mag. d-f; 400x mag.).

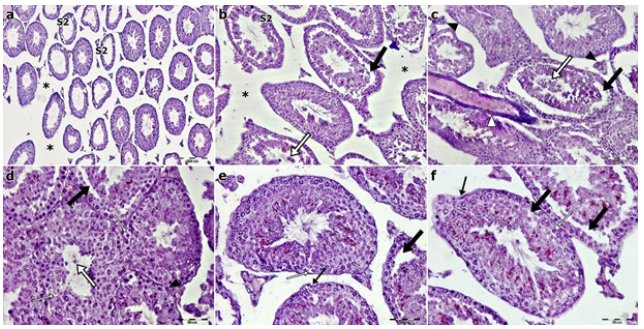

Figure 5. Photomicrographs of testis of (Group III). Photomicrographs showed disturbed testicular tissue organization. **(a,b)** Disorganized seminiferous tubules, with the widening of the interstitial space in most areas (*). Seminiferous tubules (S2) show shrinkage in germ cells layers and decreased spermatozoa in the lumen. **(c)** Congested dilated blood capillaries with a thickened walls (white arrowhead) are noticed. **(c,d)** Interstitial cells of Leydig were decreased in number and some showed dark pyknotic nuclei (black arrowhead). **(b-f)** Seminiferous tubules with spaces between spermatogenic cells (thick black arrow) and exfoliated spermatogenic cells (thick white arrow) in the lumen of seminiferous tubules are revealed. Some spermatogenic cells show vacuolations in their cytoplasm (thin white arrow). **(e,f)** Notice disturbed basement membrane (thin black arrow) (H&E stain a; 100x mag. b,c; 200x mag. d-f; 400x mag.).

and spermatocytes were seen within the recesses of the Sertoli cells. Rounded spermatids were revealed with their acrosomal caps. Spermatozoa in different phases of maturation were noticed. In addition, interstitial cells of Leydig with its rounded nucleus, lysosomes and lipid droplets in the cytoplasm were seen in between seminiferous tubules (Fig. 6).

Electron microscopic examination of testicular sections from (cold drink) group, showed areas of normal and others of disturbed testicular tissue. Some seminiferous tubules appeared with the intact basal lamina, normal spermatogenic cells and normal junctional complexes. Other seminiferous tubules showed thickened

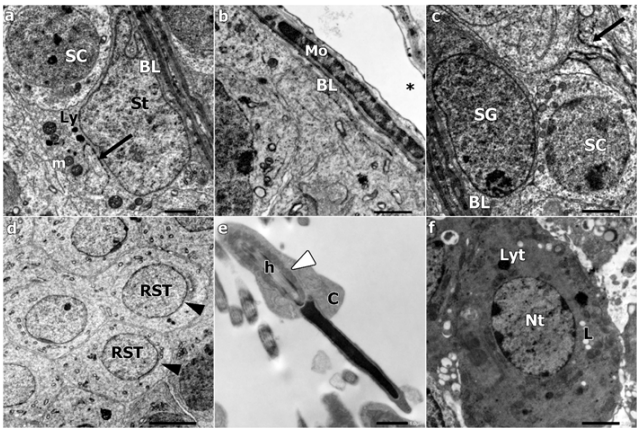

Figure 6. Electron micrographs of a mouse testis of the control groups showing,the normal ultrastructure of the testis. **(a,b)** Electron micrographs show seminiferous tubules with intact basal lamina (BL) surrounded by myoid cell (Mo). Notice the narrow interstitial spaces (*) in between seminiferous tubules. The Sertoli cell is shown with its indented nucleus (St), tubular crisate (m), lysosomes (Ly) and intact junctional complex with the adjacent Sertoli cell (black arrow). Spermatocytes (SC) are seen within the recesses of the Sertoli cells. **(c)** Electron micrograph shows normal spermatocytes (SC), spermatogonia (SG) on the intact basal lamina (BL). Notice the intact junctional complexes (black arrow). **(d)** Electron micrograph shows normally rounded spermatids (RST) with acrosomal caps (black arrowhead). **(e)** Electron micrograph shows a maturing spermatozoon notice its head (h), mitochondrial sheath (white arrowhead) in the middle piece and the excess cytoplasm (C). **(f)** Electron micrograph shows interstitial cells of Leydig with rounded nucleus (Nt), lipid droplets (L) and lysosomes (Lyt) in the cytoplasm.

irregular basal lamina. Some Sertoli cells appeared with vacuoles, vacuolated mitochondria, increased lysosomes, dilated endoplasmic reticulum, rarefaction of the cytoplasm and disturbed junctional complexes. Also, spaces in between spermatogenic cells were revealed. Furthermore, abnormal spermatocytes with dark cytoplasm were seen. While other spermatocytes showed nuclei with disturbed chromatin distribution and rarefaction of the cytoplasm. Moreover, rounded spermatids with small sizes, unapparent acrosomal caps and rarefaction of the cytoplasm were seen. In several spermatozoa middle pieces, disorganized mitochondrial sheaths were noticed. Additionally, elongated spermatids revealed some normal and other abnormal heads. The interstitial cell of Leydig appeared normal in many fields but also some degenerated interstitial cells appeared with dark irregular nuclei, increased lysosomes and increased lipid droplets were observed (Fig. 7).

Electron microscopic examination of testicular sections from (boiled drink), showed disturbed testicular tissue. Most seminiferous tubules appeared with disturbed, thickened irregular basal lamina. Abnormal spermatogenic cells were seen. Where spermatogonia showed rarefaction of the cytoplasm and disturbed nuclear envelope. Some spermatocytes had nuclei with irregular shapes and disturbed nuclear envelopes. Sertoli cells showed many vacuoles, increased lysosomes, proliferation and dilatation of endoplasmic reticulum and disturbed junctional complexes. Spaces in between cells were also noticed. In addition, small-sized rounded sper-

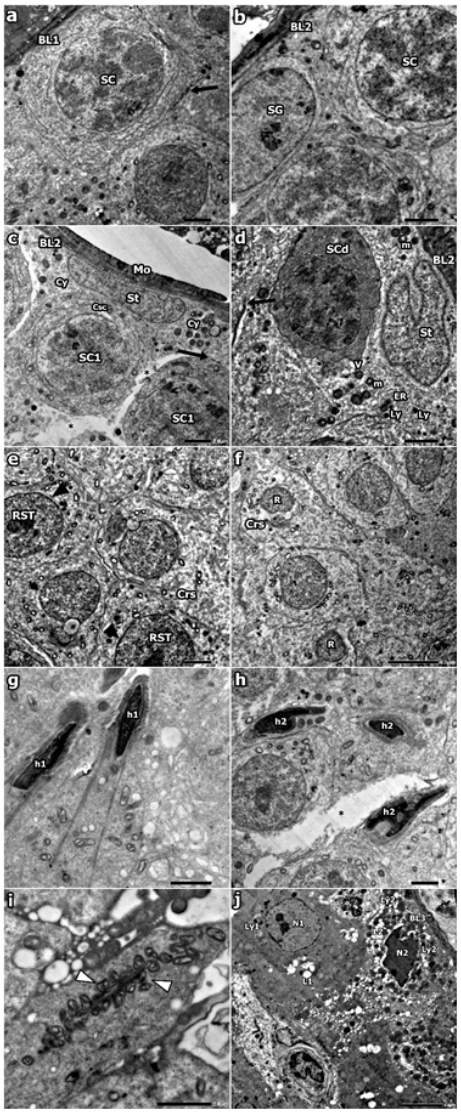

Figure 7. Electron micrographs of a mouse testis of the (Group II) showing, areas of normal and other disturbed testicular tissue. **(a)** Electron micrograph shows seminiferous tubules with intact basal lamina (BL1). Normal spermatocyte (SC) and intact junctional complexes (black arrow). **(b)** Electron micrograph shows normal spermatogonia (SG) and normal spermatocyte (SC) but a thickened irregular basal lamina (BL2) is noticed. **(c,d)** Electron micrographs show thickened basal lamina (BL2), notice the surrounding Myoid cell (Mo). Sertoli cells (St) show, vacuoles (V), vacuolated mitochondria (m), increased lysosomes (Ly), dilated endoplasmic reticulum (ER), rarefaction of the cytoplasm (Cy) and disturbed junctional complexes (black arrow). Spermatocytes show nuclei with disturbed chromatin distribution (SC1) and rarefaction of the cytoplasm (Csc). A spermatocyte with a dark cytoplasm (SCd) is revealed. Notice the spaces in between spermatogenic cells (*). **(e,f)**Electron micrograph shows normally rounded spermatids (RST) with acrosomal caps (black arrowhead). Notice small-sized rounded spermatids (R) with unapparent acrosomal caps and rarefaction of the cytoplasm (Crs). **(g,h)** Electron micrograph of elongated spermatids, normal (h1), abnormal heads (h2) and spaces in between spermatogenic cells (*) are seen. **(i)** Electron micrograph showing, middle piece of spermatozoa with disorganized mitochondrial sheath (white arrowhead). **(j)** Electron micrograph shows normal interstitial cell of Leydig with rounded nucleus (N1) and lipid droplets (L1) and lysosomes (Ly1) another degenerated interstitial cell of Leydig appears with dark irregular nuclei (N2), increased lysosomes (Ly2) and increased lipid droplets (L2). Notice irregular basal lamina (BL3).

matids with abnormal acrosomal caps were observed. Some rounded spermatids were found sloughed in the interstitial spaces. Furthermore, elongated spermatids with abnormal heads were noticed. Disorganized mitochondrial sheaths and vacuolated mitochondria were recorded in the middle piece of the spermatozoa. In addition, principal pieces appeared with disturbed fibrous sheaths. Also, degenerated interstitial cells of Leydig with increased lysosomes were revealed (Fig. 8).

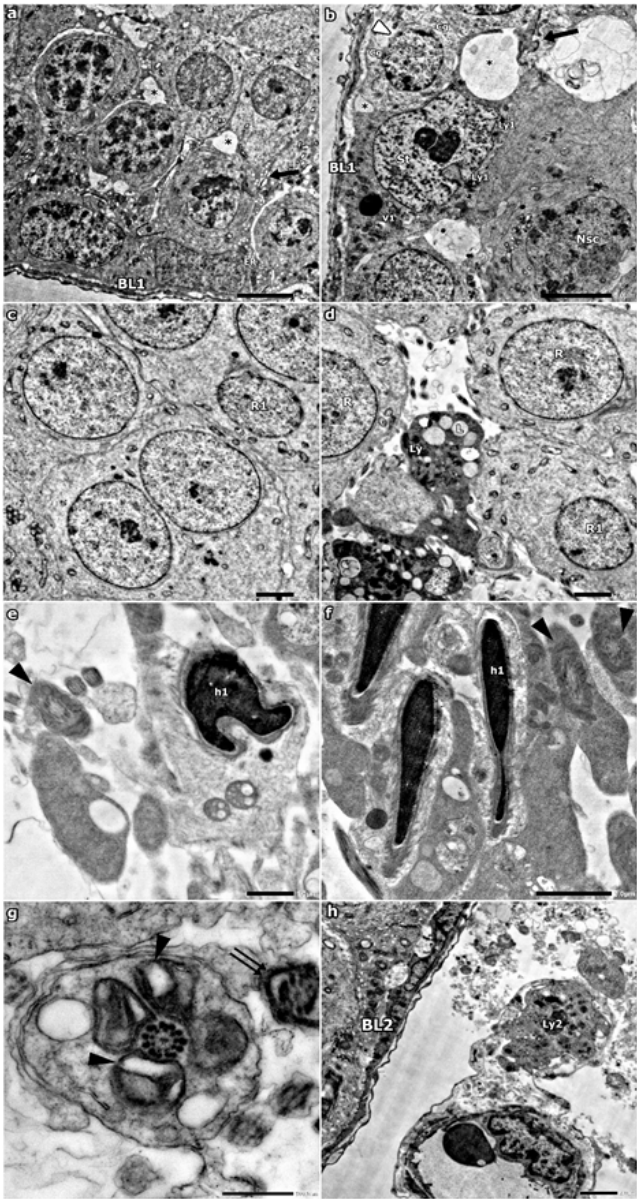

Figure 8. Electron micrographs of a mouse testis of (Group III) showing a disturbed testicular tissue. **(a,b)** Electron micrographs show seminiferous tubules with disturbed irregular basal laminae (BL1). Sertoli cells (St) show, vacuoles (V1), increased lysosomes (Ly1), proliferation and dilatation of endoplasmic reticulum (ER) and disturbed junctional complexes (black arrow). Notice spermatogonia with rarefaction of the cytoplasm (Cg) and disturbed nuclear envelope (white arrowhead). A spermatocyte with an irregular nucleus and disturbed nuclear envelope is revealed (Nsc). Notice the spaces in between cells (*). **(c,d)** Electron micrograph shows small-sized rounded spermatids (R1) with abnormal acrosomal caps. Rounded spermatids (R) are sloughed in the interstitial space. Notice interstitial cells of Leydig with increased lipid droplets (L) and lysosomes (Ly). **(e,f)** Electron micrograph of elongated spermatids with abnormal heads (h1). Notice the middle piece of spermatozoa with disorganized mitochondrial sheath (black arrowhead). **(g)** Electron micrograph showing a middle piece of spermatozoa with disorganized mitochondrial sheath and vacuolated mitochondria (black arrowhead). Notice principal piece with disturbing fibrous sheath (double black arrow) **(h)** Electron micrograph show degenerated interstitial cells of Leydig with increased lysosomes (Ly2). Notice disturbed thickened basal lamina (BL2).

Discussion

Preservative agents are important in foods and beverages. However, the effective uses of these agents are accompanied by some side effects that result from damage to normal tissue (2, 3). SO_2 is one of the most common preservatives in the food and drink industries but little data are available concerning its hazardous impact *in vivo* on Swiss albino mice as well as on human health. In this regard, the present study is designed to assess the influence of SO_2 used in the preparation of dried apricot leather namely Qamar Al-Deen. Several genotoxic endpoints and histological examinations of testis have been conducted. The presence of the high amount of SO_2 in dried apricot samples could be related to the usage of the higher concentrations of SO_2 in fruit leather processing. The mean sulphite level in dried apricot samples in this investigation was higher than those reported by the EU (18).

DNA damage over time accumulates inside the cells because of the exposure to a range of exogenous and endogenous agents. If these errors are not repaired appropriately, mutations in somatic or germline cells will occur, which are engaged in the etiology of different genetic diseases, such as cancer (19). The findings of the present study demonstrated a significant increase in the frequencies of chromosomal abnormalities in bone marrow cells of Swiss albino mice after administration of Qamar Al-Deen drink, compared with the negative control group. Meanwhile, the chromosomal abnormalities in the group that received the boiled drink are higher than that received the cold one. The cytotoxic potential of the examined drink was also evident, as shown in the decreased cellular division observed in mitotic indices in animals that received the drink, where the boiled drink group showed lower values than the group received the cold drink. It is possible to conclude that the tested drink exerts its genotoxic effect and cellular toxicity by the oxidative stress produced by the SO_2 present in it (9). These outcomes are in accordance with Uren et al. (5) who revealed that SO_2 caused significant rises in the frequency of sister chromatid exchange and micronuclei in the middle and high dose groups in human lymphocytes. It also enhanced mitotic delays and decreased mitotic index and replication index. These findings have proved that SO_2 has mutagenic impact and it can provoke genetic damage leading to malignancies.

Good quality sperm is essential for the accurate transmission of genetic material. The sperm cells analysis revealed that the tested drink administration increased the incidence of abnormal spermatozoa, which was more frequent in the boiled group. Due to different kinds of mutations can induce abnormal sperm morphology, this assay is more sensitive in evaluating germ cell mutagens than other germinal mutagenicity test (20). Sperm abnormality test is also provided as an accurate approach for determining genetic damage in

male germline cells (21). In the current investigation, the higher frequencies of sperm aberrations enhanced by Qamar Al-Deen may be an indication of the genetic alterations happened at the spermatogonial stage of the mouse germ cells. These findings are similar to Li et al. (22) who reported that mice that received SO_2 showed significant increases in sperm malformation. Similarly, Shekarforoush et al. (10) investigated the effects of sodium metabisulfite (SMB); a sulfite salt that releases SO_2, on testicular toxicity and morphometric values of the epididymis in adult male rats, revealed a decrease in the sperm characteristics.

Moreover, the level of testosterone analysis showed a significant decrease of the serum level of testosterone in the groups received the drink, where it was lower in the boiled drink group. These findings are similar to Shekarforoush et al. (10) who monitored a decrease in testosterone level in adult male Wistar ratsthat received SMB. On the other hand, Li et al. (22) revealed a decrease in testosterone concentration in SO_2-treated groups but without a significant difference, this may be attributed to the different concentrations of SO_2 and the duration of the experiment.

Besides, analysis of testicular sections from animals received the dried apricot drink, prepared by cold drink, showed many areas with normal testicular tissue organization, spermatogenic cells arrangement and normal cells structure. On the other hand, some areas showed disturbed tissue structure with the widening of the interstitial spaces. Some seminiferous tubules showed irregular distribution of the germinal epithelium, with a reduction in germ cells layers and the number of spermatozoa in the lumen of seminiferous tubules. Disturbed basement membranes were revealed. In addition, abnormalities in Sertoli cells and their junctional complexes, spermatogenic cells and interstitial cells of Leydig were recorded on the ultrastructure level. Additionally, some abnormal spermatozoa with disorganized mitochondrial sheaths and abnormal heads were present. Interestingly, all these abnormalities were very prominent and present in most areas in the testicular sections from animals that received the dried apricot drink, prepared by the boiled extract. These abnormalities may be contributed to the high level of SO_2 in the dried apricot drink. These results are in accordance with Meng and Liu, (23) findings, who stated that mice testicular tissues showed alternations in the basement membranes, seminiferous cells, spermatozoa and Sertoli cells of groups exposed to SO_2 in a dose-dependent manner. A similar pathological effect of SO_2 on the testis was also reported by Li et al. (22) who noticed that mice that received SO_2 showed atrophic seminiferous epithelia, increased intertubular gap, expanded tubular cavities and disorganized vacuolated spermatogenic cells. Higher levels of SO_2 were recorded in the boiled drink than in the cold drink, this might explain the extensive alterations in the testis and the more genotoxicity found in the animals that received the boiled drink.

The current data showed that administration of the drink (Qamar Al-Deen) prepared from dried apricot leather, by two different preparation methods (cold and boiled), increased chromosomal and sperm abnormalities and decreased the mitotic indices. Furthermore, it caused a decrease in serum testosterone level and histopathological lesions to the testis. These findings were more prominent in the groups that received the boiled drink. In addition, higher levels of SO_2 were recorded in the boiled drink than in the cold one. Thus, it can be concluded that these serious alterations induced by the examined drink could be attributed to the presence of SO_2 in the dried apricot leather.

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