Comparative Valuation of Ultrastructural and Immunoscreening Studies on the Two Food Colorants 'E100 and E110'-Triggering Testicular and Prostate Alterations in Mouse

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Abstract: Background: The synthetic food colorant E110 (Sunset Yellow; SY) caused argument due to its toxic effects. Usage of E110 is not restricted as a food dye, but it is found in drugs just for coloring. E100 (Curcumin; CU) is a natural dye utilized as a spice and traditional medicine. **Aim of the work:** The present study was designed to evaluate the cytotoxicity of E110 and E100-triggered testicular and prostate alterations using transmission electron microscopy, weighing of prostate lobes and prostate-specific antigen (PSA) test. **Material and methods:** Thirty mice were divided into control, SY-gavage E110 (30 mg/kg b.wt./day) and CU-received E100 (37 mg/kg b.wt.) groups. **Results:** The current study showed testicular ultrastructural deteriorations of SY-group, included thickening of basal laminae and degenerative changes of germ, Sertoli, and Leydig cells. Also, a significant decrease ($P \leq 0.05$) of total prostate gland weights and three cases with a positive band on the application of PSA, indicating prostate cancer in a significant difference ($P \leq 0.05$) were recorded. But the testes of CU-group illustrated relatively normal basal laminae, germ, Sertoli and Leydig cells, besides negative results on the application of PSA, and no marked variations of total prostate weight, scoring insignificant effect ($P \geq 0.05$) in comparable to control group. **Conclusion**: The present comparative ultrastructural and immunoscreening studies emphasize that, there is a need to forbid the usage of the synthetic colorant E110 in foodstuffs and drugs due to its cytotoxic effects on the testes and prostate glands. Also, the current results reinforced the inclusion of the natural dye E100 in our daily diet due to its potential safety on the testes and prostate glands.

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1. Introduction

Reproductive toxicity has been given concern and interest in contemporary decades **(Mangelsdorf *et al.*, 2003)**, as infertility and problems related to it became the major problems in the life of couples **(Aitken, 1999)**. Statistically, 35 and 25% of infertilities are related to men and women, respectively **(Carlsen *et al.*, 1992)**. The decline in male fertility has been linked to many factors which have deleterious impacts on normal spermatogenesis and sperm production, as well as the quality of formed semen **(Baker and Aitken, 2004; Haidel *et al.*, 2008)**. Among these agents are medicines, xenobiotics and environmental toxicants **(Sikka and Wang, 2008)**.

Moreover, the food manufacturing technology has considerably changed and utilization of synthetic food colorants in the form of medley or as an individual dye has greatly increased **(Sharma *et al.*, 2008)**. The synthetic food dyes are inserted into foods to substitute natural color missed during processing, and to manufacture products with brilliant color for consumer seduction **(Tripathi *et al.*, 2007)**. The synthetic food colorant E110 (Sunset yellow; SY) is an azo dye chemically known as [disodium 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalene-6-sulfonate], and is used in food processing in many countries **(EFSA, 2009; Veena and Geeta, 2013)**. It is the most synthetic food dye which is prevalent in soft drinks, liquor and beverages for adults **(Andrade *et al.*, 2014; Botelho *et al.*, 2014; Wang *et al.*, 2014)**. Moreover, SY was detected at high concentrations in cookies, chocolates, fruit juices, ice cream, sauces, seasonings, relishes, saffron, pickles, snack chips, chutney, colored rice, gelatin- and sugar-based confectionery, besides pharmaceutical pills and medicines **(Hajimahmoodi *et al.*, 2013; Wu *et al.*, 2013; Shen *et al.*, 2014)**.

The utilization of the artificial food colors is recognized as one of the most severe problems that may induce considerable toxicological influences to human beings particularly children, who are highly attracted towards the colored foods. Synthetic colors, including SY have been linked to anaphylactic trauma, vasculitis, and angioedema, as well as thromboxane synthesis suppression in humans susceptible to their constituents. Also, they can induce dangerous allergic reactions such as nettle rash (urticaria) and can reinforce aggressive behavioral changes in children **(Sardi *et al.*, 2010; Yadav *et al.*, 2013**;

Bhattacharjee, 2014). Moreover, some recent meta-analyses proposed a combination of colorectal, brain and prostate cancers with specific dietary types **(Kravchenko, 2017**).

Many researchers studied the metabolic, physiological and toxicological disorders triggered by the administration of different synthetic food colorant additives, including SY to experimental animals **(Tanaka, 2005; Sayed *et al.*, 2012; Montaser *et al.*, 2013; Ismail, 2016)**. Yet, there is limited work evaluating the ultra-cytotoxicity and immune-screening assays of the synthetic food dye "SY" on the testes and prostate glands of the mouse reproductive system.

Due to toxicological consequences linked to the utilization of artificial food coloring substances and growing consciousness concerning natural foods, an increased attention to the use of natural food additives like food preservatives and antioxidants has been reported **(Peschel *et al.*, 2006)**. In such context, the natural food supplement E100; curcumin (CU) is a polyphenol extracted from the turmeric rhizomes of *Curcuma longa* L. **(Sharma *et al.*, 2005; Bengmark *et al.*, 2009)**, and it is utilized as a condiment to give certain relish and yellow color to Curry **(Pari *et al.*, 2008)**. CU offers hope for the development of a safe alternative crucial natural food colorant instead of the synthetic food dye "SY". Also, CU has gained a great attention from numerous scientists around the world due to its numerous biological efficiencies including; anti-inflammatory, antioxidant, antiviral, antifungal, anticancer, antiangiogenic, antiproliferative, and chemopreventive activities that can be used in vast prospective applications for the treatment of several inflammatory and malignant diseases, as well as viral and fungal infections **(Weber *et al.*, 2005; Nonn *et al.*, 2007; Aggarwal and Harikumar, 2009; Moghadamtousi *et al.*, 2014; Siviero *et al.*, 2015)**. Also, preclinical studies of CU in animals revealed that 2% dietary CU (equal 1.2 g/kg b.wt.) given for 14 days to rat **(Sharma *et al.*, 2001)** or 0.2% dietary CU (equal 300 mg/kg b.wt.) administered for 14 weeks to mice **(Perkins *et al.*, 2002)** had no toxic effects.

From the previous introductory remarks, the current study was undertaken to investigate the cytotoxicity of the food colorants; the synthetic food dye E110 (SY) and the natural food color E100 (CU) on the testes of the male albino mice using the transmission electron microscopy (TEM), and also to evaluate both E110- and E100-induced prostate cancer using the prostate-specific antigen (PSA) immunoscreening test.

2. Material and Methods

2.1. Animal protocol

Thirty adult male Swiss albino mice (*Mus musculus*) ranging in weight from 24 to 26 g (~10 weeks) were used. They were purchased from the animal house of Theodor Bilharz Research Institute (TBRI), El-Giza, Egypt. Mice were preserved in clean plastic cages in a room with a relative humidity of 55 ± 5%, a temperature of 25 ± 2°C, and a 12-h light/dark cycle during the period of the study. Mice were fed with rodent pellets and water *ad-libitum*. They were left for one week prior the commencement of the experiment for adaptation and all the experimental protocol were performed taking into consideration the ethical and scientific protocols recommended by **Ferdowsian and Beck (2011)**.

2.2. Applied chemicals and dosages

Two food colorants were utilized in the current study; the synthetic dye E110 "SY" and the natural supplement E100 "CU". The artificial food colorant "SY" had a Code No. E110, and was also renowned as yellow No. 6 **(NTP, 1981)**. It was authorized as a food additive and valuated by the committees **JECFA (1982)** and **SCF (1984)** for establishing the acceptable daily intake (ADI) and marking of it. SY was purchased from El-Gomhouria Co. (Cairo, Egypt), and was orally given to the mouse at a dosage of 30 mg/kg b.wt./day.

The natural food colorant "CU" powder (Code No. E100) has been predestined by **JECFA (2004)** and **EFSA (2010)** to define its safety as a food colorant and to determine its ADI. It was obtained from Faculty of Agriculture, Ain Shams University, Cairo, Egypt, and was orally given to the mouse at a dosage of 37 mg/kg b.wt./day.

The selected doses for both food additives (SY and CU) were calculated according to the formula of dose conversion of ADIs **(Reagan-Shaw *et al.*, 2008)** from human to mouse dosages, depending upon the amended body surface area of the mouse (0.007 m^2).

2.3. Experimental design

The mice were divided randomly into three equal groups. **Group I** (Control group): Mice were orally fed with 1 ml/25 g b.wt./day drink water by gastric tube for 60 days parallel to the treated groups. **Group II** (SY-group): Mice were orally given E110 (SY) (30 mg/kg b.wt./day) dissolved in drink water (1 ml /25 g b.wt./day) by gastric tube for 60 days, and **Group III** (CU-group): Mice were orally gavage with E100 (CU) at a dose of 37 mg/kg b.wt. suspended in drink water (1 ml /25 g b.wt./daily) for 60 days.

Both control and treated-mice were fasted overnight at the final day of the experiment and then anaesthetized under light ether anesthesia. They were dissected and their left testes were removed and processed for ultrastructural preparation. Also, the whole prostates (ventral-anterior and dorsolateral lobes) were rapidly excised and weighed according to

the basic anatomical guideline by **Oliveira *et al.* (2016)**. The sera of withdrawal tail tip blood were used in immunoscreening test for PSA. Each sample of prostate-specific antigen (PSA) assay was repeated for three times at least for sure.

2.4. Ultrastructural preparations

Freshly removed testes were cut into small pieces, fixed rapidly in cold 4F1G (4% formalin + 1% glutaraldehyde, adjusted at pH 2.2) for 24 hours, and then post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer that adjusted at pH 7.3. Directly, the specimens were processed for the normal protocol of the ultrastructural investigation by TEM as explained by **Dykstra *et al.* (2002)**. Finally, the stained grids were examined and photographed by JEOL. JEM-1400-EX-ELECTRON MICROSCOPE at Electron Microscopy Department of TBRI, El-Giza, Egypt.

2.5. PSA assay

Rapid Screen Test Cassette is used to detect the prostate-specific antigen (PSA) in prostate cancer cases. This test is a direct, immediate, extremely precise and specific biosensor for the revelation of cancer biomarkers. Therefore, it is eligible in the early diagnosis and prediction of cancer **(Lu *et al.*, 2015)**. The cassette style PSA rapid screen test is a chromatographic immunoassay *in vitro* diagnostic test for the qualitative disclosure of PSA tumor markers in the serum. It is provided from Distinct, Rapid Test Devices, Germonizer, Premier Distributor & Dealer of CBRN products, for *in vitro* diagnostic use.

PSA is the most ordinary tested tumor biomarker for the diagnosis of prostate gland diseases **(Ottawa, 2013)**. The test procedure is performed as the following steps: Removing the test cassette and disposable pipette, then placing the test cassette on a flat surface with the test window and sample well facing up. Clean the tail tip of the mouse with an alcohol swab. After 30-45 seconds, prick tail tip with the sterilized scalpel to get a large or "hanging" drops of blood, and obtain the serum by the pipette. Wait 30 seconds until the sample was absorbed completely, and then add two drops of the PSA buffer solution to the specimen well, observing the emigration of the test sample across the test window. The interpretation of results is the following: Potent positive results represent as a burgundy colored test (T) band in the test (T) region can be seen within 2-3 minutes, whereas weak positive results take a longer time that can rise to 10 minutes. If the T-region is positive, the time elapsed prior the T-band observed is an indication of how concentrated beyond 4 ng/ml PSA is in the system, as showing in Figure 1.

Fig. 1. Revealing the interpretation of the prostate-specific antigen (PSA) Rapid Screen Test Cassette to detect the PSA in both E100- and E110-induced prostate cancer in mice as the three following cases; negative, positive or invalid result of diseased by the prostate cancer:

Negative	**Positive 4ng/ml**	**Invalid**
No line appears in the T-region during 8-10 min. Absence of a T-band illustrates that PSA levels are less than 4 ng/ml.	A T-band evolves in the T-region. This line may be weaker or stronger than the control (C) line indicating a positive result where PSA level is 4 ng/ml^{+}. Any line develops in T-region, whatever how faint is considered a positive result.	Absence of a C-line denotes an invalid test. Causes are insufficient sample or buffer and ⁄or user error. No test interpretation should be made and it must repeat the test.

2.6. Statistical analysis

Statistical analysis of the data, which reported as means and Std. Deviation (±SD) of both immunoscreening test for PSA and whole prostate weights were estimated by *t*-test, SPSS statistics 17.0. Differences were considered significant when $P \leq 0.05$ and were used to compare the measurements recorded for each treatment with the respective control.

3. Results

3.1. Ultrastructural results

3.1.1 Group I: Control group

Transmission electron microscopic examination showed a normal fine structural architecture of the testes of control mice with germ, Sertoli and Leydig cells revealing cellular characteristics similar to those observed in active spermatogenesis (Figs. 2A - 2F). The spermatogonia rest upon the basal lamina of the seminiferous tubule. They possess scattered mitochondria, lysosomes, few stacks of rough endoplasmic reticulum (RER), cisternae of smooth endoplasmic reticulum (SER) and nuclei with one or two nucleoli and homogeneous nucleoplasm (Fig. 2A). The primary spermatocytes are large spherical cells containing granular cytoplasm with few mitochondria, RER and SER. They also possess prominent spherical nuclei which have nucleoli and homogeneous chromatin materials (Fig. 2B). Spermatids appear in varied forms during spermatogenesis as revealed in Figures (2C & 2D); they are small ovoid or spherical in shape with ovoid or spherical nuclei which possess finely granular chromatin materials. Their cytoplasm contain few stacks of RER, cisternae of SER, vacuolated mitochondria, lysosomes and well-developed Golgi apparatus which forms the acrosomal cap that adhered to the anterior pole of the nucleus. Sertoli cells exhibit distinguished nuclei and cytoplasmic characteristics proportionate with an active secretory state. As seen in Figure (2E), Sertoli cells rest upon the basal lamina of the seminiferous tubule, prolong towards the lumen and possess irregular nuclei containing prominent nucleoli, homogeneous chromatin materials and surrounded by nuclear envelopes exhibited deep indentations. Their cytoplasm characterized by the presence of mitochondria with tubular cristae, cisternae of SER, free ribosomes, lysosomes, and few phagosomal bodies. The interstitial tissues revealed normal Leydig cells being oval in shape with their cytoplasm contain mitochondria, lysosomes, and SER, as well as lipid droplets. Each cell has a nucleus formed of a prominent nucleolus, euchromatin and an intense rim of heterochromatin adhered to the inner surface of its nuclear envelope (Fig. 2F).

3.1.2. Group II: SY-Group

The electron micrographs of the testes of mice treated with SY revealed severe and extensive fine structural alterations (Figs. 3A - 3F). The basal laminae of the boundary tissues of several seminiferous tubules appeared irregular and thickened (Figs. 3A, 3E & 3F). Most of the spermatogenic cells, including spermatogonia, primary spermatocytes and spermatids manifested with altered structures. Spermatogonia lost their regular architecture, being star or irregular in shape with their nuclei showing irregular nuclear envelopes and condensed chromatin materials. Their cytoplasm appeared with electron-dense mitochondria and fragmented cisternae of SER (Fig. 3A). Accordingly, the intercellular spaces between these altered spermatogonia were dilated as seen in Figure (3A). The primary spermatocytes decreased in size, revealed distinctly irregular contours and possessed vacuolated mitochondria in their cytoplasm and condensed chromatin materials in their nuclei which surrounded with irregular nuclear membranes (Fig. 3B). As illustrated in Figures (3C & 3D), the spermatids appeared distinctly deteriorated with irregular contours and possessed deformed nuclei with fragmented chromatin materials. Their cytoplasm exhibited electron-dense mitochondria, fragmented RER and SER, lipid droplets, lysosomes, condensed acrosomal granules and acrosomal caps covered the anterior hemisphere of their nuclei.

Marked alterations in Sertoli cells were seen in the testes of mice treated with SY. As seen in Figure (3E), Sertoli cells were decreased in size. Their cytoplasm showed degenerative mitochondria, fragmented SER, and lysosomes. Irregularly-shaped nuclei with highly indented nuclear envelopes and electron-dense chromatin bodies were also observed. The integrity of the tight junctional complexes between Sertoli cells was detached giving rise to increased irregular spaces between the distorted spermatogenic cells.

The interstitial tissues of testes of SY-treated mice exhibited depletion in the number of Leydig cells, most of them being decreased in size and characterized by increased number of lipid droplets, electron dense mitochondria, many phagosomal bodies, SER disappearance, and pyknotic nuclei (Fig. 3F).

3.1.3. Group III: CU-Group

Examination of testicular specimens from mice treated with CU revealed relatively normal fine structural characteristics as seen in Figures (4A - 4F). The basal laminae of the seminiferous tubules, as well as Sertoli, spermatogenic and Leydig cells appeared similar to those seen in control mice, except for some abnormalities. The irregularity and thickening of the basal laminae of some tubules were seen (Figs. 4A & 4B). Spermatids showed well-defined nuclear and cytoplasmic configuration (Figs. 4C & 4D). The integrity of the tight junctional complexes between Sertoli cells was maintained (Fig. 4E). Furthermore, few Leydig cells with irregular nuclear membrane contours and deformed mitochondria were observed in this group (Fig. 4F).

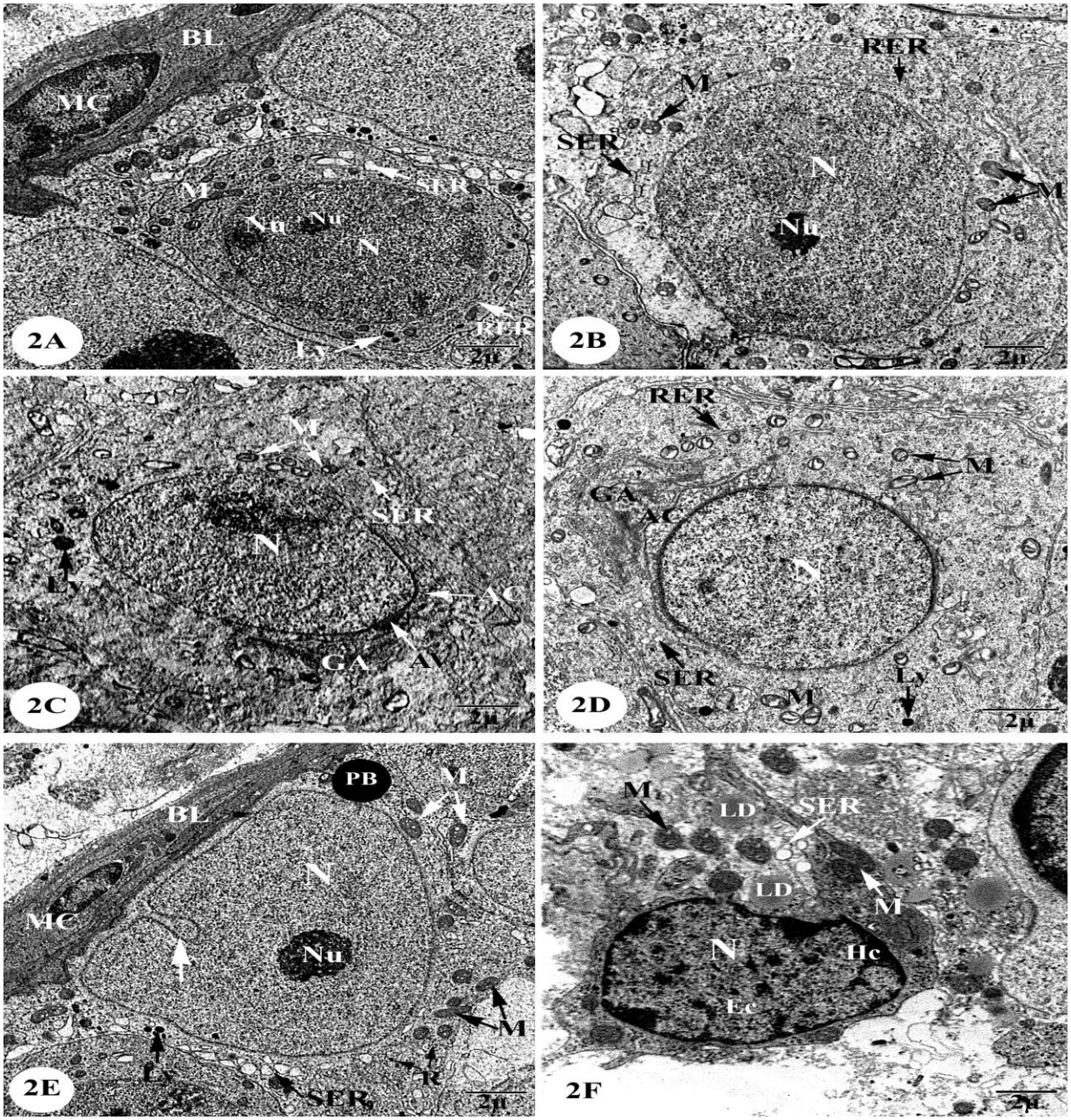

Fig. 2. Electron micrographs of testes of control mice showing: **(A)** Myoid cell (MC), spermatogonium rests upon thin basal lamina (BL) and possesses mitochondria (M), lysosomes (Ly), RER, SER, and a spherical nucleus (N) with double nucleoli (Nu). **(B)** Primary spermatocyte has a large rounded nucleus (N) with a finely granular nucleoplasm and a nucleolus (Nu), and the cytoplasm contains rounded mitochondria (M) and few elements of RER and SER. **(C & D)** Spermatids have acrosomal granule (AG) and acrosomal cap (AC) cover the anterior hemisphere of the nucleus (N). The cytoplasm contains relatively vacuolated mitochondria (M), few stacks of RER, cisternae of SER, Golgi apparatus (GA) and lysosomes (Ly). **(E)** Sertoli cell possesses an irregularly shaped nucleus (N) with a deep indentation (arrow) and a prominent nucleolus (Nu). The cytoplasm contains mitochondria (M), SER, free ribosomes (R), lysosomes (Ly) and phagosomal bodies (PB). **(F)** Leydig cell possesses a relatively extended nucleus (N) having clumps of heterochromatin (Hc) and fine granulated euchromatin (Ec), besides the cytoplasm reveals several lipid droplets (LD), mitochondria (M) and SER.

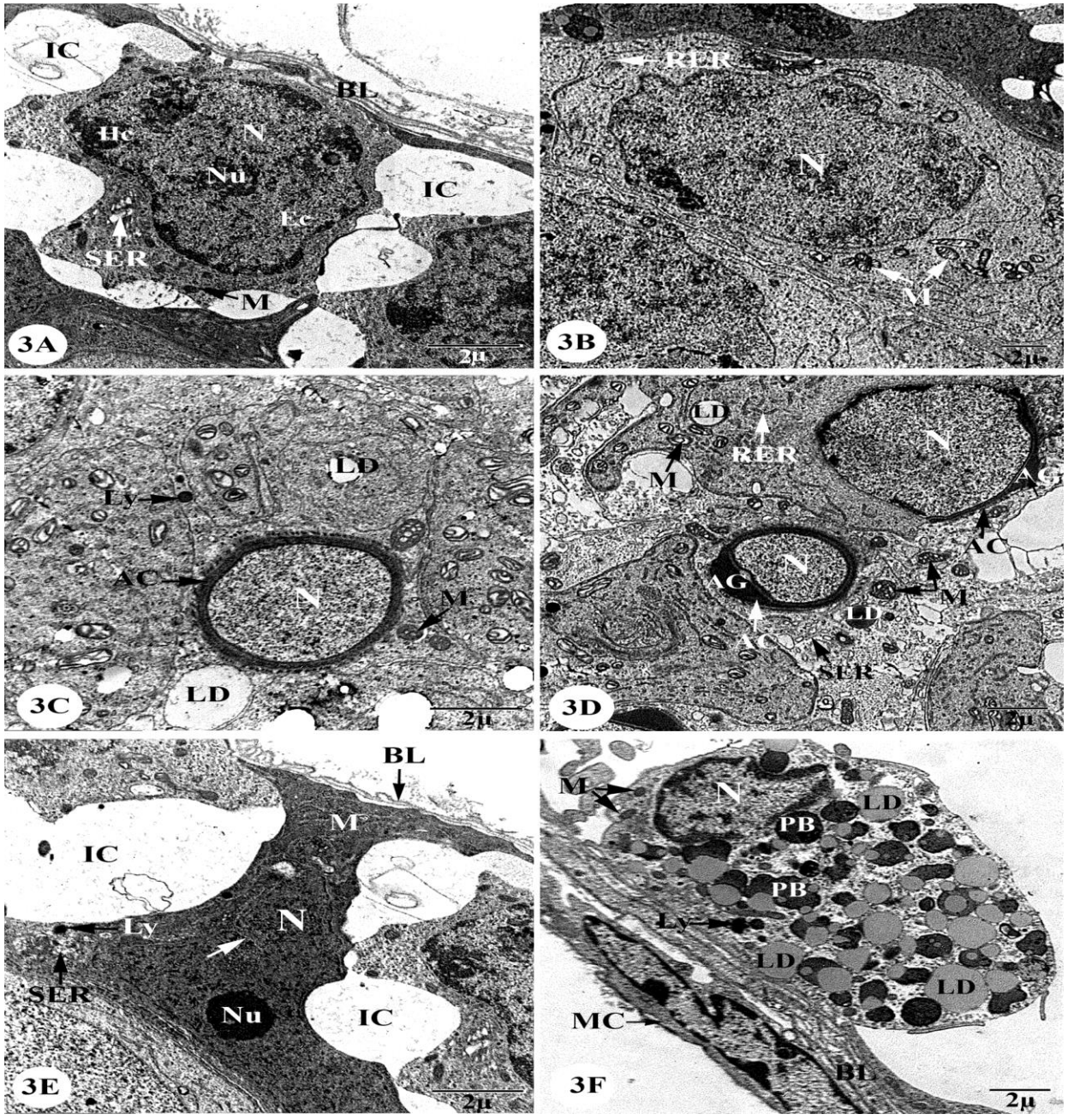

Fig. 3. Electron micrographs of testes of SY-treated mice showing: **(A)** An irregular or star-shaped deformed spermatogonium with irregular basal lamina (BL), tortuous nucleus (N) has nucleolus (Nu), electron-dense heterochromatin (Hc) and euchromatin (Ec), besides electron-dense mitochondria (M) and fragmented cisternae of SER in its cytoplasm. Dilated intercellular spaces (IC) are also seen. **(B)** A deteriorated primary spermatocyte has a distinctly serrated nucleus (N), vacuolated mitochondria (M) and fragmented RER. **(C & D)** Deteriorated configured spermatids appear with distinctly irregular and deformed nuclei (N) and their cytoplasm exhibiting condensed acrosomal granules (AG), acrosomal caps (AC), electron-dense mitochondria (M), lipid droplets (LD), lysosomes (Ly), fragmented RER and SER. **(E)** A degenerated Sertoli cell contains dense corrugated-shaped nucleus (N) with dense nucleolus (Nu) and nuclear indentation (arrow), deformed mitochondria (M), fragmented SER and intensive lysosomes (Ly). Dilated intercellular spaces (IC) and ruptured basal lamina (BL) are also markedly detected. **(F)** A deformed Leydig cell reveals pyknotic nucleus (N), numerous lipid droplets (LD), phagosomal bodies (PB), electron-dense mitochondria (M), and intensive lysosomes (Ly). Thickened basal lamina (BL) with a tucked Myoid cell (MC) is also observed.

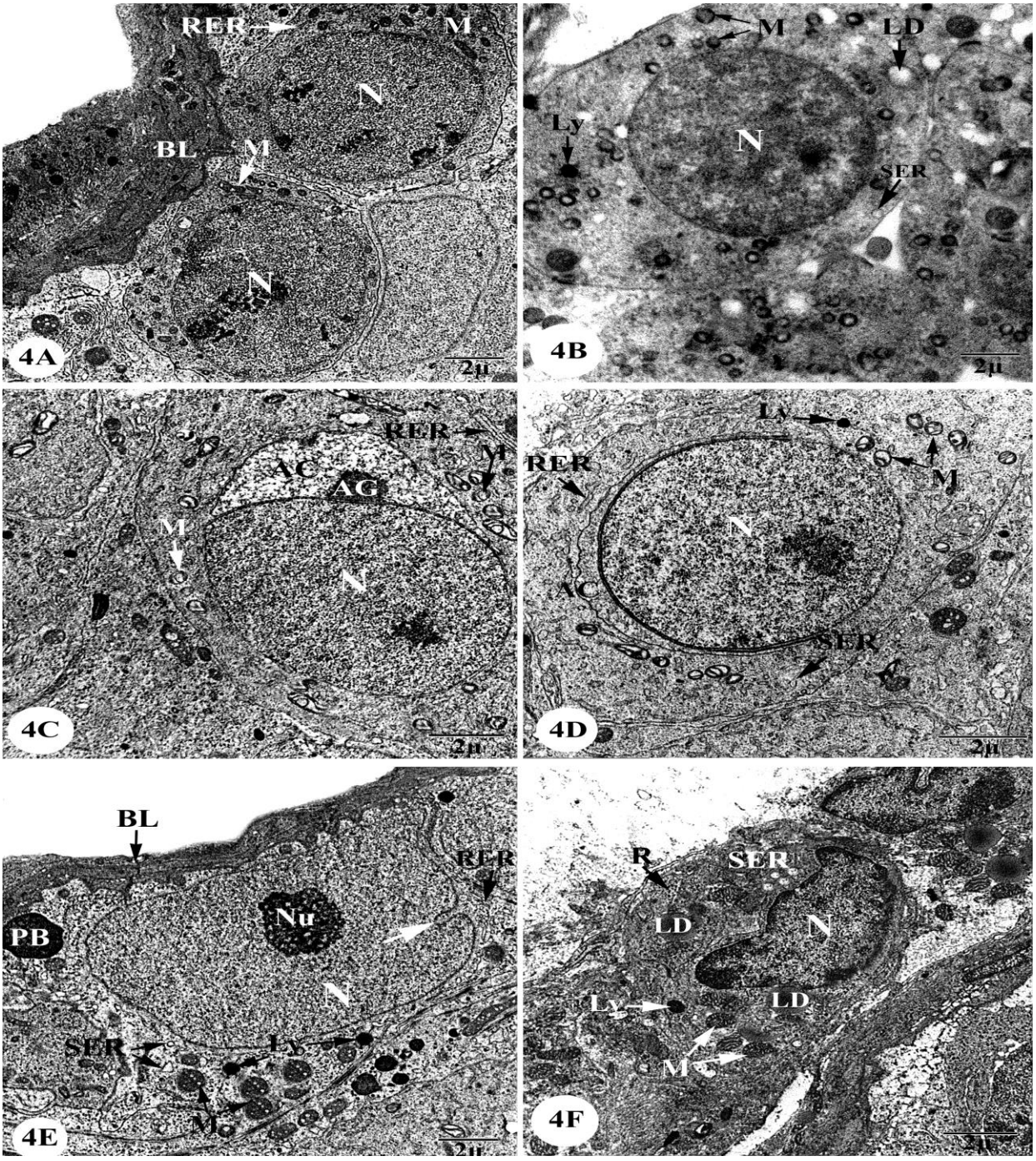

Fig. 4. Electron micrographs of testes of CU-treated mice showing: **(A)** Two normal spermatogonia rest on a thick basal lamina (BL) and contain spherical nuclei (N), mitochondria (M) and RER. **(B)** Nearly normal primary spermatocyte with large rounded nucleus (N), vacuolated mitochondria (M), lysosomes (Ly) and few lipid droplets (Li). **(C & D)** Normal spermatids with acrosomal granule (AG) and acrosomal cap (AC) cover the anterior hemisphere of the nucleus (N). Vacuolated mitochondria (M), few stacks of RER, cisternae of SER and lysosomes (Ly) are seen in the cytoplasm. **(E)** Intact Sertoli cell rest upon a thin basal lamina (BL) and possesses its characteristic shaped nucleus (N) with a nuclear indentation (arrow) and a prominent nucleolus (Nu), mitochondria (M), RER and SER, lysosomes (Ly) and phagosomal bodies (PB). **(F)** A Leydig cell with a relatively irregular nucleus (N), mitochondria (M), free ribosomes (R), many lipid droplets (LD) and SER.

3.2. Immunoscreening test for PSA

The present results of the application of PSA rapid screen test cassette to detect PSA in prostate cancer cases in both E100- and E110-induced prostate cancer of mice exhibited that all controls$^{n=10}$ and CU (E100)-orally fed mice$^{n=10}$ gave a negative results (Fig. 1 & Table 1). But in the cases of SY (E110)-orally fed mice$^{n=10}$, three only diseased animals were recorded which were evidenced by positive bands in the PSA immunoscreening test (Fig. 1 & Table 1), indicating that just three animals from total ten mice were diseased with prostate cancer. However, such group (SY-treated mice) manifesting a mean (±SD)$^{n=10}$ of 0.3 (±0.483) was a statistically significant increase (P = 0.033, i.e., $P \leq 0.05$) as detected in Table 1.

The current results of weights of the total prostate glands (ventral-anterior & dorsolateral lobes) of male mice ingested with the synthetic food dye E110 (SY) recorded a statistically significant decrease ($P \leq 0.05$) as detected in Table 2. But in the administration of the natural food colorant E100 (CU), they have not manifested any marked alterations in weights of the total prostate glands, scoring insignificant effect ($P \geq 0.05$), in comparable with their respective control ones (Table 2).

Table 1. Means and Std. Deviation (±SD)$^{n=10}$ of positive immunoscreening test for prostate-specific antigen (PSA)$^{+}$ for male mice orally fed with the equivalent ADI dose of either 30 mg/kg b.wt. of Sunset yellow-E110 (SY-group) or with 37 mg/kg b.wt. of curcumin-E100 (CU-group) daily for 60 days, and their respective control group.

Experimental Animal Groups (10 Mice / each group)	**Mean (±SD)$^{n=10}$ of Positive PSA^{+} Rapid Screen Test Cassette**	***P*- values**
Control-Group	**0.0 ± 0.0**	**-**
SY-Group (fed on E110)	**0.3 ± 0.483**	**0.033***
CU-Group (fed on E100)	**0.0 ± 0.0**	**-**

Level of significance:
$P \geq 0.05$ is a statistically non-significant. *$P \leq 0.05$ is a statistically significant.

Table 2. Weights, Means, and Std. Deviation (±SD)$^{n=10}$ of whole prostate glands (ventral-anterior & dorsolateral lobes) of male mice (aged ~18 weeks) oral gavage with the equivalent ADI dose of either 30 mg/kg b.wt. of Sunset yellow-E110 (SY-group) or with 37 mg/kg b.wt. of curcumin-E100 (CU-group) daily for 60 days, and their respective controls.

Measured Criteria	**Experimental Animal Groups**		
	Control-Group	**SY-Group (fed on E110)**	**CU-Group (fed on E100)**
Weights & Means (± SD) of prostate glands (mg) in 10 mice / each experimental group	(49, 53, 50, 51, 48, 47, 51, 48, 52, 49) **49.8 ± 1.93**	(25, 65^, 24, 29, 72^, 21, 26, 23, 68^, 27) **38 ± 21.10**	(46, 48, 47, 52, 50, 51, 49, 48, 47, 53) **49.1 ±2.33**
P-values	**-**	**0.047*↓**	**0.23**

Level of significance:
$P \geq 0.05$ is a statistically non-significant. ***$P \leq 0.05$ is a statistically significant.
↓ represents a significant decrease in weight.
^Corresponds to the three detected positive specimens of prostate-specific antigen (PSA)$^{+}$ in E110-induced prostate cancers in male mice.

4. Discussion

Recently, the synthetic food colorant SY (E-110) has caused argument as to its toxic effects. SY was reported to have mutagenic and carcinogenic effects in mammals **(Feng *et al.*, 2012)**. Usage of E-110 is not limited only as a food additive dye, but it is found out in several human drugs merely for coloring, as in effervescent multi-vitamin powders and tablets **(Ottó *et al.*, 2005)**. Also, E-110 is found in 15% of the studied pharmaceutical excipients and the information on drug labels of cough and cold, mucoregulatory, analgesic/antipyretic, antihistamine, bronchodilator, antimicrobial, corticosteroids, anti-inflammatory and diabetic medications, with disregarding and inaccuracy of its adverse reactions **(Balbani *et al.*, 2006)**.

On the other hand, CU (E100) is an active yellow-colored pigment obtained from turmeric roots.

It has been utilized for many years as a condiment and traditional medicine in Africa and Asia. CU is also used as an antiseptic, anti-inflammatory, antioxidant, hypocholesterolemic and anticarcinogenic agents **(Maheshwari *et al.*, 2006; Aggarwal and Harikumar, 2009)**.

Both food additives "E110 and E100" have almost the same texture and coloring when dissolved. Whilst, **Chuah *et al.* (2014)** reinforced the bioefficacy and bioavailability of an amorphous solid dispersion of CU to provide it as a more powerful, effective formula helping in masking the taste, color, and smell that actually limit its application as a functional food additive. Therefore, the present study aimed to find a safe alternative colorant to the synthetic dye "SY", by using a natural additive such as CU as a beverage, food or drug colorant to eschew its cytotoxicity on the male reproductive system. Therefore, the current study had been designed to focus the light on the ultrastructural and immunoscreening comparative evaluation between the two food colorants E100 and E110-induced testicular-prostate alterations in adult male albino mice.

The testis is the most significant organ in the male reproductive system since it performs two important functions; the synthesis and secretion of steroid hormones, as well as the formation of spermatozoa **(Carreau *et al.*, 2002)**. Complicated interactions between estrogen and androgen regulate prostatic development and physiology **(García-Flórez *et al.*, 2005)**. The circulating androgens are dihydrotestosterone (DHT) and testosterone which is greatly synthesized (~95%) by the testis and fewer (~5%) by the adrenal gland **(Coffey, 1992)**.

Prostate-specific antigen (PSA), biochemically, is a 33 kDa glycoprotein belonging to the kallikrein family of proteases, a subgroup of serine proteases. It is formed by the epithelial cells that line the prostate gland and is concentrated in the prostatic tissue **(Onozawa *et al.*, 2001)**. PSA is secreted by the prostate in the semen where its function is to liquefy the semen post ejaculation **(Balk *et al.*, 2003**). The majority of PSA produced by the prostate gland is transported out from the body in the semen, while an extremely few amount escapes into the blood stream, approximately (4.0–10.0 ng/ml) of PSA is normally found in the blood **(Chen *et al.*, 2015)**. Disruption of the normal prostatic structure via numerous prostatic diseases permits major amounts of PSA to get in the general circulation. Elevated serum PSA level has been affirmed as a useful tumor marker for prostate cancer in man and rats **(Onozawa *et al.*, 2001**).

In more confirmation, **Nna (2013)** reported that many candidate biomarkers for diagnosis of prostate cancer have been investigated, but PSA testing remains the frontline test and it is cost-effective, analytically reliable, and flexibly high throughput for both mass screening and individual clinical testing. Considering that PSA testing is required as a part of a screening battery, when the test performance of positive predictive value with a cutoff figure of 4.0 ng/ml, in particular, appeared rather low <30% **(Tokudome *et al.*, 2016)**. So, the present study used PSA rapid screen test cassette to detect the PSA in prostate cancer cases to evaluate both E100- and E110-triggered prostate cancer in male mice. Furthermore, the mouse is an excellent model for studying the prostate and has been used as a surrogate for finding new medications to treat human patients suffered from prostate cancer **(Taft *et al.*, 2006; Valkenburg *et al.*, 2016)**.

The results of the current investigation showed several ultrastructural changes of the testicular tissues including, irregularity and thickening of the basal laminae and the boundary peritubular tissues, as well as degenerative changes of germ, Sertoli and Leydig cells. The basal lamina performs a vital role in maintaining transportation of substances between spermatogenic epithelia and the interstitial tissue, and in maintaining the structural and functional integrity of the tissues **(Richardson *et al.*, 1998)**. Interactions between different cells; Sertoli, germ, Leydig and peritubular Myoid cells are essential for keeping normal spermatogenesis. These interactions are communicated through the extracellular matrix of the basal lamina. **Dobashi *et al.* (2003)** reported that type IV collagen is the essential component of mammalian basal laminae which is produced by Sertoli and Myoid cells. The observations of many authors in their researches verified that over expression of the subtype IV collagen is related to thickening of the basement membrane which correlates with spermatogenic dysfunction occurred in human and other mammals **(Hager *et al.*, 2005; Mattias *et al.*, 2005)**. Severe functional disruption of the testis has been correlated with alteration of the architecture of basal lamina. This deterioration may subsequently influence transportation of nutrition, oxygen, metabolites and hormone **(Zheng *et al.*, 2008)**.

Sertoli cell damage was evident post treatment with SY. Widened intercellular spaces and absence of contact between spermatogenic cells appeared as a result of Sertoli cell disruption. This ultimately caused loss of germ cells, devastation of testicular tissues and finally infertility as reported by **Monsees *et al.* (2002)**. Sertoli cells initiated the development of germ cells and maintained their viability via secreting nutritive and hormonal agents into the blood-testis barrier; a specialized compartment formed by the tight junctions between the neighboring germ and Sertoli cells **(Richburg, 2000; Sawada and Esaki, 2003)**. Also, **Mesbah *et al.* (2008)** reported that Sertoli cells play a

vital role in maintaining spermatogenesis which is a complicated dynamic process leading to the continual formation of spermatozoa since Sertoli cells are responsible for orchestrating the germ cells along the consecutive phases of mitosis, meiosis, and differentiation.

Different defects including shrinkage and lyses of spermatogenic cells were also illustrated in SY-treated mice in the current study. Spermatogonia lost their normal appearance, revealing features of necrotic cells. Also, primary spermatocytes and spermatids decreased in size. Abnormal acrosomal structures in spermatids were also observed. **Sepaniak *et al.* (2006)** reported that germ cells are more vulnerable to toxic substances than Leydig and Sertoli cells due to their rapid cell division. As described by **De Rooij and Russell (2000)**, spermatogonia are susceptible to toxic substances due to their mitotic activity, since spermatogenesis is started with spermatogonia, then the population of germ cells is greatly increased as one spermatogonium approximately goes through 8 to 9 divisions before differentiation into spermatocytes. So, defects of spermatogonia are reflected in the development of the subsequent cells of spermatogenesis.

In the current investigation, the observed changes in spermatocytes and spermatids following SY administration could be attributed to the disturbance in the micro-environment of Sertoli cells, which influences the protein synthesis machinery important to germ cell differentiation. Since proteins are secreted at their highest levels in the testis during spermatid elongation and spermiation **(Manivannan *et al.*, 2009)**.

Leydig cells were also affected post-SY-treatment. They exerted remarkable depletion in size and number, with increased lipid droplets inside their cytoplasm. Such abnormalities occurred in Leydig cells may be related to the intimate relationship between these cells and blood vessels, which put Leydig cells under hazards of any exogenous toxicants causing spermatogenesis arrest due to the reduction of testosterone secretion as reported by **Spaliviero *et al.* (2004)**.

One of the most important ultrastructural alterations found in almost all spermatogenic, Sertoli and Leydig cells was the mitochondrial degeneration. Such degeneration of mitochondria was found to be more sensitive to the formation of reactive oxygen species (ROS) that impair the antioxidant system after treatment with SY. The imbalance between ROS formation and ROS removal by the antioxidant system can induce oxidative stress in the tissues **(Kelly, 2003; Turner and Lysiak, 2008)**. Therefore, such oxidative stress produced by SY impairs testicular structure and function and has effects on sperm morphology, motility and count. So, it is important to increase the antioxidant capacity of the testis against oxidative damage because the testis possesses high metabolic activity and cell replication **(Cocuzza *et al.*, 2007; Turner and Lysiak, 2008)**.

On the other hand, electron microscopic examinations of the testes of CU-treated mice illustrated that the basal laminae, seminiferous epithelium and Leydig cells appeared relatively normal in comparable with those of the control mice.

Also, the obtained results of the current study exhibited that all control and CU (E100)-orally fed mice gave negative results on the application of PSA rapid screen test cassette. But, SY (E110)-orally fed mice scored three only diseased cases in a significant increase ($P \leq 0.05$), indicating that just three mice from total ten animals were diseased with prostate cancer. In addition, the present investigation of weights of the total prostate glands (ventral-anterior & dorsolateral lobes) of male mice ingested with the E110 (SY) recorded a statistically significant decrease ($P \leq 0.05$), but in the administration of E100 (CU), they were not manifested any marked alterations of weights of the total prostate glands, scoring insignificant effect ($P \geq 0.05$), in comparable with their respective control ones. In this concern, **Ismail (2016)** recorded a significant decrease ($P \leq 0.05$) in the testes weights of mice post treatment with E110 (SY-group), and a non-significant difference ($P \geq 0.05$) in the set of E100 (CU-group) in comparable to their respective control group. In the same manner, the same author also found that the oral feeding with E110 for 60 days caused a significant decrease ($P \leq 0.05$) in the level of testosterone in serum compared with the control group after application of the analysis with testosterone rat/mouse ELISA. Such phenomenon may be the main cause of weight deficiency for whole-prostate glands of mice in the present investigation. Whilst, the other three specimens in the present study, were detected as a positive criterion of PSA^{+} of that E110-induced prostate cancers in male mice were corresponded to a markedly increase in the weight of E110-prostate glands. Prostate cancer represents the most considerably diagnosed non-cutaneous malignancy and the major cause of cancer mortality in men **(Ramalingam *et al.*, 2017)**.

Parallel with our results, several researchers proved that CU provides support to the antioxidant enzyme system of the testes of the experimental animals, in order to prevent lipid production and oxidative stress, so as to protect spermatogenesis and steroidogenesis against toxic substances **(Aktas *et al.*, 2012; Khorsandi *et al.*, 2013; Głombik *et al.*, 2014; Coşkun *et al.*, 2016)**.

Recently, **Harisa *et al.* (2017)** and **Moldes *et al.* (2017)** announced that some nutraceuticals (e.g., CU)

are natural substances that can be extracted from various plant sources and act as antioxidants, anticancer and antitumor when included in the food to protect us from different diseases. In more corroboration, the results by **Fehl and Ahmed (2017)** suggested that CU sensitizes prostate cancer cells to the oncolytic effects of vesicular stomatitis virus by modulating antiviral responses and components of the intrinsic apoptotic pathway. However, **Singh *et al.* (2017)** provided an insight into the present regulations, which have not been completely effective in the prevention of food contamination and adulteration, such as by using the synthetic food additives.

In conclusion, the obtained results of the present comparative valuation of ultrastructural and immunoscreening studies emphasize that, there is a need to forbid the usage of E110 (SY) as an artificial food colorant in foodstuffs and drugs due to its cytotoxic effects to the testes and prostate glands of the male reproductive system in adult mice. Moreover, the current results reinforce the use of E100 (CU) as a nutritional supplement and its inclusion in our daily diet due to its enormous potential for healthy improvement and protection from oxidative stress, especially of the male reproductive system.

Conflict of interest statement

The authors announce that there is no conflict of interest.

References

1. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol. 2009; 41:40–59.
2. Aitken RJ. The Amoroso Lecture. The human spermatozoon - a cell in crisis? J Reprod Fertil. 1999; 115:1-7.
3. Aktas C, Kanter M, Erboga M, Ozturk S. Anti-apoptotic effects of Curcumin on cadmium-induced apoptosis in rat testes. Toxicol Ind Health. 2012; 28:122-30.
4. Andrade FI, Guedes MI, Vieira IG, Mendes FN, Rodrigues PA, Maia CS, Avila MM, de Matos Ribeiro L. Determination of synthetic food dyes in commercial soft drinks by TLC and ion-pair HPLC. Food Chem. 2014; 157(15):193-8.
5. Baker MA, Aitken RJ. The importance of redox regulated pathways in sperm cell biology. Mol Cell Endocrinol. 2004; 216:47-54.
6. Balbani AP, Stelzer LB, Montovani JC. Pharmaceutical excipients and the information on drug labels. Braz J Otorhinolaryngol. 2006; 73(3):400-6.
7. Balk SP, Ko YJ, Bubley GJ. Biology of prostate-specific antigen. J Clin Oncol. 2003; 21(2):383–91.
8. Bengmark S, Mesa MD, Gil A. Plant-derived health: the effects of turmeric and curcuminoids. Nutr Hosp. 2009; 24: 273–81.
9. Bhattacharjee M. Evaluation of mitodepressive effect of sunset Yellow using *allium sativum* assay. Internat J Sci Environ Technol. 2014; 3(3):1120 – 30.
10. Botelho BG, de Assis LP, Sena MM. Development and analytical validation of a simple multivariate calibration method using digital scanner images for sunset yellow determination in soft beverages. Food Chem. 2014; 159 (15):175-80.
11. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. BMJ. 1992; 305(6854):609-13.
12. Carreau S, Bourguiba S, Lambard S, Galeraud-Denis I, Genissel C, Levallet J. Reproductive system: aromatases and estrogens. Mol Cell Endocrinol. 2002; 139: 137-43.
13. Chen R, Xie L, Cai X, Huang Y, Zhou L, et al. Percent free prostate-specific antigen for prostate cancer diagnosis in Chinese men with a PSA of 4.0–10.0 ng/ml: Results from the Chinese Prostate Cancer Consortium. Asian J Urol. 2015; 2 (2):107-13.
14. Chuah AM, Jacob B, Jie Z, Ramesh S, Mandal S, Puthan J, Deshpande P, Vaidyanathan V, Gelling R, Patel G, Das T, Shreeram S. Enhanced bioavailability and bioefficacy of an amorphous solid dispersion of curcumin. Food Chem. 2014; 156:227-32.
15. Cocuzza M, Sikka SC, Athayde KS, Agarwal A. Clinical relevance of oxidative stress and sperm chromatin damage in male infertility: an evidence based analysis. Int Braz J Urol. 2007; 33: 603-21.
16. Coffey DS. The molecular biology, endocrinology and physiology of the prostate and seminal vesicles. In: Walsh PC, Retik AB, Stamey TA, Vaughn JED, editors. Campbell's Urology. Six ed. W.B. Saunders Co., Philadelphia, PA, USA; 1992. p. 1381-428.
17. Coşkun G, Özgür H, Doran S, Polat S. Ameliorating effects of curcumin on nicotine-induced mice testes. Turk J Med Sci. 2016; 46: 549-60.
18. De Rooij DG, Russell LD. All you wanted to know about spermatogonia but we afraid to ask. J Androl. 2000; 21(6):776-98.
19. Dobashi M, Fujisawa M, Naito I. Distribution of type IV collagen subtype in human testes and

their association with spermatogenesis. Fertil Steril. 2003; 80:755- 60.
20. Dykstra MJ, Mann PC, Eiwell MR, Ching SV. Suggested standard operating procedures (SOPs) for the preparation of electron microscopy samples for toxicology/pathology studies in a GLP environment. Toxicol Pathol. 2002; 30(6): 735-43.
21. EFSA (European Food Safety Authority). Panel on Food Additives and Nutrient Sources added to Food. Scientific Opinion on the re-evaluation of Sunset Yellow FCF (E 110) as a food additive. EFSA J. 2009; 7(11):1330.
22. EFSA (European Food Safety Authority). Scientific opinion on the re-evaluation of curcumin (E100) as a food additive. EFSA Panel on Food Additives and Nutrient Sources added to Food. EFSA J. 2010; 8 (9): 1679- 725.
23. Fehl DJ, Ahmed M. Curcumin promotes the oncoltyic capacity of vesicular stomatitis virus for the treatment of prostate cancers. Virus Res.2017; 228:14–23.
24. Feng J, Cerniglia CE, Chen H. Toxicological significance of azo dye metabolism by human intestinal microbiota. Frontiers in Biosci (Elite Edition). 2012; 1(4):5680-6.
25. Ferdowsian HR, Beck N. Ethical and Scientific Considerations Regarding Animal Testing Research. PLoS One. 2011; 6 (9): e24059.
26. García-Flórez M, Oliveira CA, Carvalho HF. Early effects of estrogen on the rat ventral prostate. Braz J Med Biol Res. 2005; 38(04): 487-97.
27. Głombik K, Basta-Kaim A, Sikora-Polaczek M, Kubera M, Starowicz G, Styrna J. Curcumin influences semen quality parameters and reverses the di(2-ethylhexyl)phthalate (DEHP)-induced testicular damage in mice. Pharmacol Rep. 2014; 66: 782–7.
28. Häger M, Gawlik K, Nyström A, Sasaki T, Durbeej M. Laminin {alpha}1 chain corrects male infertility caused by absence of laminin {alpha}2 chain. Am J Pathol. 2005; 167(3):823-33.
29. Haidel G, Schuppe HC, Kohn FM, Leiber C. Evidence-based drug therapy for male infertility. Urol A. 2008; 47(12):1555-6.
30. Hajimahmoodi M, Afsharimanesh M, Moghaddam G, Sadeghi N, Oveisi MR, Jannat B, Pirhadi E, ZamaniMazdeh F, Kanan H. Determination of eight synthetic dyes in foodstuffs by green liquid chromatography. Food Addit Contam. Part A: Chem Anal Control Expo Risk Assess. 2013; 30(5): 780-5.
31. Harisa P, Marya V, Aparnaa P, Dileepc KV, Sudarsanakuma C. A comprehensive approach to ascertain the binding mode of curcumin with DNA. Spectrochem Acta A Mol Biomol Spectrosc. 2017; 175: 155–63.
32. Ismail MA. Molecular and cytochemical comparative assessment between the two food additives; sunset yellow and curcumin-induce testicular toxicity in mice. J Biosci Applied Res. 2016; 2(7): 509-23.
33. JECFA (Joint FAO/WHO Expert Committee on Food Additives). Toxicological evaluation of certain food additives. Twenty six Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series. 1982; No. 17.
34. JECFA (Joint FAO/WHO Expert Committee on Food Additives). Evaluation of certain food additives and contaminants. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 2004; 922. Geneva. Available at: http://whqlibdoc.who.int/trs/who_trs_922. pdf.
35. Kelly F. Oxidative stress: its role in air pollution and adverse health effects. Occup Environ Med. 2003; 60:612-6.
36. Khorsandi L, Mirhoseini M, Mohamadpour M, Orazizadeh M, Khaghani S. Effect of Curcumin on dexamethasone-induced testicular toxicity in mice. Pharm Biol. 2013; 51: 206-12.
37. Kravchenko JS. Diet and Cancer. In: Reference Module in Biomedical Sciences, from International Encyclopedia of Public Health (2nd Ed.); 2017. p. 294-304.
38. Lu N, Gao A, Dai P, Mao H, Zuo X, Fan C, Wang Y, Li T. Ultrasensitive Detection of Dual Cancer Biomarkers with Integrated CMOS-Compatible Nanowire Arrays. Anal Chem. 2015; 87 (22): 11203-8.
39. Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. Life Sci. 2006; 78: 2081-7.
40. Mangelsdorf I, Buschmann J, Orthen B. Some aspects relating to the evaluation of the effects of chemicals on male fertility. Reg Toxicol Pharmacol. 2003; 37: 356-9.
41. Manivannan B, Mittal R, Goyal S, Ansari AS, Lohiya NK. Sperm characteristics and ultrastructure of testes of rats after long-term treatment with the methanol subfraction of Carica papaya seeds. Asian J Androl. 2009; 11(5):583-99.
42. Mattias H, Kinga G, Alexander N. Laminin {alpha} 1 chain corrects male infertility caused by absence of laminin [alpha} 2 chain. Am J Pathol. 2005; 167:823-33.
43. Mesbah SF, Shokri S, Karbalay-Doust S, Mirkhani H. Effects of Nandrolone decanoate on

ultrastructure of testis in male adult rats. Iran J Med Sci. 2008; 33: 94-100.
44. Moghadamtousi SZ, Kadir HA, Hassandarvish P, Tajik H, Abubakar S, ZandiK. A review on antibacterial, antiviral, and antifungal activity of curcumin. BioMed Res Int. 2014; 1-12.
45. Moldes AB, Vecino X, Cruz JM. Nutraceuticals and food additives, in: Current Developments in Biotechnology and Bioengineering. Food and Beverages Industry: Elsevier BV; 2017. p. 143–64.
46. Monsees TK, Franz M, Gebhardt S, Winterstein U, Schill WB, Hayatpour J. Sertoli cells as a target for reproductive hazards. Andrologia. 2002; 32: 239-46.
47. Montaser MM, Mohamed E, Alkafafy ME. Effects of Synthetic Food Color (Carmoisine) on Expression of Some Fuel Metabolism Genes in Liver of Male Albino Rats. Life Sci J. 2013; 10(2): 2191-8.
48. Nna E. The end of the road for prostate specific antigen testing? Niger J Clin Pract. 2013; 16(4): 407-17.
49. Nonn L, Duong D, Peehl DM. Chemopreventive anti-inflammatory activities of curcumin and other phytochemicals mediated by MAP kinase phosphatase-5 in prostate cells. Carsinogenesis. 2007; 28:1188–96.
50. NTP (National Toxicology Program). Carcinogenesis Bioassay of FD & amp; C Yellow No. 6 (CAS No. 2783-94-0), Natl Toxicol Program Tech Rep Ser. 1981; 208 PMID: 12778227.
51. Oliveira DS, Dzinic S, Bonfil A, Saliganan AD, Sheng S, Bonf D. The mouse prostate: a basic anatomical and histological guideline. Bosn J Basic Med Sci. 2016; 16(1): 8–13.
52. Onozawa M, Fukuda K, Watanabe M, Ohtani M, Akaza H, Sugimura T, Wakabayashi K. Detection and cloning of a protein recognized by anti-human prostate-specific antigen (PSA) antibody in the rat ventral prostate. Jpn J Cancer Res. 2001; 92(8):863-8.
53. Ottawa ON. Canadian Agency for Drugs and Technologies in Health. Rapid Response Reports. 2013; PMID: 24741718.
54. Ottó D, Bicanic D, Ajtony Z, Koehorst R. Determination of sunset yellow in multi-vitamin tablets by photoacoustic spectroscopy and a comparison with alternative methods. Food Addit Contam. 2005; 22 (6): 503-7.
55. Pari L, Tewas D, Eckel J. Role of curcumin in health and disease. Arch Physiol Biochem. 2008; 114: 127-49.
56. Perkins S, Verschoyle RD, Hill KA. Chemopreventive efficacy and pharmacokinetics of curcumin in the Min/+ mouse, a model of familial adenomatous polyposis. Cancer Epidem Biomar Prevent. 2002; 11: 535-40.
57. Peschel W, Sanchez-Rabaneda F, Dieckmann W, Plescher A, Gartzia I, Jimenez D. An industrial approach in the search of natural antioxidants from vegetable and fruits wastes. Food Chem. 2006; 97: 137-50.
58. Ramalingam S, Vidya P, Ramamurthy VP, Njar VC. Dissecting major signaling pathways in prostate cancer development and progression: Mechanisms and novel therapeutic targets. J Steroid Biochem Mol Biol. 2017; 166: 16–27.
59. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J. 2008; 22(3): 659-61.
60. Richardson LL, Kleinman HK, Dym M. Altered basement membrane synthesis in the testis after tissue injury. J Androl. 1998; 19: 145-55.
61. Richburg JH. The relevance of spontaneous- and chemically-induced alteration in testicular germ cell apoptosis to toxicology. Toxicol Lett. 2000; 112-113:79-86.
62. Sardi M, Haldemann Y, Nordmann H, Bottex B, Safford B, Smith B, Tennant D, Howlett J, Jasti PR. Use of retailer fidelity card schemes in the assessment of food additive intake: Sunset yellow a case study. Food Addit Contam. 2010; 27(11): 1507-15.
63. Sawada H, Esaki M. Electron microscopic observation of 137Cs-irradiated rat testis: production of basal laminae for germ cells, despite their absence. J Electron Microsc. (Tokyo). 2003; 25: 391-7.
64. Sayed HM, Fouad D, Ataya FS, Hassan NH, Fahmy MA. The modifying effect of selenium and vitamins A, C and E on the genotoxicity induced by sunset yellow in male mice. Mutation Res. 2012; 744 (2): 145-53.
65. SCF. Reports of the Scientific Committee for Food (14thSeries), opinion expressed. 1984; 6.
66. Sepaniak S, Forges T, Gerard H, Foliguet B, Bene MC, Monnier-Barbarino P. The influence of cigarette smoking on human sperm quality and DNA fragmentation. Toxicology. 2006; 223: 54-60.
67. Sharma RA, Gescher AJ, Steward WP. Curcumin: the story so far. Eur J Cancer. 2005; 41: 1955–68.
68. Sharma RA, Ireson CR, Verschoyle RD. Effects of dietary curcumin on glutathione S-transferase and malondialdehyde-DNA adducts in rat liver and colon mucosa: relationship with drug levels. Clin Cancer Res. 2001; 7: 1452-8.
69. Sharma S, Goyal RP, Chakravarty G, Sharma A. Toxicity of tomato red, a popular food dye blend

on male albino mice. Exp Toxicol Pathol. 2008; 60(1): 51-7.
70. Shen Y, Zhang X, Prinyawiwatkul W, Xu Z. Simultaneous determination of red and yellow artificial food colourants and carotenoid pigments in food products. Food Chem. 2014; 157(15): 553-8.
71. Sikka SC, Wang R. Endocrine disruptors and estrogenic effects on male reproductive axis. Asian J Androl. 2008; 10:134-45.
72. Singh SP, Kaur S, Singh D. Toxicological profile of Indian foods—ensuring food safety in India. In: Food Safety in the 21st Century, Chapter 9; 2017. p. 111-27.
73. Siviero A, Gallo E, Maggini V, Gori L, Mugelli A, Firenzuoli F, Vannacci A. Curcumin, a golden spice with a low bioavailability. J Herbal Med. 2015; 5:57–70.
74. Spaliviero JA, Jimenez M, Allan CM, Handelsman DJ. Leuteinizing hormone receptor-mediated effects on initiation of spermatogenesis in gonadotropin-deficient (hpg) mice are replicated by testosterone. Boil Reprod. 2004; 70: 32-8.
75. Taft RA, Davisson M, Wiles MV. Know thy mouse. Trends in Genetics. 2006; 22(12): 649–53.
76. Tanaka T. Reproductive and neurobehavioural toxicity study of trartrazine administration to mice in the diet. Food Chem Toxicol. 2005; 5: 16–25.
77. Tokudome S, Ando R, Koda Y. Discoveries and application of prostate-specific antigen, and some proposals to optimize prostate cancer screening. Cancer Manag Res. 2016; 8: 45-7.
78. Tripathi M, Khanna SK, Das M. Surveillance on use of synthetic colours in eatables vis a vis Prevention of Food Adulteration Act of India. Food Control. 2007; 18(3): 211-9.
79. Turner TT, Lysiak JJ. Oxidative stress: a common factor in testicular dysfunction. J Androl. 2008; 29: 488- 98.
80. Valkenburg KC, Amend SR, Pienta KJ. Murine prostate micro-dissection and surgical castration. J Vis Exp. 2016; 11(111).
81. Veena BK, Geeta B. Toxicological Effect of Disodium6-Hydroxy-5-[(4-Sulfophenyl)azo]-2 Naphthalene sulfonate on Behavior of Albino Rats. Adv Biol Res. 2013; 7(6): 223-7.
82. Wang M, Zhang, J, Gao Y, Yang X, Gao Y, Zhao J. Determination of Sunset Yellow in soft drinks at attapulgite modified expanded graphite paste electrode. J Eelectrochem Soc. 2014; 161(3): H86-91.
83. Weber WM, Hunsaker LA, Abcouwer SF, Deck LM, Vander Jagt DV. Anti-oxidant activities of curcumin and related enones. Bioorg Med Chem. 2005; 13: 3811–20.
84. Wu H, Guo JB, Du LM, Tian H, Hao CX, Wang ZF, Wang JY. A rapid shaking-based ionic liquid dispersive liquid phase microextraction for the simultaneous determination of six synthetic food colourants in soft drinks, sugar- and gelatin-based confectionery by high-performance liquid chromatography. Food Chem. 2013; 141(1): 182-6.
85. Yadav A, Kumar A, Tripathi A, Das M. Sunset yellow FCF, a permitted food dye, alters functional responses of splenocytes at non-cytotoxic dose. Toxicol Letters. 2013; 217(3): 197-204.
86. Zheng Y, Zhang X, Zhou J, Cheng F, Rao T, Yao Y. The effects of artery-ligating and artery-preserving varicocelectomy on the ipsilateral testes in rats. Urology. 2008; 72: 1179-84.

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