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Effect of Colorant on Cytogenetic, Biochemical and Histochemical Parameters and Quality Changes during Storage of some Commercial Fruit Drinks

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Abstract

In this research, several physical and chemical properties were identified for some commercial fruit drink samples (pear, cherry, strawberries and red grape) stored at 4°C for six months. Color, pigment, tannin contribution and anthocyanin analyses were done monthly. Commercial fruit drinks were ingested into mice to evaluate its influences on chromosomal aberrations and biochemical contents (glucose content, GOT and GPT). Also, the influence of these drinks on histochemical and histopathological in the liver of mice was evaluated. Statistical analyses of obtained results of studied properties revealed that the effects of storage time on L*, a*, b*, C*, H*, BI, polymeric color, color density and total anthocyanin were found to be high correlation coefficient in commercial drink. The total anthocyanin values decreased with increasing storage time in pear and strawberry drinks, but increased with increasing storage time in cherry and red grape drinks. The results show that the all commercial fruit drink caused a highly significant increase in structural chromosomal aberrations more than control. GOT and GPT activity and glucose content in both plasma and liver were also more stimulated by commercial drink. Moreover, results showed a considerable increase in all parameters compared to the control of especially the commercial fruit drink. The results were discussed with particular references to the evaluation of cytogenetic studies on fruit drink in general. In the present study, the histological examination of liver treated with commercial drink revealed liver tissue mostly normal. Also, pigment of commercial drink or food coloring agent has toxicological and histochemical harmful effects on liver.

Keywords: Pear; Cherry; Strawberries; Red grape; Drink; Color; Anthocyanin; Storage; Mice; Marrow cell; Spermatocyte cell; GOT; GPT; Histochemical; Ag Nors; DNA ploidy

Introduction

Anthocyanins are responsible for the attractive color of some fruit drinks like blackberry nectars [1]. Rommel reported that the effect of processing and storage period on color and pigment (anthocyanins) during the storage of the blackberry juice [1]. They have been hypothesized as important antioxidants. However anthocyanins dissappear as monomeric compounds and are transformed into polymeric forms. This transformation results in a color change to a more brownish shade [2,3]. Storage temperature is the main responsible factor for anthocyanin loss. Degradation rate increases as corresponding with food properties and anthocyanins compositions. Many researchers [1,2,4-7] reported anthocyanin losses in different foods during storage. Anthocyanin loss can be easily determined by pigment, color analysis, and also tristimulus colorimetry. Tristimulus colorimetry in color is a valuable tool for discriminating changes in color due to both Mailard reaction and anthocyanin degradation [8]. Storage time is the main responsible factor for anthocyanin loss. Degradation rate increases as corresponding with food properties and anthocyanins compositions. Many researchers [9,10] reported anthocyanin losses in different foods during storage. The importance of the assessment of the effect of colorants (anthocyanine drinks) on metabolism and upon the genetic make up of mankind is obviously a critical issue in the use of these chemicals. An increasing number of commercial drink, which are used as food colorants (drinks) long periods are shown to exhibit genotoxic effects. The human exposures to such agents are very wide, and feeding over long periods is likely. Therefore, they possessed potential hazards to the human health.

Previous studies showed changes in cytogentic assays of feeding mice with food colorants relative to control mice. Further investigations were carried out on both traditional and commercial hibiscus drink to evaluate the acceptability and antagonistic effects of these food colorants drinks as foodstuff for human and animal feeding [11-13].

Also, anthocyanin pigments may play a role in the prevention of oxidative damage in living systems. However, anthocyanin and PCA have been shown to have antioxidant activity and to offer protection against atherosclerosis and cancer [14], DNA is a vital molecule in the cell activities and was the main target for chemicals induced cell injury. The commercial pigment or coloring agent has been implicated in several clinccal conditions, but most experimental work has concentrated on childhood hyperactivity, articaria, asthma [15]. Wojewodzka et al. [16] consider inter individual variability important; it can be detected by the analysis of parameters in the comet assay. They found considerable intra-individual homogeneity, and high inter individual variability, suggesting that the extent of the damage, as well as the decrease in the capacity of DNA damage repair, constantly induced by endogenous or

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exogenous factors, may be involved in the variability of the individual responses found.

This research was conducted to identify the effect of storage time for 6 months at refrigerator (4°C) on quality characteristics, color characteristics, anthocyanine and quality pigment contents during the storage of some commercial fruit drinks, as well as to evaluate their biotoxic and mutagenic influences on adult mice. Also, the quantitative histochemical and histopathological influences of some commercial fruit drinks in the liver of mice were aimed.

Materials and Methods

Commercial fruit drinks

Commercial fruit drinks (pear - *'Alexander Lucas'*, cherry - *(Prunus cerasus L.*), strawberry - (*Fragaria x ananassa Duch.*) and red grape - *Vitis vinifera L*) with colorant (# RC1539) were obtained from the International Futura Labs Co. in - Industrial Zone – 6th October city, Egypt. The fruit drinks had been filled into 200 ml glass bottles, stored and evaluated as the other commercial drinks. Upon production, the samples arriving to the laboratory were obtained several physical and chemical properties and the half of the fruit drinks samples were stored at refrigerator temperature (approximately +4°C) for six months in darkness. Color and pigment analyses were done monthly within 6 months.

Physical and Chemical analyses

The pH of drink samples was measured using a digital pH-meter (HANNA, HI 902 meter, Germany). The percent of Total Soluble Solids (TSS), expressed as °Brix (0-32), was determined with a Hand refractometer (ATAGO, Japan). Titratable acidity was determined according to the method reported by Tung-Sun et al. [17]. The viscosity measurements were carried out using HAAKE viscometers (HAAKE, Mess-Technik Gmbhu. Co., Germany) with thermostatic bath to control the working temperature within the temperature of 25°C. Results of viscosity were expressed in centipoise (cP) according to the method of Ibarz et al. [18] Sedimentation was measured as sedimentation/2hours (ml/100 ml) by the method of Krop and Pilnik [19].

Assay of pectinmethylesterase enzyme activity

Pectinmethylesterase enzyme (EC 3.1.1.11) activity was assayed according to the method of Arreola et al. [20] using a 0.1 N NaOH solution.

Non-enzymatic browning determination

Non-enzymatic browning was measured spectrophotometrically by 4054 UV/Visible spectrophotometer, (LKB-Biochrom Comp., London, England), as absorbance at 420 nm using ethanol as blank according to the method of Birk et al. [21].

Color characteristics and parameters determinations

Hunter a*, b* and L* parameters were measured with a color difference meter or the color of commercial fruit drinks (pear, cherry, strawberries and red grape) was measured using a spectro-colorimeter (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Color Standard (LX No.16379): X=72.26, Y=81.94 and Z=88.14 (L*=92.46; a*=-0.86; b*=-0.16) [22]. Color difference, Delta E, was calculated from a*, b* and L* parameters, using Hunter-Scotfield's equation [23] as follows.

Delta E=(delta a^2+ delta b^2 + delta L^2) $^{1/2}$

where: a-ao, b-bo and L-Lo; subscript «o» indicates color of control or untreated sample.

The Hue (H)*, Chroma (C)* and Browning Index (BI) was calculated according to the method of Palou et al. [24] as follows:

$$H^* = \tan^{-1}[b^*/a^*] \quad (1)$$

$$C^* = \text{square root of } [a^{2*} + b^{2*}] \quad (2)$$

$$BI = \left[100\left(x - 0.31\right)\right]10.72 \quad (3)$$

$$X = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$$ Where:-

Pigment and anthocyanin analyses: Polymeric color (PC), color density (TCD) and tannin contributions (CDT) were determined using spectral methods described by Spayd et al. [25]. For total color density (TCD), a 2 ml sample was diluted 2 mL distilled water and absorbance 420, 515 (at the absorbance maximum), and 700 nm on the spectrophotometer. TCD was calculated as follows:

$$TCD = \left(A420 + A515\right) - 2\left(A700\right) \quad (4)$$

For polymeric color (PC), a 2 ml sample was treated with 2 ml 20% K2S2O5. Samples were held 1 hour at 20°C under commercial light prior to measurement of absorbance 420, 515 and 700 nm. Polymeric color was calculated as follows:

$$PC = \left(A420 + A515\right) - 2\left(A700\right) \quad (5)$$

Percent of color due to tannin (% CDT) was calculated as

$$\%\ CDT = \left(PC / TCD\right) \times 100 \quad (6)$$

Concentration of total anthocyanins (TACN) was determined by the pH differential method as described by Wrolstad [26]. Absorbance was measured in the spectrophotometer at 515 nm and at 700 nm in buffer pH 1.00 and pH 4.50, using A=(A515-A700) pH1.00- (A515-A700) pH 4.50. Results were expressed as milligrams of cyanidin-3-glucoside equivalent per liter of fresh weight using an extinction coefficient of 29600 and molecular weight of 445.2 g mol^{-1}.

Animals and experimental Design

60 Male mice weighting about (25-30 g) obtained from a closed random-bred colony at the National Research Center, were used. Food and water were provided addlibitum (means regularly sufficient food and water) according to the method of Lane-Peter and Pearson [27]. The used dose 0.8 and 2.4 mg/Kg of traditional and commercial drink was ingested sub actually (means administration of the drink orally for mice at two dose levels for 3 weeks to study the accumulation of its effect) for consecutive days and mice were killed 24 hours after the last dose. An untreated group acted as control.

Slide preparation and scoring

For studying chromosomal aberration, both treated and control animals were injected i.p. with colchicines 2.5 hours before killing. Bone marrow cells were prepared according to Yosida and Amano [28] and spermatocyte metophases were prepared according to Brewen and Preston [29].

The Biochemical Evaluation

Glucose determination

Glucose content (mg/dL) was enzymatic determined in blood serum according to Trinder [30] using Kit (RTU, bio Merieux) and recorded at spectrophotometer with wavelength 505 nm.

GPT and GOT determination

The activities of GOT (μ/ml) and GPT (μ/ml) were adapted to determine in blood serum according to the method of Young [31].

Histochemical evaluation

Small pieces of liver from each animal were removed and fixed in 10% formal saline and processed to prepare 5 Um-thick paraffin sections. Sections were stained with: Hematoxlin and eosin stain for histological examination, Feulgen technique for DNA demonstration [32] and Argyrophilic silver stain for Nucleolar Organizer Region (Ag-NORs) Staining and quntitation [33].

Quantitative Studies

Ag Nors Counts

100 nuclei per group were counted, using oil immersion lens at magnification of x1000, by careful focusing, only well- defined and sharply stained intra- and extra-nucleolar Ag-Nors dots were included in the counting regime, as well as larger dots representing the total nucleolus where AgNOR dots were wholly aggregated.

DNA Ploidy

Quantitative measurement was achieved by using computerized image analyzer (Leica Qwin 500 image) in Image Analyzer Unit, pathology Department and National Research Center. DNA content analysis was performed on sections stained by Feulgen technique. For each group 100-120 cells were randomly measured. The result is presented as histogram, in which normal diploid cells (2c) are separated clearly from aneuploidy cells (>4c).

Statistical Analysis

All commercial fruit drinks studied samples were conducted at least in duplicate and each sample was analyzed in duplicate. The results of color and anthocyanine parameters were analyzed statistically using analysis of variance, least significant difference (LSD) and correlation coefficient (square root) as described by Richard and Gouri [34]. The results of the chromosomal aberrations in somatic and germ cells analyzed using the chi-square test but biochemical results were statistically analyzed by *t*-test (P>0.05) according to Sendecor and Cocheran [35]. The means of the AgNORs count in different groups were compared statistically using the student *t*-test at *P*-value 0.05 was considered statistically significant [36]. Statistical analysis and graphic illustration were performed using MS Excell XP software.

Results and Discussion

Histochemical and Histopathological evaluations of some commercial fruit drinks

The microscopic examination of control liver section showed normal architecture of liver lobules and hepatocytes with round nuclei and granular cytoplasm (Figure1).

The microscopic examination of liver sections of mice treated with different doses of some synthetic food colorings (pear, cherry, strawberry and red grape drinks) showed different pictures of nuclear damage as well as cellular degeneration comparing to normal structure of hepatic lobules in which the hepatocytes arranged in cords radiating from the central vein (Figure 1). Commercial pear drink at dose 0.8 mg/kg revealed vacuolar degeneration and hepatocellular necrosis associated with edematous which surrounded by aggregation of inflammatory cells, proliferation and dilation of bile duct and signs of fibrosis could be seen (Figure 2). While the dose of 2.4 mg/kg commercial pear drink exhibited hepatocyte ballooning and hydropic degeneration, dilation of blood vessels, increase in mononuclear inflammatory cells observed

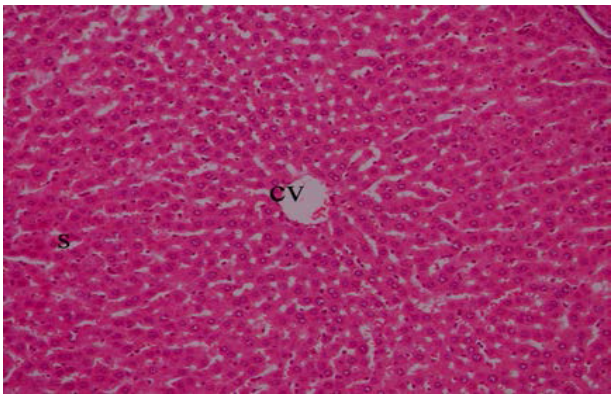

Figure 1: Section of liver of control animal showing normal structure of hepatic lobule, central vein (cv) and blood sinusoids (s) (HX&E x200).

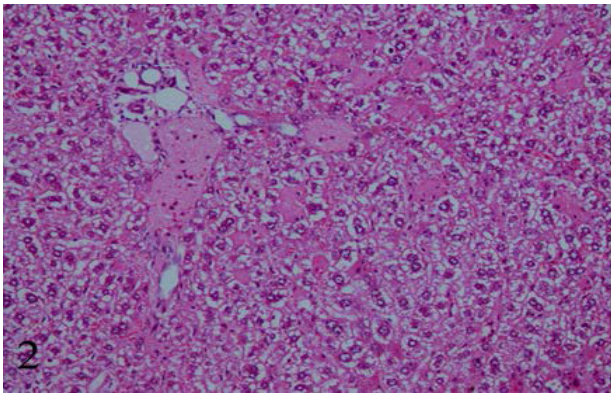

Figure 2: A section in the liver drenched Commercial pear drink at dose (0.8 mg/kg b.w./day) for 3week showing vacuolar degeneration and hepatocellular necrosis associated with edematous which surrounded by aggregation of inflammatory cells, proliferation and dilation of bile duct, signs of fibrosis could be seen (Hx&E x200).

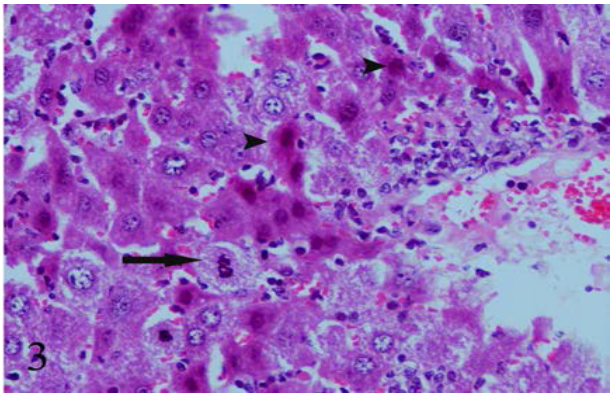

Figure 3: A section of liver drenched pear drink at dose (2.4 mg/kg b.w./day) for 3week showing hepatocytev ballooning and hydropic degeneration. Dilation of blood vessels, increase in mononuclear inflammatory cells observed in sinusoids.Sings of nuclear degeneration in the form of necrosis(N) ,keryolysis,apoptosis (arrow) and increased basophilia (HX&Ex400).

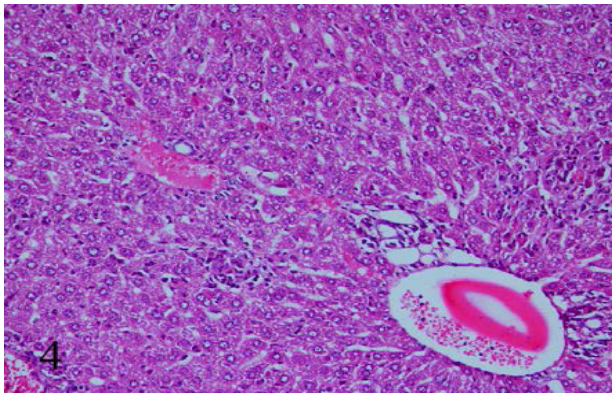

Figure 4: A section of liver drenched cherry drink at dose (0.8 mg/kg b.w./day) for 3week showing nodules of aggregated of inflammatory cells and minimal fibrous. Minute vacuolar degeneration in the bile duct around the dilated portal tract (HX&Ex200).

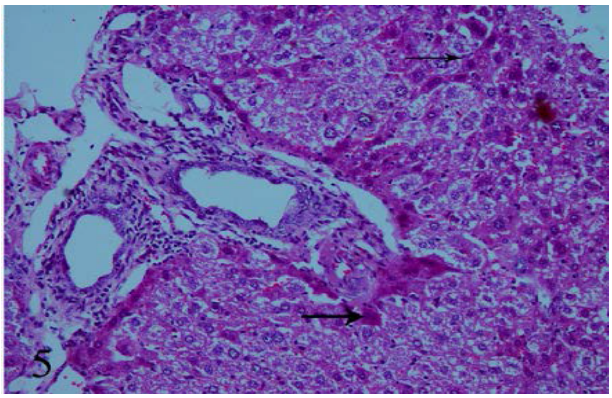

Figure 5: A section of liver drenched cherry drink at dose (2.4 mg/kg b.w./day) for 3week showing necrotic areas, Proliferation in bile ducts and necrosis in their epithelial cells, eosinophilic cytoplasm in hepatocytes around portal area (HX&Ex400).

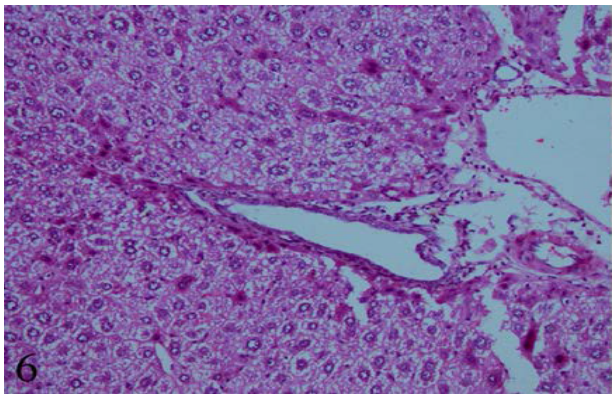

Figure 6: A section of liver drenched strawberry drink at dose (0.8 mg/kg b.w./day) for 3week showing marked dilatation in portal tract, proliferation in the bile duct epithelial cells.Hepatocytes revealed vacuolar degeneration and peripheral chromatin clumping (Hx &E x200).

in sinusoids, sings of nuclear degeneration in the form of necrosis, keryolysis, apoptosis and increased basophilia (Figure 3).

The liver of mice subjected to commercial cherry drink at dose 0.8 mg/kg.bw for 3 weeks showed nodules of aggregated of inflammatory cells and minimal fibrous beside minute of vacuolar degeneration in the bile duct around the dilated portal tract (Figure 4). While, 2.4 mg/kg commercial cherry drink showed necrotic areas, proliferation in bile ducts and necrosis in their epithelial cells, eosinophilic cytoplasm

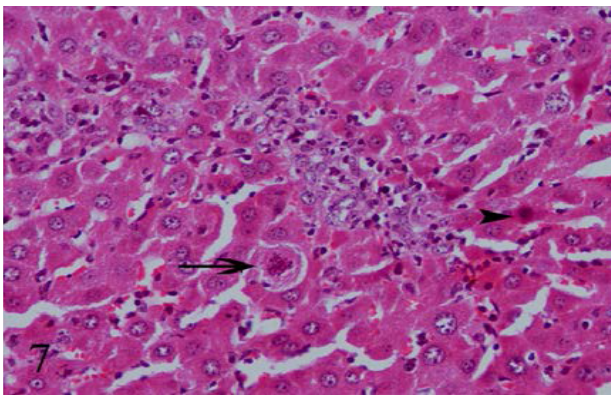

Figure 7: A section of liver drenched strawberry drink at dose (2.4 mg/kg b.w./day) for 3week showing – aggregation of inflammatory cells. Some nuclei are apoptotic (arrow), others pyknotic and karyolysis (Hx&Ex400).

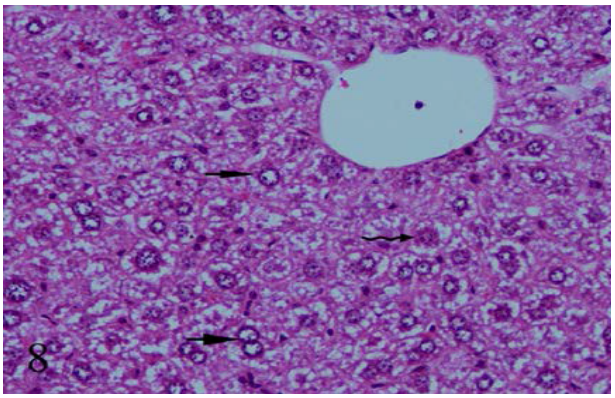

Figure 8: A section of liver drenched commercial Red grape drink at dose (0.8 mg/kg b.w./day) for 3week showing vacuolar degeneration, and peripheral chromatin clumping and focal necrosis.Sings of degeneration in the form of karyorrhexis and karyolysis (Hx&E x40).

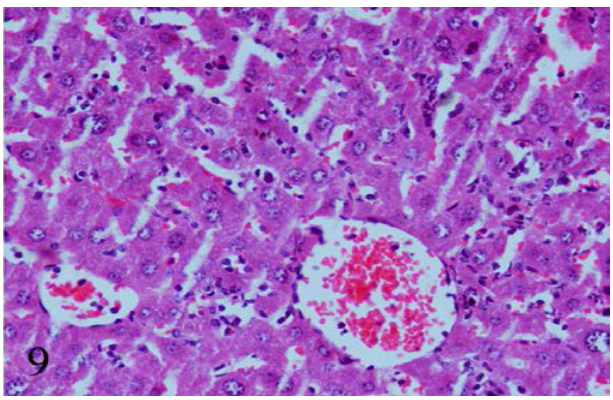

Figure 9: A section of liver drenched commercial Red grape drink at dose (2.4 mg/kg b.w./day) for 3week showing hepatic necrosis, diffusion of inflammatory cells and congestion of blood vessels. (Hx&E x40).

in hepatocytes around portal area (Figure 5). Also, the commercial strawberry drink at dose 0.8 mg/kg b.w./day for 3week showing marked dilatation in portal tract, proliferation in the bile duct epithelial cells. Hepatocytes revealed vacuolar degeneration and peripheral chromatin clumping (Figure 6). The liver of mice received food color of strawberry drink at dose 2.4 mg/kg b.w./day showing aggregation of inflammatory cells, nuclear degeneration in the form of apoptosis, pyknotic and karyolysis (Figure 7). Commercial Red grape drink at dose 0.8 mg/kg b.w./day for 3week showed vacuolar degeneration, and peripheral chromatin clumping and focal necrosis in hepatocyte. Sings of degeneration in the form of karyorrhexis and karyolysis (Figure 8). Meanwhile, the dose of 2.4 mg/kg b.w./day Red grape drink showed beside the hepatocyete degeneration, diffusion of inflammatory cells and congestion of blood vessels also present (Figure 9).

Discussion

Synthetic food dye has been suspected of being toxic or carcinogenic [37]. The daily intake of artificial food colorants that included the aromatic azo compounds may impair hepatic functions and lead to change in liver and kidney histopathological structure [38-40]. In the present study, pear color drink caused vacuolar and ballooning degeneration and hepatocellular necrosis associated with edematous which surrounded by aggregation of inflammatory. These result go in agreement with Nabela et al. (2011) who found that food color TiO showing vacuolar, hydropic degeneration and cell death of some hepatic cells and steatosis. Also, Soltan and Shehata [40] found that the food colors caused lymphocytic infiltration around central veins. Klastskin and Oconn [41] attributed the dilatation and congestion of hepatic vessels and sinusoids to the direct toxic effect of the toxins leading to hepatocellular damage. Visweswaran and Krishnamoorthy [39] mentioned that the pathological changes observed in liver may attributed to elevated lipid peroxidation level and ROS. Our result showed that the treated mice with food color of cherry drink at doses (0.8 mg/kg b.w./day) and (2.4 mg/kg b.w./day) for 3week showed nodules of aggregated of inflammatory cells and minimal fibrous, minute vacuolar degeneration in the bile duct around the dilated portal, necrotic areas, proliferation in bile ducts and necrosis in their epithelial cells and eosinophilic cytoplasm in hepatocytes around portal area. These results were in agreement with Mahmoud [42] who found that the synthetic food dye brilliant blue revealed histopathological alterations in liver of rat. This alteration include: focal necrosis of hepatocytes, infiltration and vacuolation Sarkar and Ghosh [37] reported that the chronic exposure of rat to food color (metanil yellow) caused an extensive degeneration of hepatocytes, diminish in cytoplasmic content, appearance of pycnosis of nuclei and damage occurred in the central vein regions.

The results of the present study revealed that liver mice treated with food color of strawberry drink at dose (0.8 mg/kg b.w./day) and (2.4 mg/kg b.w./day for 3week showed marked dilatation in portal tract, proliferation in the bile duct epithelial cells, vacuolar degeneration and peripheral chromatin clumping aggregation of inflammatory cells. Nuclear degeneration (apoptotic, others pyknotic and karyolysis). The obtained in this study are in accordance with results recorded by Aboel-Zahab [43] who observed brown pigment deposition in the portal tracts and Kupffer cells of the liver as well as in the interstitial tissue and renal tubular cells of the kidney. Congested blood vessels and areas of haemorrhage in both liver and renal sections were revealed in rats receiving mixture B. Our results are also in accordance with data reported by Rus et al. [44] who described changes in the liver when guinea pigs received Tartrazine in drinking water in concentrations of 1, 2 and 3% for 3 weeks. The concentration of 1% the changes consist of slight congestion, in both intralobular and extralobular vessels and discrete perivascular edema. In the external third of lobules they observed some apoptotic hepatocytes. For the concentration of 2%, the liver vascular phenomena are more pronounced; capillary congestion is present in many lobules, determining a slight compression atrophy of hepatocyte cords. Hepatocytes in various stages of apoptosis were observed in small numbers within the liver lobe, the number of hepatocytes in apoptosis being greater at the lobe periphery.

The present results of liver drinking Red grape drink color at dose (0.8 mg/kg b.w./day) and (2.4 mg/kg b.w./day)for 3week showed vacuolar degeneration, diffusion of inflammatory cells, and peripheral chromatin clumping,necrosis, as well as, sings of degeneration in the form of karyorrhexis and karyolysis. These results may be due to synthetic color caused oxidation of fatty acids in biological membranes lead to increase free radicals is able to caused auto-oxidation of the hepatic cells [40]. These results are in accordance with Hernández [45]: food additive causes, ballooning degeneration, hepatocellular necrosis, aggregation of chromatin material around the periphery of the nuclear envelope.

Sasaki et al. [46] who studied the genotoxicity of 39 chemicals currently in use as food additives. They treated groups of four male ddY mice once orally with each additive at up to half its LD50 or the limit dose (2000 mg/kg bw) and performed Comet assays on glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow, 3 and 24 hours after treatment. Tartrazine induced dose- related DNA damage in the glandular stomach, colon, and/or urinary bladder. All 7 food dyes tested induced DNA damage in the gastrointestinal organs at low doses (10 or 100 mg/kg). Among them, Amaranth, Allura Red, New Coccine, and Tartrazine induced DNA damage. Sharma et al. [47] reported that synthetic color has adverse effect on vital organs. At low dose synthetic color, the liver revealed adisruption of hepatic cells near the central vein and hepatocelluler damaged [40].

Quality evaluations of some commercial fruit drinks during storage at refrigerator (4°C)

The following discussion of the quality characteristics of commercial fruit drinks are shown in Table 1.The pH of fresh commercial fruit drink were 12.8-14.60 to 14-15 in pear, cherry, strawberry and red grape after 6 months storage at 4°C, which showing an increase in pH values of commercial drink sample after storages. TSS (oBrix) of stored commercial fruit drinks was greater than the fresh commercial fruit drink. Whereas, the increase of TSS in stored commercial fruit drink was obvious with increasing of sugar concentration content. This increasing of TSS was attributed to the greater degree of tissue breakdown, releasing more components that contribute to soluble solids [17]. The TSS/acid ratio is the major analytical measurement for quality in fresh and stored commercial fruit drink. The TSS/acid ratio of stored commercial fruit drink was increased by increasing of sugar concentration. TSS/acid ratio was shown to be correlated with sweetness but not so closely with flavour [48]. Titratable acidity of fresh commercial fruit drink was higher than stored commercial fruit drink, which may due to enzymatic desertification and increased pH resulting in an increased of total acid. The viscosity (cP) was selected as a measure of drink quality. However, the stored commercial fruit drink was (0.36-0.58 cP) higher than fresh commercial fruit drinks (0.28-0.47 cP), respectively. No sedimentation occurred and also

Fruit drinks	pH	TSS	Total acidity (%)*	TSS / Tottal acidity	Sedimentation / 2hr (ml/100ml) (n=5)	PME (unit /ml)	Viscosity (cP) (n=5)
At zero time and before storage							
Pear	13.8	2.64	0.2112	65.34	70 ± 0.33	0	0.47 ± 0.55
Cherry	13.8	2.32	0.384	35.94	00 ±	0	0.34 ± 0.41
Strawberry	12.8	2.91	0.2592	49.38	30 ± 0.18	0	0.40 ± 0.52
Redgrape	14.6	2.27	0.3072	47.53	0 ± 0	0	0.28 ± 0.40
After 1 months storage at 4^{0}C							
Pear	14	2.86	0.192	72.92	70 ± 0.28	0	0.49 ± 0.35
Cherry	13.6	2.32	0.384	35.42	0	0	0.39 ± 0.11
Strawberry	13	2.88	0.2496	52.0	30 ± 0.19	0	0.43 ± 0.42
Redgrape	14	2.23	0.3072	45.57	0	0	0.29 ± 0.33
After 2 months storage at 4^{0}C							
Pear	14	2.78	0.1152	121.53	50 ± 0.24	0	0.53 ± 0.19
Cherry	13.2	2.37	0.192	68.75	0	0	0.41 ± 0.21
Strawberry	13.2	2.86	0.1344	98.21	50 ± 0.17	0	0.45 ± 0.32
Redgrape	14.8	2.3	0.1152	128.47	0	0	0.32 ± 0.09
After 3 months storage at 4^{0}C							
Pear	14	4.96	0.144	97.22	50 ± 0.29	0	0.54 ± 0.15
Cherry	13.8	4.6	0.192	71.88	0	0	0.43 ± 0.28
Strawberry	13.2	4.85	0.1632	80.88	50 ± 0.31	0	0.46 ± 0.34
Redgrape	15	4.53	0.3072	48.83	0	0	0.33 ± 0.17
After 4 months storage at 4^{0}C							
Pear	14.2	4.9	0.144	98.61	50 ± 0.26	0	0.56 ± 0.41
Cherry	14	4.59	0.192	72.92	0	0	0.44 ± 0.19
Strawberry	13.8	4.95	0.1632	84.56	40 ± 0.18	0	0.47 ± 0.22
Redgrape	15	4.5	0.3072	48.83	0	0	0.35 ± 0.24
After 5 months storage at 4^{0}C							
Pear	14.4	4.24	0.144	100	50 ± 0.34	0	0.56 ± 0.11
Cherry	14	4.05	0.192	72.92	0	0	0.45 ± 0.21
Strawberry	13.6	4.36	0.1632	83.33	40 ± 0.28	0	0.49 ± 0.32
Redgrape	15	4.03	0.3072	48.83	0	0	0.36 ± 0.64
After 6 months storage at 4^{0}C							
Pear	14.5	4.48	0.144	100.70	50 ± 0.42	0	0.58 ± 0.55
Cherry	14	4.24	0.192	72.92	00 ±	0	0.46 ± 0.52
Strawberry	14	4.72	0.1632	85.78	40 ± 0.61	0	0.51 ± 0.50
Redgrape	15	4.28	0.3072	48.83	0 ± 0	0	0.36 ± 0.53

Data represent the mean value of triplicate samples
*Titratable acidity as citric acid

Table 1: Quality characteristics of some commercial fruit drinks during storage at at refrigerator (4°C).

no pectinmethylesterase (PME) was detected in some fruit drinks like cherry and red grape. Whereas, the PME-activity was inhibited by boiling extraction in commercial fruit drink or pasteurization in commercial drink, as seen in extraction and processing of fruit drink [49]. Sedimentation of pear and strawberry were 70 and 30% at fresh samples but were 50 and 40% in stored samples respectively (Table1).

Color characteristics, pigment and anthocyanin contents of some commercial fruit drinks during storage at refrigerator (4°C)

Color is only part of the overall appearance, but is probably a major quality factor in juices and drink products. Color characteristics measurement directly in the drink samples with a Hunter Lab Ultra Scan revealed that color changed in some commercial fruit drink sample (Table 2). In this case, brightness (L*-values) decreased, redness (a*-values) increased and yellowness (b*-values) decreased. However, anthocyanin loss can be easily determined by pigment, color analysis, and also tristimulus colorimetry. Tristimulus colorimetry in color is a valuable tool for discriminating changes in color due to both Milard reaction and anthocyanin degradation [8]. The results of periodically examined properties of the commercial fruit drinks are shown in Table 2 and Figures 10-13.

In this study, the effects of refrigerated storage on L* value (lightness index), a* value (redness index), b* values (yellowness index), H*, C*, browning index (BI), Delta E, A 420 nm (NE), polymeric color, color density, tannin contribution and total anthocyanin were statistically significant found ($p<0.01$) in stored commercial fruit drink, but it was no significant in fresh commercial fruit drink. L* values were generally decreased in commercial fruit drink during storage at refrigerator temperature, that is, the color was to clear up. The decrease in L values point to loss of the color. Loss of the color was result of anthocyanin degradation. Main and Morris obtained decrease for grape juice wines, while Rommel et al. and Pilando et al. [1,50] obtained an increase.

Until the 1th months of refrigerated storage, a* values increased and then generally decreased in commercial fruit drink. The a*-values obtained increased in commercial fruit drink samples during storage. During storage, the changes of a*- values are similar to results reported by Pilando et al. [50] however they were different from the

Fruit drink samples	Months storage periods	L*	a*	b*	C*	H*	BI	Δ E	A_{420nm} (NE)
Pear	0	28.09	-1.78	-0.36	1.82	11.43	-10.61	64.41	5.34
	1	27.65	-1.52	-0.52	1.61	18.89	-10.53	46.97	5.25
	2	25.84	-1.46	-0.91	1.72	31.93	-13.52	25.90	4.80
	3	29.09	-1.31	3.18	3.44	67.61	14.45	63.38	4.72
	4	27.43	-1.4	1.38	1.97	44.59	2.33	65.08	4.75
	5	29.9	-1.11	2.9	3.11	69.06	13.11	62.70	5.14
	6	25.57	-1.31	0.32	1.35	13.73	-4.56	66.96	4.44
Cherry	0	0.42	1.69	0.72	1.84	23.08	1041	91.75	0
	1	0.78	2.86	1.09	3.06	20.86	805	91.93	0.04
	2	0.53	2.1	0.91	2.29	23.43	1048	92.35	0
	3	0.26	1.41	0.44	1.48	17.33	955	92.15	0
	4	0.48	2.08	0.83	2.24	21.75	1034	92.06	0
	5	1.2	3.06	1.67	3.49	28.62	819	91.43	0.05
	6	1.25	2.7	1.04	2.89	21.07	458	91.36	0.1
Strawbery	0	16.74	14.4	6.82	15.93	25.34	197.8	77.58	1.73
	1	17.38	14.39	7.09	16.04	26.23	194.6	77.12	1.79
	2	14.05	13.69	7.43	15.58	28.49	248.7	77.18	1.25
	3	16.31	18.32	10.95	21.34	30.87	316.7	79.23	1.24
	4	15.94	17.13	10.04	19.86	30.37	295.6	79.29	1.32
	5	16.73	17.63	10.78	20.66	31.44	299.4	78.78	1.37
	6	17.1	16	9.51	18.61	30.73	254.6	77.90	1.53
Redgrape	0	0.76	2.08	1.3	2.45	32.0	1156	91.79	0
	1	1.76	2.24	1.7	2.81	37.2	476	90.93	0.09
	2	0.85	2.35	1.46	2.77	31.8	1163	91.33	0
	3	0.45	1.83	0.78	1.99	23.1	1056	91.97	0
	4	0.3	1.38	0.52	1.47	20.6	1022	92.22	0
	5	0.3	1.25	0.51	1.35	22.2	1019	92.26	0
	6	2.25	2.13	1.6	2.66	36.9	317	90.05	0.18

Data presented as an average of three replicated samples
(NE)=Non-enzymatic browning

Table 2: Colour characteristics and non-enzymatic browning of some commercial fruit drinks during storage at refrigerator (4°C).

results of Garzon and Wrolstad and De Rosso Veridiana Vera and Adriana Z Mercadante [51,52]. The increase of a*- value may stem from polymerization of anthocyanins and browning. The b*- values of commercial fruit drink samples stored at refrigerator temperature more increased while decreased in the commercial fruit drink samples. Rommel et al. [1,5] reported similar results. The red colour intensity also decreased during storage accompanied by the tonality changes from red to yellow colour (b*-values), as the C* and H* values decreased during the storage time (Table 2). In addition, since the L*, b*, a*, C*, H*, Delta E and BI values of commercial fruit drink indicate that anthocyanin degradation can be easily perceived by human eyes [53], commercial fruit drink showed visual colour changes approximately after 2 months of storage. Hunter hue angle, saturation index (chroma) and browning index (BI) were increased in commercial fruit drink samples, while decreased in the commercial fruit drink samples stored in refrigerator. Delta E remained almost constant in both fresh and commercial fruit drink samples, which changed due to particle precipitation, (Table 2). These results are in good agreement with those of Palou et al., De Rosso Veridiana Vera and Adriana Z Mercadante and Genovese et al. [24,52,54].

As shown in Table 2, browning could not be noticed in the commercial fruit drinks samples; whereas commercial fruit drink samples did not brown to an extent that would be objectionable. For example, the A420 nm of traditional hibiscus drink during storage for 6 months at 4°C was 5.43-4.44 in pear and strawberries compared to 0.00–0.1 in case of the commercial cherry and red grape drink samples. Crandall et al. [55] concluded that two measures of browning were used, color a* or L* and absorbance at 420 nm where the higher numbers indicate increased absorbance due to the formation of brown pigments.

The correlation coefficient of color characteristics and storage time (except non-enzymatic browning values of commercial fruit drink samples stored at refrigerator temperature) also negative. Whereas, R^2 0.80 in case of non-enzymatic browning. But during storage period of commercial fruit drink, the non-enzymatic browning values was observed that the R^2 0.73 through six months storage time, while all other values were positive.

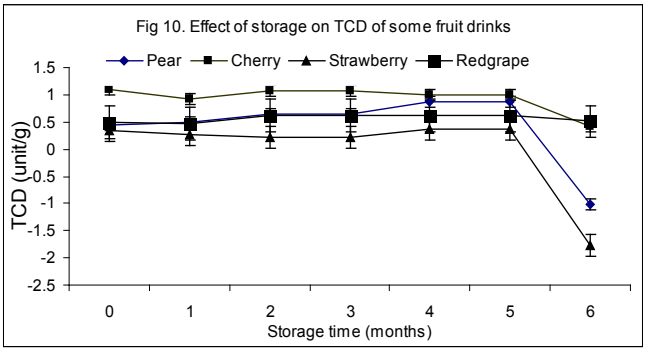

Figure 10: Pigment and anthocyanin contents of some commercial fruit drinks during storage time (months) at refrigerator (4 oC).

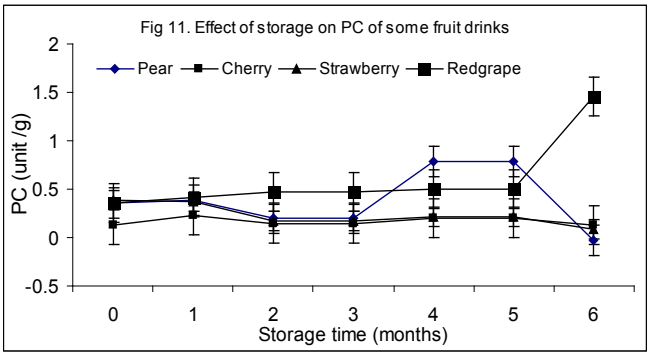

Figure 11: Pigment and anthocyanin contents of some commercial fruit drinks during storage time (months) at refrigerator (4 oC).

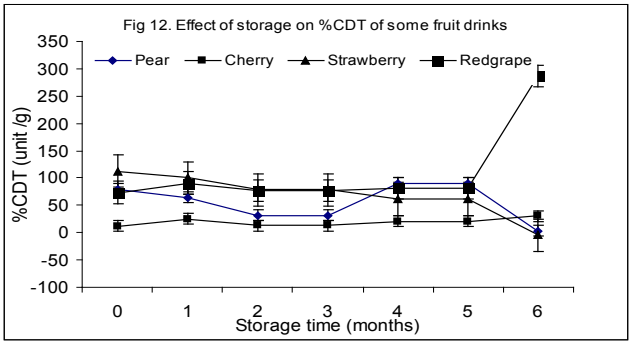

Figure 12: Pigment and anthocyanin contents of some commercial fruit drinks during storage time (months) at refrigerator (4°C).

Anthocyanin pigments, being most heat sensitive, may preferably be used as an index of food product quality. The excellent linear correlation between Hunter color parameters (a*, b*, L*, C*, H*, BI and Delta E) and anthocyanins (Table 2 and Figures 10-13) inferred that the Hunter color parameters may also be used instead of anthocyanins. The advantage of using the visual Hunter color parameters is that it may be measured as on-line quality control parameters during heating of food industry. These results are similar to results of Zhendong et al. [56]

Total anthocyanin loss was 69% and 100% for stored after 6 months in refrigerator commercial pear and strawberry fruit drink samples (Figures 10-13). This result showed the importance of storage periods on total anthocyanin retention. The total anthocyanin values decreased with increasing storage time in pear and strawberry drinks, but increased with increasing storage time in cherry and red grape drinks (Figures 10-13). This manner was also reported by several researchers [1,4-6,57,58]. These results showed that the importance of storage temperature on total anthocyanin retention. Total anthocyanin contents in commercial fruit drink samples stored at refrigerator temperature were in agreement with the findings of several researchers [1,6,9,10,59-61]. The anthocyanin loss in stored samples at refrigerator temperature was more than the results belongs to Cemeroglu and Artik and Tosun and Yuksel [57,59]. This difference may due to different composition and anthocyanin profiles.

The other color characteristics and parameters (except Delta E value of commercial fruit drink samples stored at refrigerator temperature) also decreased. But during storage time of commercial fruit drink, the anthocyanin was observed that the Delta E value decreased through six months storage time, while all other values increased. As shown in Figures 10-13, the degradation of anthocyanins in some commercial fruit drink during storage time showed high and positive correlation with C* (R^2 90.71), BI (R^2 78.03), a* (R^2 77.53) and b* (R^2 75.32), while the degradation of anthocyanins showed high and negative correlation with L* (R^2 65.91) and Delta E (R^2 56.07). These results are similar to those of Zhendong et al. [56]

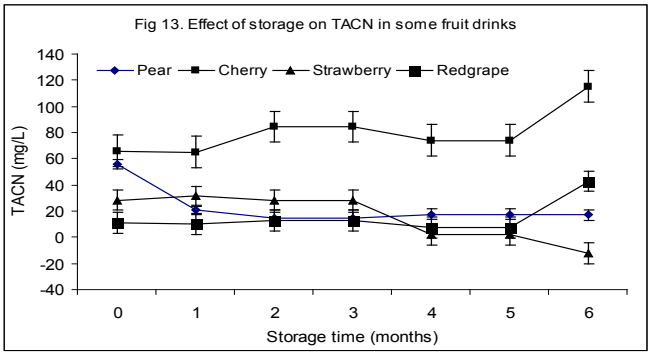

Figure 13: Pigment and anthocyanin contents of some commercial fruit drinks during storage time (months) at refrigerator (4 oC).

In this study, it was evaluated correlation coefficients between the other parameters and total anthocyanin values (Table 2 and Figures 10-13). According to the obtained result of correlation analyses, It was found that there was only positive correlation between L*, a* and b* values and total anthocyanin values in fresh commercial fruit drink, but negative in stored commercial fruit drink. The correlation coefficient was highly negative for total anthocyanin, tannin contribution and polymeric color with storage periods in fresh commercial fruit drink, but highly positive in stored commercial fruit drink. Rommel et al. [1,5] found negative correlation between total anthocyanin and L*, b*, and tannin contribution and our results agree with these reports.

Cytogenetic and biochemical evaluations of some commercial fruit drinks

The results of the cytological examination of bone marrow and spermatocyte cells of mice, ingested orally with some commercial fruit drinks like pear, cherry, strawberries and red grape (0.8 and 2.4 mg/Kg) were listed in Tables 3 and 4.

The structural aberrations induced in both types of cells were highly significant (P>0.05) in the case of the commercial fruit drink like pear, cherry and strawberries than in the commercial fruit drink like red grape. They were represented by gaps, deletion and centromeric attenuations. Following the recommendation of Brusick [62], gaps were not counted in the total aberrations. The effect of commercial fruit drink on both types of cells (Tables 3 and 4), showed that the maximum number of aberrated cells was reached after using the commercial fruit drink. Studies of numerical aberrations showed higher increase in pear, cherry and strawberries commercial fruit drink than in red grape commercial fruit drink.

All commercial fruit drinks caused a highly significant increase in chromosome aberration in both bone marrow and spermatocyte cells. Sub acute treatment caused high percentage of aberrant cells due to the accumulation effect of the commercial fruit drinks. Deletion is the main type of chromosomal aberrations in both types of examined cells (i.e. loss of the DNA content). Since DNA is considered as constant genetic component of every cell in all organs, the decrease of DNA content may be due to the presence of colorants in commercial fruit drinks, which caused cell hyperplasia or cell enlargement. This suggestion is in agreement with those found by Borzelleca and Hagan [63] who showed

Drink samples	Dose (mg/Kg body weight)	Gap	defetion	Fragment	C.fusion	aberations	normal cells	Hypop	Hyper
Control	0.8	1 ± 0.5	2 ± 0.5	10.5 ±	1 ± 0.5	50.7 ±	245 ± 0.7	0 ± 0	0 ± 0
	2.4	2 ± 0.5	1 ± 0.4	1 ± 0.4	2 ± 0.5	6 ± 1	244 ± 1	10.4 ±	0 ± 0
Pear	0.8	391.3 ±	79 ± 1.8	44 ± 1.3	54 ± 1.9	216 ± 3.5	34 ± 3.5	10.4 ±	2 ± 0.5
	2.4	34 ± 0.8	42 ± 1.1	47 ± 1.7	80 ± 3.2	203 ± 3.1	47 ± 3.1	10.4 ±	10.4 ±
Cherry	0.8	220.5 ±	45 ± 1	31 ± 1.3	35 ± 1	133 ± 1.8	117 ± 1.8	0 ± 0	10.4 ±
	2.4	88 ± 2.9	40 ± 1	53 ± 1.1	64 ± 0.8	245 ± 2.2	5 ± 2.2	60.8 ±	80.5 ±
Strawberry	0.8	180.9 ±	34 ± 0.8	25 ± 0.8	73 ± 0.4	1501.9 ±	100 ± 1.9	60.4 ±	40.4 ±
	2.4	23 ± 0.9	57 ± 2.1	62 ± 1.1	74 ± 1.6	216 ± 4	34 ± 4	2 ± 0.5	10.4 ±
Red grape	0.8	140.8 ±	290.8 ±	21 ± 0.8	16 ± 0.4	80 ± 1.9	170 ± 1.9	10.4 ±	00 ±
	2.4	16 ± 0.8	26 ± 0.4	30 ± 1	21 ± 0.8	93 ± 1.3	157 ± 1.3	00 ±	1 ± 0.4

Table 3: Effect of some commercial fruit drinks on chromosomal aberrations in bone marrow cells of mice treated with commercial fruit drinks.

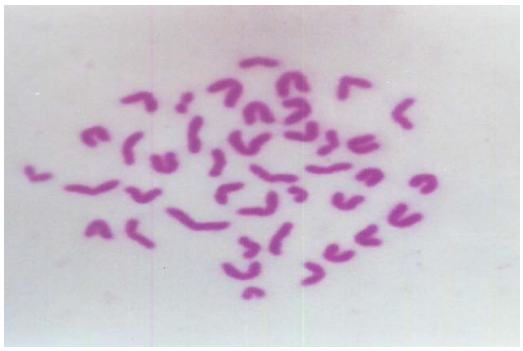

Figure 14: Normal chromosomal of bone-marrow.

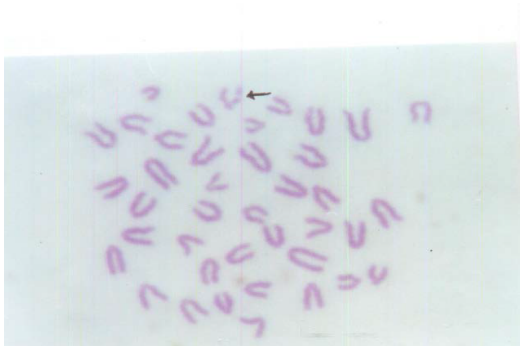

Figure 15: Gap chromosomal aberration of bone-marrow.

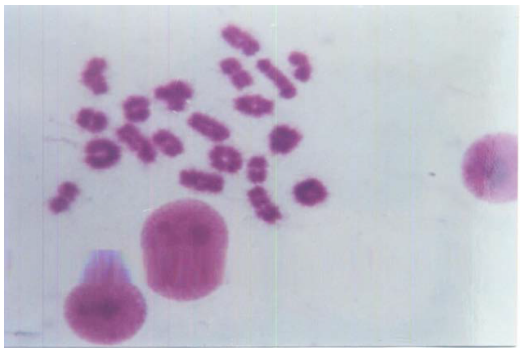

Figure 16: Norma chromosomal of spermatocytes.

that erythrosine, brilliant black and indigo carmine caused tumors in the different organs of rat and mice relative to control. Haveland and Combes [64] studied the genetic effects of 25 colorants dyes belonging to 6 major structural classes and proved their abilities to cause DNA damage and mutations in bacterial assay systems.

In this study, Figures 14-16 showed that the normal chromosomal of bone marrow and spermatocytes. Also, Figures 15 and 17 showed that the gap chromosomal aberration of bone marrow and X-Y univalent chromosomal aberration of spermatocytes.

Data presented in Table 5 show that GOT and GPT activity and glucose in both plasma and liver of all experimental mice ingested with all commercial fruit drinks was stimulated relative to control. It is of great interest however, to illustrate that among process commercial fruit drinks gave the highest and significant stimulations in the activity of plasma and liver glucose and GPT, but lowest in the activity of plasma and liver of GOT. In addition, some commercial fruit drinks ingestion slightly and non significant stimulate these enzymes in both plasma and liver like red grape. In contrast, some commercial fruit drinks like pear drinks increased the enzymes stimulation and the activity, which it was about similar to that of control mice. These results confirmed the finding of Hamama et al. and Abdel-Rahim et al. [65,66],who indicated that either the synthetic food colorants or the other commercial ones ingestion stimulated the activity of transaminases of both plasma and liver.

The present findings were in agreement with those of Abdel-Rahim et al. [66] who found that plasma and liver Glucose and GPT activity were enhanced under the induction of food colorants. The present results could be rendered to the difference in the chemical structure between the commercial fruit drinks. Consequently, it can be suggested

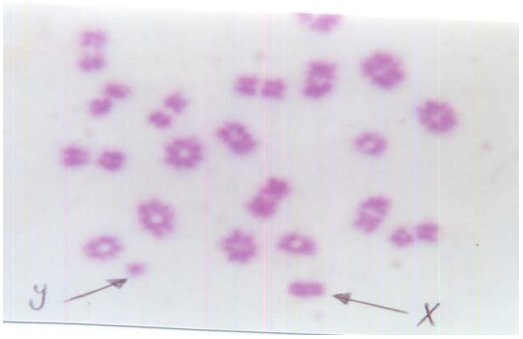

Figure 17: X-Y univalent chromosomal aberration of spermatocytes.

Drink samples	Dose (mg/ Kg body weight)	chain	autosomal	x-y	T.aberration	Normal aberration
Control	0.8	2 ± 0.5	3 ± 0.5	2 ± 0.5	7 ± 0.9	243 ± 0.9
	2.4	3 ± 0.5	2 ± 0.5	1 ± 0.4	6 ± 0.8	244 ± 0.8
Pear	0.8	7 ± 0.5	25 ± 0.7	20 ± 1	52 ± 1.5	198 ± 1.5
	2.4	18 ± 1.5	22 ± 1.3	11 ± 0.8	51 ± 2.8	199 ± 2.8
Cherry	0.8	5 ± 0	13 ± 0.5	12 ± 0.5	30 ± 0.7	220 ± 0.7
	2.4	25 ± 1.6	45 ± 0.7	6 ± 0.4	76 ± 1.3	174 ± 1.3
Strawberry	0.8	6 ± 0.4	18 ± 0.5	15 ± 0.7	39 ± 0.8	211 ± 0.8
	2.4	10 ± 0	18 ± 1.5	9 ± 0.4	37 ± 1.7	213 ± 1.7
Red grape	0.8	2 ± 0.5	7 ± 0.5	4 ± 0.4	13 ± 0.5	237 ± 0.5
	2.4	15 ± 1	36 ± 1.5	3 ± 0.5	54 ± 0.8	196 ± 0.8

Table 4: Effect of some commercial fruit drinks on chromosomal aberrations in spermatocyte cells of mice treated with commercial fruit drinks (n=5).

Drink samples	Dose (mg/Kg body weight)	GOT (µ/ml)	GPT (µ/ml)	Glucose (mg/ dL)
Control	0.8	217 ± 2.1	96 ± 2.3	84 ± 2.8
	2.4	21493.4 ±	954 ± 8.7	436 ± 4.6
Pear	0.8	13803.7 ±	462 ± 3.3	104 ± 2.2
	2.4	1930 ± 3.7	5293.1 ±	4855.7 ±
Cherry	0.8	3151.9 ±	206 ± 2.3	138 ± 1.9
	2.4	15844.6 ±	720 ± 3.3	693 ± 4.2
Strawberry	0.8	2042.2 ±	416 ± 5.4	113 ± 4
	2.4	1674 ± 4.1	489 ± 5.1	5155.7 ±
Red Grape	0.8	200 ± 4.1	128 ± 1.5	96 ± 3.1
	2.4	1071 ± 3.7	5603.7 ±	417 ± 4.2

Table 5: Effect of some commercial fruit drinks on plasma activity of GOT, GPT and glucose of mice treated with commercial fruit drinks (n=5).

that, the higher effect of commercial fruit drinks may be rendered to their uptake and accumulation by the body. Effect of some commercial fruit drinks on plasma as well as the activity of GOT, GPT and glucose of mice treated with some commercial fruit drinks (pear, cherry, strawberries and red grape) (Table 5).

Conclusion

Total anthocyanin contents of commercial fruit drinks decreased during six months of storage at refrigerator temperature of 4°C and the colors of samples changed. According to the results and statistical analysis, the degradation of anthocyanins and color parameters (a*- values, C*, H*, BI, TCD and total anthocyanin) showed positive correlation coefficient with storage time in most commercial fruit drinks, while the degradation of anthocyanins and color parameters showed negative correlation coefficient with storage time.

The obtained results showed that the most commercial fruit drinks caused a highly significant increase in structural chromosomal aberrations more than some commercial fruit drinks like red grape. GOT and GPT activity and glucose content in both plasma and liver were stimulated by commercial fruit drink, but it was higher for pear commercial drink. Also, results showed a considerable increase in all parameters compared to the control especially for commercial fruit drinks. In the present study, the histological examination of liver treated with commercial drink revealed liver tissue mostly normal. Also, pigment of commercial drink or food coloring agent has toxicological and histochemical harmful effects on liver. However, the commercial drinks with that synthetics coloring had a cumulative effect on long way after commercial drinks intake.

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