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Histological Study of the Effect of Semicarbazide on Testicular Seminiferous Tubules of Juvenile Albino Rat

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Abstract Semicarbazide (SEM) is a by-product of azodicarbonamide used to improve the properties of the plastic seals of the glass jar lids of the canned food such as baby food. This work aimed to evaluate the effect of semicarbazide (SEM) on the morphology of testicular seminiferous tubules of juvenile albino rat. Twenty juvenile male albino rats aged 4 weeks were divided into two main groups: group I (control), group II (treated with SEM 40 mg/kg oral daily for 4 weeks). Specimens from the testes were processed for different histological examinations, histochemical staining with Periodic Acid Schiff (PAS) and immunohistochemical study using antibodies against Bcl-2 was carried out in addition to morphometrical and statistical analysis. SEM-treated group showed some vacuolated spermatogenic and Sertoli cells, discontinuity of germinal epithelium, some exfoliated spermatogenic cells in the lumen in addition to focal loss of the basement membrane. A statistically significant decrease in both mean epithelial height and area percentage was recorded in group II. A statistically significant decrease of PAS reaction of the basement membrane was observed. A statistically significant decrease in Bcl-2 positive cytoplasmic immunoreaction in cells of seminiferous tubules was detected. Electron microscopic examination revealed swollen mitochondria, dilated rough endoplasmic reticulum, vacuolated cytoplasm and areas of cytoplasmic loss in spermatogenicand Sertoli cells. The nuclei of some primary spermatocytes and spermatids appeared dark and shrunken or fragmented. Thickened basement membrane with collagen deposition was detected. It could be concluded that SEM causes significant structural changes in juvenile seminiferous tubules of rat testis.So, it is recommended to use alternative safe substance in the sealing process of glass jar lids.

Keywords | Semicarbazide, seminiferous tubules. Rat, Bcl-2, electron microscope

Introduction

Semicarbazide (SEM) is a chemical used in making the plastic gaskets that seal the jar lids of multiple products, including baby food, fruit juice, jams, honey, sauces, mustard and ketchup to prevent leakage and microbiological contamination of the jar contents (Abramsson and Svensson, 2005; De La Calle and Anklam, 2005). SEM represents a decomposition by-product of azodicarbonamide. The latter is used in food processing and as flour treatment agent to improve the baking properties. SEM is suspected to be toxic (European Food Safety Authority, 2003). The risk of SEM toxicity is much higher in infants than in adults due to the infants' small body weight beside their relative high consumption of products packed in glass jars as compared to other consumers (Vlastos et al., 2010).

In young age, SEM causes severe osteochondral lesions including thickening and disarrangement of the epiphyseal cartilage and deformation of articular cartilage, but these effects decrease during the recovery period. Although SEM causes osteochondral lesions also in adult, yet reduction of severity was not obvious during the recovery period (Okasha and Elbakary, 2011).

Moreover, SEM causes alternations in the appearance of elastic lamina of blood vessels, exerts many genetic pleiotropic effects on several organs and causes teratogenic effects such as induction of cleft palate and aortic aneurysms (Gongetal., 2006; Takahashi et al., 2010). Reproductive system toxicity at juvenile age was also reported (Ramos et al., 2012). Spermatogenesis in particular was reported to be affected by many factors as chemical agents such as medications and toxic elements in environmental pollution (Yano *et al*., 2002).

This work aimed to evaluate the effect of semicarbazide (SEM) on the morphology of testicular seminiferous tubules of juvenile albino rat using different histological, histochemical and immunohistochemical techniques.

Materials and methods

Experimental design

The present study was carried out on 20 juvenile male albino rats aged 4 weeks with an average weight of 25 - 40 g each. All the animals were housed in suitable clean properly ventilated cages at room temperature (22–25°C) under similar conditions and were fed on a similar commercial laboratory diet and water**.** The experiment was approved by the Local Ethics Committee and was held at the animal house of the department of Histology, Tanta faculty of Medicine, Egypt. The animals were divided into two main groups:

Group I (juvenile control group): included 10 juvenile male albino rats that were divided equally into two subgroups; **Subgroups (Ia):** Animals of these subgroups were held without any treatment for 4 weeks. **Subgroup (Ib):** Animals of this subgroup received distilled water orally by gavage once daily for 4 weeks.

Group II (Juvenile semicarbazide-treated group): This group included 10 juvenile male albino rats, which were administered semicarbazide hydrochloride (SEM) (S2201,Sigma-Aldrich Medical Company, St. Louis, Missouri, USA) orally by gavage once daily at a dose of 40 mg/kg body weight dissolved in 0.5 ml distilled water for 4 weeks (Maranghi et al., 2009).

Light microscopic fixation and staining

After 24 h from the last administration, the animals were euthanized by an intraperitoneal injection of sodium pentobarbital as anesthetic agent (50mg/kg) (Gaertner et al., 2008). Both testes were carefully dissected. One part of the right testis was immediately fixed in aqueous Bouin's solution to be processed for hematoxylin and eosin staining for morphological examination and the other part was fixed in formol saline for histochemical staining with Periodic Acid and Schiff (PAS) for detection of neutral mucopolysaccharides and immunohistochemical processing (Wulff and Advisor, 2004; Chandler and Roberson, 2009).

Bouin's solution was used for preservation of the morphologic details of testes for histopathological evaluation, while formol saline was used for better immunohistochemical staining (Janssen et al., 1994; Latendresse et al., 2002).The left testis was fixed in 2.5 % phosphate buffered glutaraldehyde for transmission electron microscopy processing.

Immunohistochemicalstaining

Sections of 5 µm thick were dewaxed, rehydrated, and washed with phosphate buffered saline (PBS). The sectionswere incubated overnight in humid chamber at 4°C with the primary antibody; a rabbit polyclonal anti-Bcl-2 (ab59348 Abcam; Cambridge, Massachusetts, USA). The sections were washed in PBS and incubated with goat anti rabbit secondary antibody for one hour at room temperature, then incubated with streptavidin peroxidase for 30-60 min and rinsed in PBS buffer. Drops of Diaminobenzidine (DAB) hydrogen peroxidewere applied to the sections to visualize the immunoreaction. Sections were counterstained with Mayer's hematoxylin.Positive reactions expressed as a brown cytoplasmic coloration.The negative control sections were prepared by excluding the primary antibody (Kiernan, 2000).

Electron microscopic examination

The testis specimens were immediately fixed by immersion in 2.5% phosphate-buffered glutaraldehyde (pH 7.4) at 4˚C for 24 hours, washed in PBS and fixed in 1% osmium tetroxide, and dehydrated in alcohol and embedded in epoxy resins. Semithin sections (1 µm thick) were obtained and stained with 1% toluidine blue for light microscopy examination. Ultrathin sections (50–60 nm) were cut on a Leica Ultramicrotome (Leica Microsystems, Vienna, Austria), mounted on copper grids, and stained with uranyl acetate, followed by lead citrate (Kuo, 2007). The grids were examined using a JEOL-JEM-100 SX Transmission Electron Microscope (Jeol, Tokyo, Japan) at the Electron Microscopic Unit of the Faculty of Medicine, Tanta University (Tanta, Egypt)**.**

Morphometric study

An Olympus light microscope (Tokyo, Japan) coupled to Olympus digital camera (E-420)was used for image acquisition at the department of Histology, Tanta faculty of Medicine, Egypt. "Image J"software(National Institute of Health, Bethesda, Maryland, USA) was used for image analysis. Ten different non-overlapping randomly selected fields from each slide were examined at a magnification of 400. Specimens were examined for:

1- The mean epithelial height (µm) and the mean epithelial area percentage of the germinal epithelium lining seminiferous tubules (in H&E stained sections).

2- The mean color intensity of PAS histochemical reaction (in PAS-stained sections).

3- The mean color intensity of Bcl-2 immunohistochemical reaction (in DAB–stained sections).

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by student t test as post comparison test using Microsoft office Excel 2010 for windows (Microsoft corporation, Redmond, Washington, USA). All values were expressed as mean ± standard deviation (SD). Differences were regarded as significant if probability (P) values were less than 0.05 and highly significant if P values were less than 0.001 (**Dawson and Trapp, 2001**).

Results

Light microscopic examination

H&E-stained sections

Group I (Control group):

H&E–stained sections from the testes of both control subgroups showed the normal juvenile histological structure represented by densely packed seminiferous tubules lined with Sertoli cells with elongated pyramidal nucleus and stratified germinal lining including spermatogonium type A with ovoid

nuclei, spermatogonium type B with spherical nuclei, primary spermatocytes with large spherical dark nuclei, few round spermatids with areas of condensed chromatin in their nuclei and some late spermatids. The seminiferous tubules were surrounded by well-defined basement membrane and ensheathed by myoid cells with flat nuclei. Some Leydig cells with central nuclei and acidophilic cytoplasm could be detected in between the seminiferous tubules **(Fig. 1).**

Group II (SEM-treated group)

Examination of some sections obtained from the testes of this group showed vacuolated homogenous acidophilic material and congested blood vessels within the interstitial space. Some tubules were lined with few spermatogenic cells while some cells were exfoliated in the lumen **(Fig. 2a)**, other tubules showed disorganized and discontinuous germinal epithelium with loss and disruption of the basement membrane.Moreover, focal detachment of the germinal epithelium from the underlying basement membrane was also observed **(Fig. 2b).** Somespermatogonia and primary spermatocytesrevealed vacuolated cytoplasm **(Fig. 2c)**. Other primary spermatocytes showed dark shrunken irregular nuclei and acidophilic cytoplasm **(Fig. 2d).**

Morphometrical and statistical analysis revealed a highly significant decrease in the mean epithelial height in group II (SEM-treated group) as compared with the control subgroups (Table 1 and histogram 1). Moreover, a highly significant decrease in the mean epithelial area percentage in group II as compared with the control subgroups was observed (Table 1 and histogram 1).

Periodic Acid Schiff´s reaction (PAS) staining:

Examination of PAS–stained sections from the control subgroups revealed an apparent strong PAS positive reaction in the basement membranes of the seminiferous tubules **(Figs. 3a)**. Whereas PAS-stained sections obtained from group II depicted an apparent focal weakness of the PAS reaction of the basement membrane along with a deposition of vacuolated homogenous PAS positive material inbetween the seminiferous tubules of some specimens **(Fig. 3b).**

Morphometrical and statistical analysis revealed a highly significant reduction in the mean color intensity of PAS reaction in group II (SEM-treated group) as compared with the control subgroups (Table 1 and histogram 1).

BCL-2 immunohistochemical staining:

Bcl-2-immunostained sections obtained from the testes of control subgroups have similarly exhibited an apparent moderate Bcl-2 positive cytoplasmic reaction in all cells of seminiferous tubules in the form of brown coloration **(Fig. 4a)**. On the other hand, sections obtained from the testes of group II (SEM-treated group) showed an apparent weak Bcl-2 positive cytoplasmic immunoreaction in the cells of seminiferous tubules **(Fig. 4b).**

Morphometrical and statistical analysis revealed a highly significant decrease in the mean color intensity of BCL2 immunoreaction in group II (SEM-treated group) as compared with the control subgroups **(Table 1 and histogram 1).**

Electron microscopic results

Group I (control group)

Electron microscopic examination of the ultrathin sections of the testes obtained from both control subgroups showed the normal histological structure of juvenile testis. The seminiferous tubules rested on regular basement membranes which were enclosed by myoid cells**.** The basal compartment of the seminiferous tubules were formed of three types of cells; **Sertoli cell**s with euchromatic pyramidal nuclei **(Fig 5a)**, **Spermatogonium type A (pale type)** with ovoid nuclei **(Fig. 5b)** and **spermatogoniumtype B** with almost rounded nuclei and condensed chromatin arranged peripherally and around the nucleolus **(Fig 5c).** While the adluminal compartment showed primary spermatocytes, spermatids. **The primary spermatocytes** had rounded to oval nuclei with fine chromatin **(Fig. 5d).** The secondary spermatocytes were scarcely seen. The **early round spermatids** were identified by their characteristic acrosomal caps, large rounded and euchromatic nuclei with fine granular chromatin, while their cytoplasm showed peripherally arranged vesicular mitochondria **(Fig. 5e). Late spermatids** appeared with their elongated nuclei and acrosomalcaps **(Fig. 5f).**

Group II (SEM-treated group)

Ultrathin sections obtained from the testes of this group revealed thickened basement membrane with collagen deposition **(Fig. 6a).**The **Sertoli cells** showed areas of cytoplasmic loss, dilated rough endoplasmic reticulum, heterogenous electron dense bodies, lipid droplets and swollen mitochondria **(Fig. 6b).** Some **spermatogoniatype A**showed swollen mitochondria in their cytoplasm **(Fig. 6c).**While some **spermatogniatype B** depicteddilated perinuclear space, areas of vacuolationand cytoplasmic loss **(Fig. 6d).** Some of the **primary spermatocytes** appeared with dark shrunken irregular nuclei **(Fig. 6e).** Some **round spermatids** and few **elongating spermatids** showed shrunken nuclei **(Fig. 6f).**

Table 1: Morphometrical and statistical analysis of different studied groups.

Parameters	sG Ia	sG Ib	G II
Mean epithelial height (µm)	43.98±8.13	44.69±7.94	31.48±9.83*
Mean epithelial area percentage (%)	71.05±8.71	71.86±6.94	56.50±8.43*
Mean color intensity of PAS reaction	75.56±8.97	74.7±8.79	33.78±8.69*
Mean color intensity of Bcl-2 immunoreaction	76.92±13.47	75.91±15.61	18.82±3.27*

*Data are expressed as mean±SD, * p< 0.001 indicates high significance versus control.*

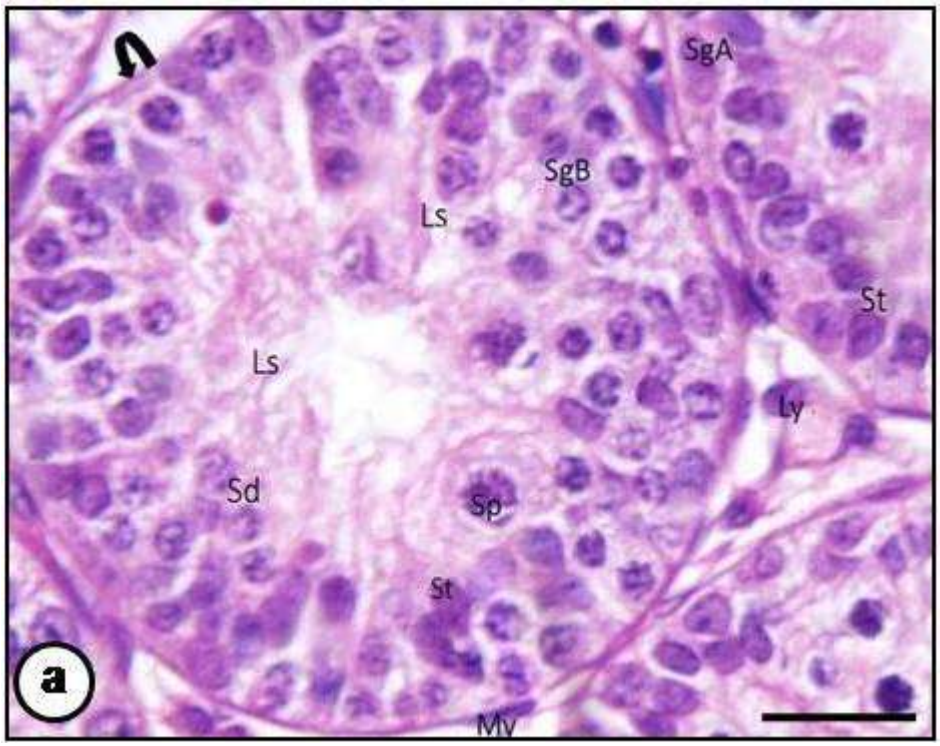


Fig. 1: H&E staining of control subgroup Ia illustrating seminiferous tubules containing Sertoli cell (St) with elongated pyramidal nucleus and different stages of spermatogenesis including spermatogonium type A (SgA) with an ovoid nucleus, spermatogonium type B (SgB) with a spherical nucleus, primary spermatocytes (Sp) with a large spherical nucleus and condensed chromatin. Few round spermatids (Sd) are detected with their nuclei containing areas of condensed chromatin. Some late spermatids (Ls) are also observed. The seminiferous tubules are surrounded by well-defined basement membrane (curved arrow) and ensheathed by myoid cells (My) with flat nuclei. Notice Leydig cells (Ly) with their central nuclei and acidophilic cytoplasm present in between the seminiferous tubules. [H& E, magnification x 1000, scale bar=25 µm]

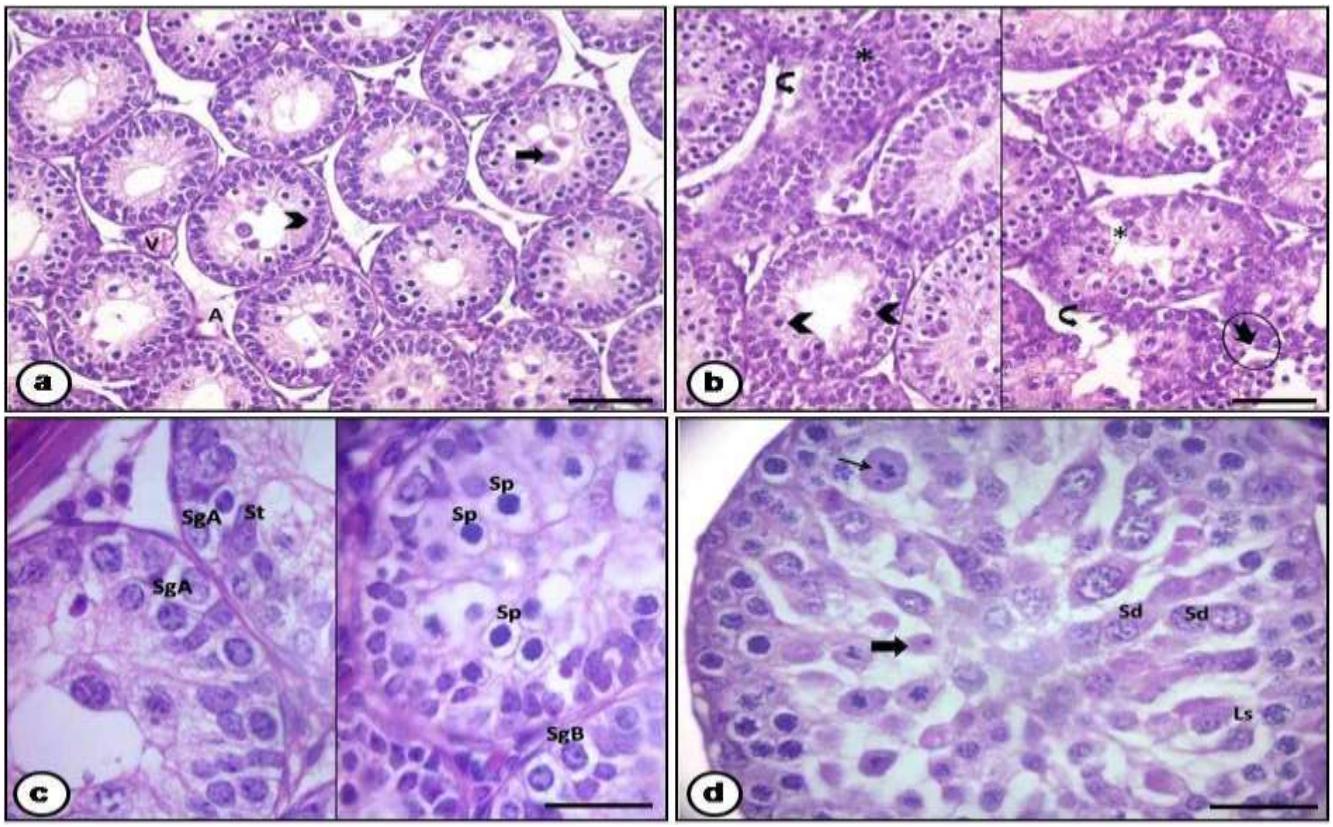


Fig. 2: H&E staining of group II (SEM-treated) (a) showing some tubules lined by few spermatogenic cells (arrow head). Some cells are exfoliated in the lumen (arrow). Notice a congested blood vessel (V) and vacuolated homogenous acidophilic material (A) in the interstitial space. (H& E x 400, scale bar=50 µm). (b) showing a seminiferous tubule lined by disorganized and discontinuous germinal epithelium (asterisks) with loss and disruption of the basement membrane (curved arrows). Focal separation of the germinal epithelium from the underlying basement membrane is noticed (notched arrow with a circle). (H& E x 400). (c) showing spermatogonia type A with vacuolated cytoplasm (SgA), spermatogonia type B with vacuolated cytoplasm (SgB). Primary spermatocytes with vacuolated cytoplasm (Sp) are observed. Notice Sertoli cell (St) with its pyramidal nucleus. (H& E x 1000). (d) showing primary spermatocytes with dark shrunken nuclei and acidophilic cytoplasm (thick arrow). Some primary spermatocytes appear with irregular and fragmented nuclei and acidophilic cytoplasm (thin arrow). Notice the presence of round spermatids (Sd) and late spermatids (Ls). [H& E, magnifications; (a, b x 400, scale bar=50 µm), (c, d x 1000 scale bar=25 µm)]

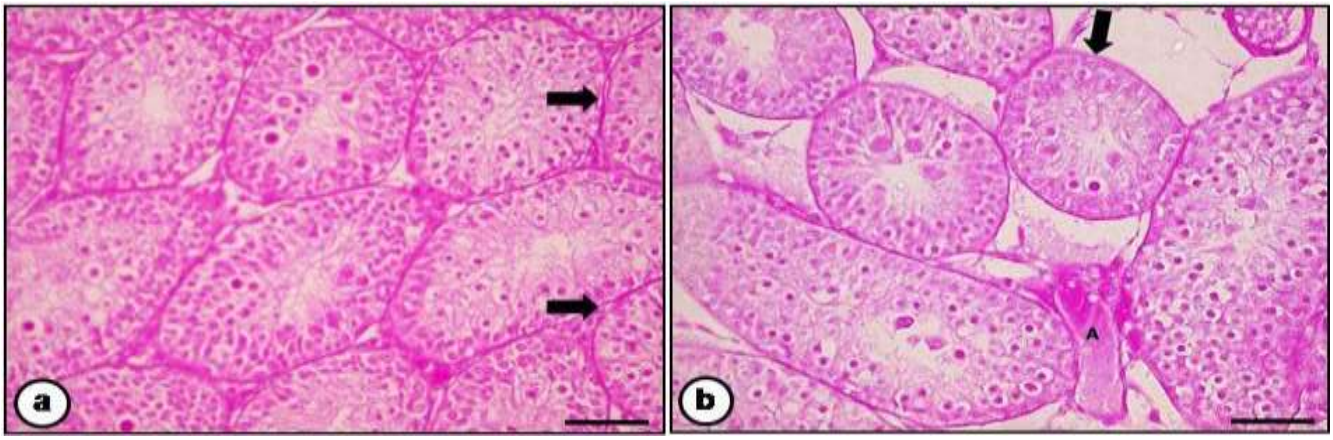


Fig. 3: PAS histochemical staining (a) Control subgroup Ia showing an apparent strong PAS positive reaction of the basement membranes (arrows). (b) Group II showing apparent weak PAS positive reaction in the basement membranes (arrow). Vacuolated homogenous PAS positive acidophilic substance is present in between the tubules (A). [PAS, magnifications a, b x 400, scale bar =50 µm]

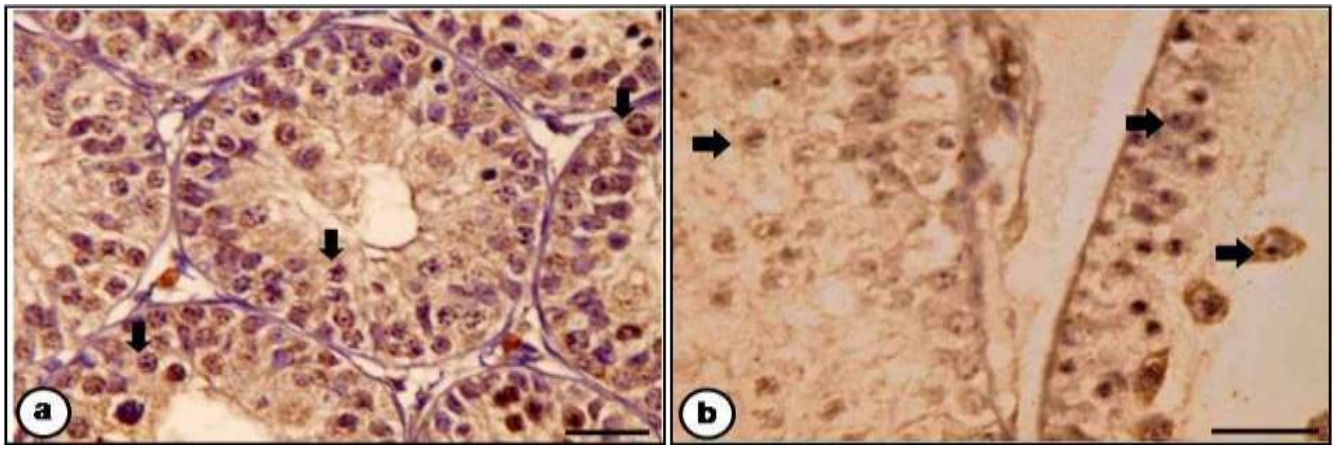


Fig. 4: BCL2 immunohistochemical staining (a) control subgroup Ia showing an apparent moderate Bcl-2 positive cytoplasmic immunoreaction in the cells of the seminiferous tubule (arrows). (b) Group II showing apparent weak Bcl-2 positive cytoplasmic immunoreaction in the cells of the seminiferous tubules (arrows). [Bcl-2 immunostaining, magnifications a-d x 400, scale bar=50 µm]

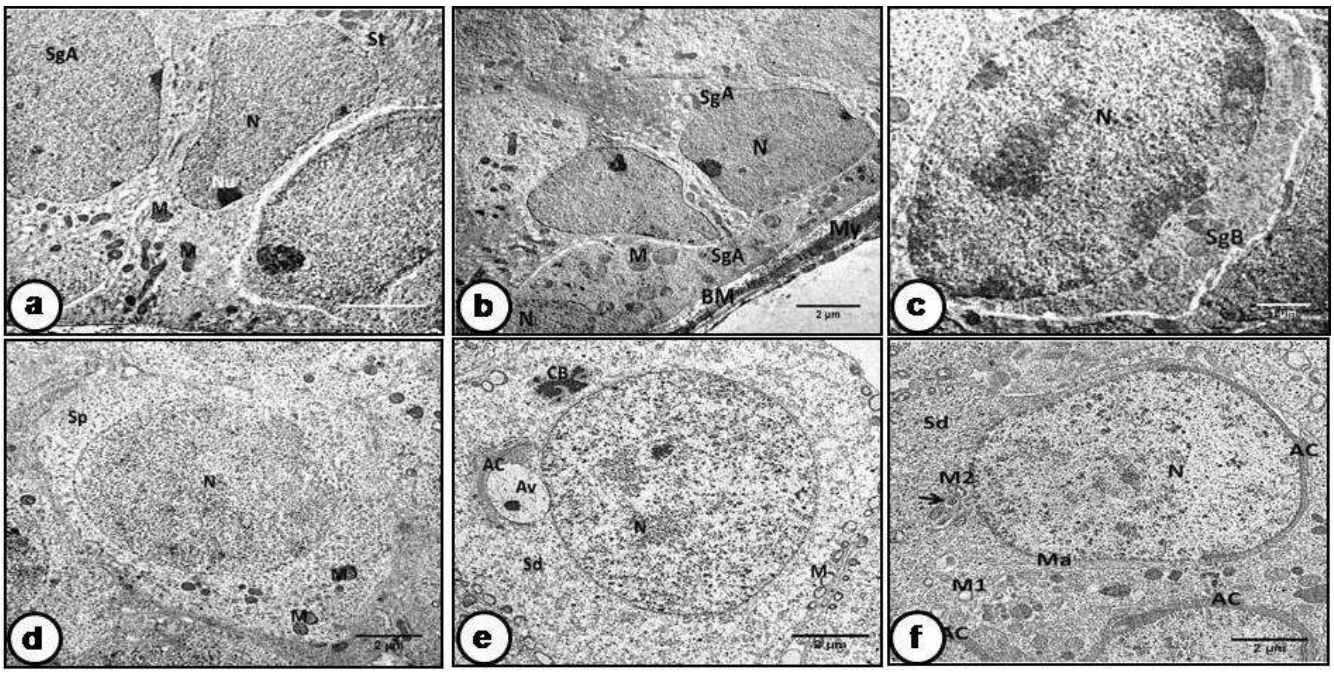


Fig. 5: TEM examination of control subgroup Ia; (a) showing a Sertoli cell (St) having a large indented euchromatic nucleus (N) with a prominent nucleolus (Nu). Multiple mitochondria (M) can be seen in the cytoplasm. Spermatogonia type A is also seen with its ovoid nucleus (SgA) (b) showing two spermatogonia type A (SgA) with ovoid nuclei (N) and multiple mitochondria (M) in their cytoplasm. They are resting on a straight regular basement membrane (BM) that is enclosed with myoid cell (My) (c) showing a spermatogonium type B (SgB) having an almost ovoid nucleus (N) with condensed chromatin arranged peripherally and around the nucleolus. (d) showing a primary spermatocyte (Sp) having a rounded to ovoid nucleus (N) with fine chromatin. Multiple mitochondria (M) can be seen in the cytoplasm(e)showing a round spermatid (Sd) with a characteristic acrosomal vesicle (AV) and incomplete acrosomal cap (AC) formation. The nucleus (N) is large rounded and euchromatic with fine granular chromatin, while the cytoplasm shows peripherally arranged vesicular mitochondria (M).An electron dense body is seen in the cytoplasm (chromatoid body) (CB).(f) showing an elongating spermatid with its characteristic acrosomal cap (AC). Its nucleus (N) is large, elongated and euchromatic with fine granular chromatin, while the cytoplasm shows peripherally arranged vesicular mitochondria (M1). The manchette (Ma) is seen. The centriole (thin arrow) is noticed at the caudal end of the nucleus preparing for flagellum formation. Some mitochondria (m2)are seen around the centriole. Notice the visible parts of acrosomal caps (Ac) of two other spermatids. [TEM, magnifications (a x2000, scale bar=2 µm), (b, d, e, f x 1500, scale bar=2 µm), (c x 2500, scale bar=1 µm)]

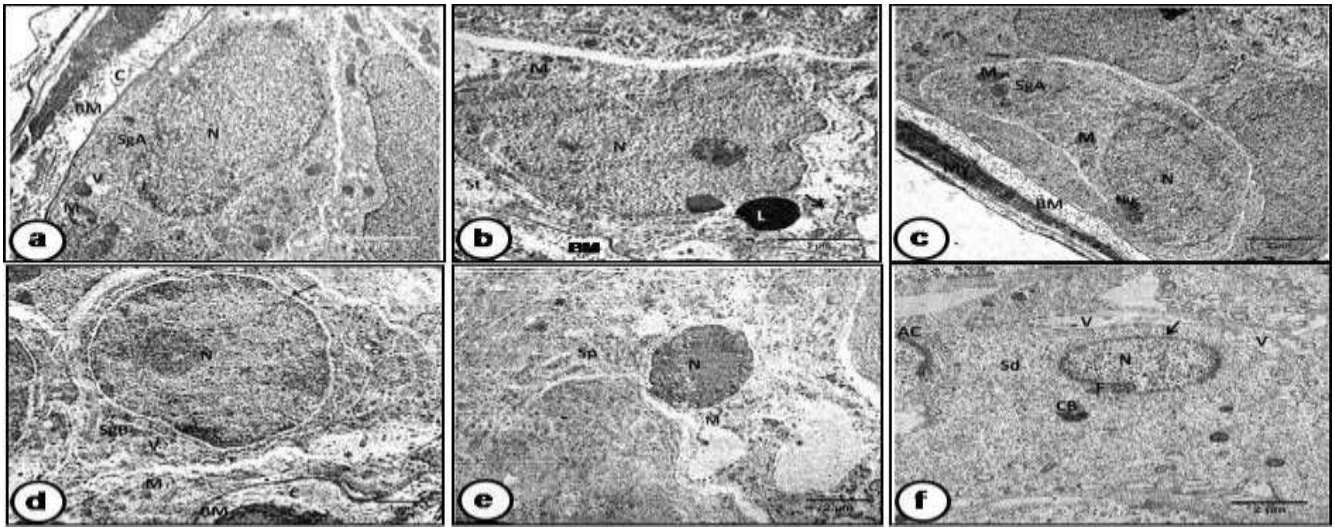
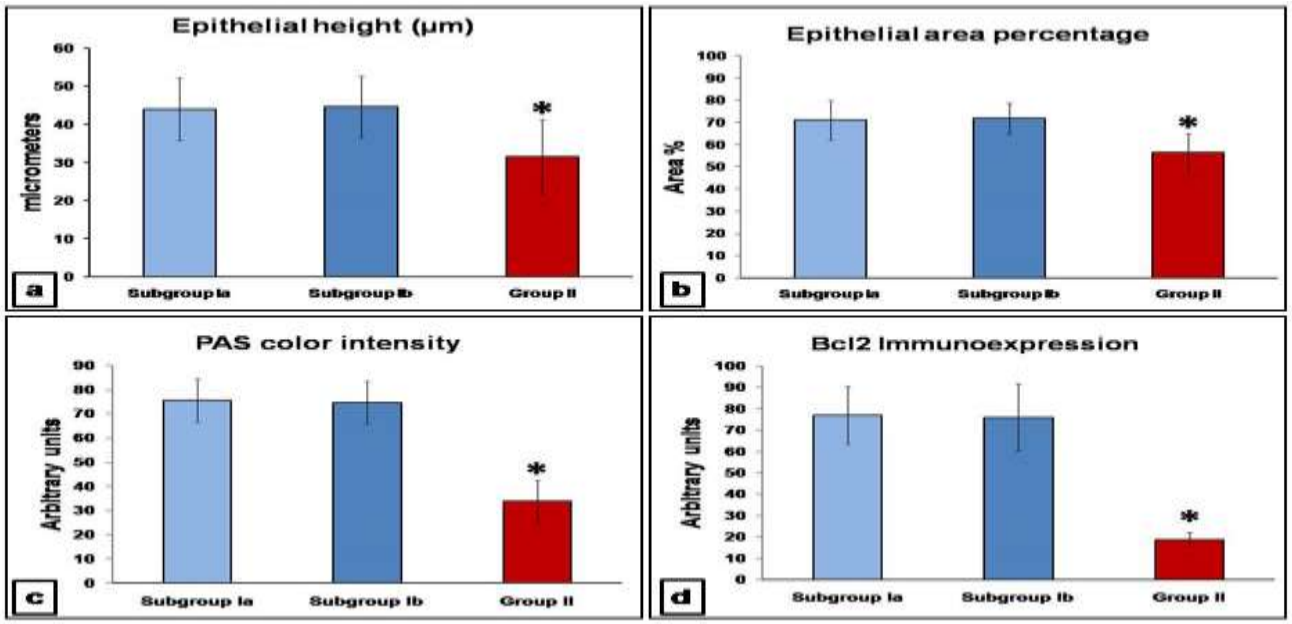


Fig. 6: TEM examination of group II (SEM-treated); (a) showing a type A spermatogonium (SgA) with ovoid nucleus (N) and cytoplasmic vacuolation (V). Swollen mitochondria are noticed (M). Notice the thickened basement membrane (BM) with collagen deposition (C). (b) showing a Sertoli cell (St) having an indented nucleus (N) and areas of cytoplasmic loss (*), a large lipid droplet (L), smooth endoplasmic reticulum (arrow) and mitochondria (M), in its cytoplasm. It rests on a thickened basement membrane (BM). (c) showing a spermatogonium type A (SgA) having an ovoid nucleus (N) with marginal nucleolus (Nu) and some swollen mitochondria (M). Type A spermatogonium (SgA) is resting on a thickened basement membrane (BM) enclosed with myoid cell (My). (d) showing a spermatogonia type B (SgB) with an almost rounded nucleus (N) and dilated peri-nuclear space (arrow). The cytoplasm shows areas of cytoplasmic loss (*), cytoplasmic vacuolation (V) and swollen mitochondria (M). Notice the irregular thickened basement membrane (BM) with collagen deposition (C). (e) showing a primary spermatocyte (Sp) having a dark, shrunken and irregular nucleus (N) and areas of cytoplasmic loss (*). (f) showing an elongating spermatid (Sd) with shrunken nucleus (N). The initial part of the flagellum (F) is seen. The cytoplasm shows an electron dense body (chromatoid body) (CB) and multiple vacuoles (V). Notice the absence of acrosomal cap in this spermatid (arrow). A part of acrosomal cap (AC) of another spermatid is seen. [TEM, magnifications; (a, b x2000, scale bar=2 µm), (c, e, f x 1500, scale bar=2 µm), (d x2500, scale bar=1 µm)]



Histogram 1:Morphometric and statistical analysis o f the different studied groups; (a) Mean epithelial height (µm) (b) Mean epithelial area percentage (c) Mean color intensity of PAS histochemical reaction (d) Mean color intensity of Bcl-2 immunohistochemical reaction. * p< 0.001 indicates high significance versus control

Discussion

Environmental factors represent about 40 % of the male infertility cases. The spermatogenic epithelium is a highly sensitive structure due to its high rate of mitotic activity, making the testis more vulnerable to environmental hazards than other tissues (Inborn, 2007).The immature elimination systems at infancy including the immature metabolizing enzymes and/or renal function increase the potential toxic effect of SEM (Schwenk, 2003). Therefore, this work aimed to study the effect of SEM on juvenile testicular morphology of albino rat.

In the present work, light and electron microscopic examination of sections obtained from SEM-treated group revealed many morphological changes in the seminiferous tubules, where sections from SEM-treated animals showed wide intertubular spaces which might be due to the seminiferous tubules atrophy (Hassan and Abdel Moneium, 2001). An intertubular homogenous acidophilic material detected in the current study could be attributed to different mechanisms;it might be due to excess lymphatic exudates of degenerating lymphatic vessels (Paniagua et al., 1991) or to an increase in the vascular permeability (Salama et al., 2003). SEM causes impairment of cross-linking reactions of collagen and elastin, which are essential for connective tissuematuration, including blood and lymphatic vessels thus might affect their permeability and result in excessive exudate (Mercier et al., 2009). Nevertheless, inter-tubular congested blood vessels were also observed in the present study. SEM administration was argued to cause tissue ischemia followed by reperfusion during which the interstitial spaces are infiltrated by different cells such as macrophages and leukocytes. Thereafter, macrophages secrete cytokines in the peritubular zones causing congestion of blood vessels (De Rooij and Russell, 2000).

Moreover, disorganized germinal epithelium, its discontinuity, scarcity of germ cells, together with diminished epithelial height of the germinal epithelium were detected. The decrease in the number of spermatogenic cells and seminiferous tubule atrophy could be indicators of spermatogenesis failure (Cameron et al., 1985). SEM was suggested to exert its adverse effects on juvenile rats by altering the normal testicular architecture and normal successive stages of spermatogenesis (Ramos et al., 2012) as was similarly reported in rats treated with phthalates (Kondo et al., 2006), dye blend (tomato red)(Sharma et al., 2008) and olanzapine (Soliman et al., 2014). However, it was suggested that the adverse effects of SEM on testis of juvenile Sprague-Dawley rats were caused by altering the percentage of testicular tissue programmed for spermatogenesis without affecting spermatogenesis itself (Maranghi et al., 2009).

Shrunken spermatogenic cells with irregular and fragmented nuclei and acidophilic cytoplasm were revealed in the current study. These findings might be due to increased constitutive levels of germ cell degeneration and apoptosis by SEM (Tripathi et al., 2009). The separation and loosening of cell to cell connections detected in the present study could be attributed to the shrinkage of both Sertoli and germ cells. Loss of cell contact might be also due to destruction of the Sertoli cell processes that fill the spaces between germ cells (Ross and Pawlina, 2011).The toxic effects of SEM on the reproductive system was suggested to result from its enzymatic inhibitory action on superoxide dismutase and glutathione peroxidase, which are two major enzymes that scavenge harmful reactive oxygen species (ROS) in the male reproductive system (Agarwal et al., 2006). Increased ROS such as hydrogen peroxide (H_2O_2), hydroxyl radicals, and superoxide radical ions, lead to an increase in lipid peroxidation, intercellular stability change, DNA damage and consequently cell death (Bruner et al., 2000; El-Shahat et al., 2009).

Moreover, a statistically significant decrease in PAS reaction observed in the SEM-treated group might be explained by the SEM-induced ROS effect on the basement membrane (Sakr et al., 2003). The current study revealed thickened basement membrane with collagen deposition. Similar findings were reported in irradiated rats (Sawadaand Esaki, 2003) and in rats treated by olanzapine (Soliman et al., 2014) and were attributed to increased collagen production by Sertoli or myoid cells or decrease in the turnover of the basement membrane proteins (Nicholls and Mandel, 1989).

The B- cell lymphoma 2 (BCL-2) family of proto-oncogenes (apoptosis inhibiting gene products) encodes specific proteins, responsible for regulation of programmed cell death under different physiological and pathological conditions (Antonsson and Martinou, 2000). Bcl-2 is present mainly on the outer membrane of the mitochondria, acting as an antiapoptosis factor, through preventing the release of cytochrome C thus preventing the activation of the caspase, which is responsible for cell apoptosis (Preedy, 2016). It was suggested that there is a physiological early apoptotic wave in the testis which is coincident with a temporary high expression of the apoptosis-promoting proteins, This apoptotic wave disappears at sexual maturity and is necessary for normal mature spermatogenesis to develop, as it maintains a critical cell number ratio between Sertoli cells and some germinal cell stages to allow perfect development (Rodriguez et al., 1997).

In the present work, there was moderate Bcl-2 positive cytoplasmic reaction in the cells of seminiferous tubules in control group. This could be explained by Chen et al., (2004) who stated that Bcl-2 is an apoptosis-related molecule that was shown to be down-regulated during the juvenile age leading to programmed cell death. A highly significant decrease in the immunohistochemical expression of Bcl-2 protein in the SEM-treated group. A similar finding was reported by Al-Azemi et al. (2010) who related it to the apoptotic

effect of the cadmium on the rat testis through caspase activation.

The present study also revealed vacuolation of germ cells which might result from metabolic disturbance in these cells and change in their morphology (Manivannan et al., 2009). Moreover, ROS resulting from SEM could lead to damage of the cell membrane as well as membranes of the cellular organelles that increase their permeability and cause disturbance of the ion concentration in the cytoplasm and in the organelles thus affecting the volume regulation of the cell (Emanuel, 2001). It was suggested that this vacuolation might be due to dilated smooth endoplasmic reticulum (SER) as a trial to detoxify the noxious agent affecting the cell (Creasy, 2001). The dilated rough ER detected in this study was suggested to be a sign of cellular degeneration caused by SEM whereas focal cytoplasmic loss might be a result of degeneration and loss of cellular organelles, leaving their sites empty, or it may be a sign of cellular degeneration caused by SEM (Hirakawa et al., 2003).

Nevertheless, mitochondrial swelling depicted in the current work could be considered as a pre-apoptotic sign. It was stated that the oxidative stress causes the opening of the mitochondrial permeability transition pores (PTP) resulting in the formation of high conductance channels in the inner mitochondrial membrane leading to mitochondrial swelling. Additionally, degenerating cells that are phagocytosed by the Sertoli cells could result in the accumulation of lipid droplets and electron dense bodies in the cytoplasm of Sertoli cells (Soliman et al., 2014) as was similarly observed in the present work.

The chromatoid bodies detected in the spermatogenic cells were suggested to be fibro-granular structures formed by mitochondriaand it disappears during the end stages of spermiogenesis (Wang et al., 2015; Pauli, et al., 2017). Peruquettiet al. (2008) stated that the chromatoid body observed near the axonema of late spermatids might result from disintegration of spermatid nucleoli at the onset of spermatogenesis then a fraction of this nucleolar material migrates to the cytoplasm in the form of chromatoid body participating in some parts of the rodent spermiogenesis process.

Taken altogether, it could be concluded that SEM causes significant structural changes in the testicular seminiferous tubules of juvenile albino rat. It can be recommended that using alternative substances in the sealing process will be better or to use concomitant antioxidant. Further studies on SEM effect on adult testis and its effect on serum testosterone arealso recommended.

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الملخص العربى

دراسة هستولوجية لتأثير السيميكاربازيد على الأنابيب المنوية الدقيقة فى خصية الجرذ الأبيض في سن الحداثة

أسماء محمد على منصور و مروة عوض عبد الحميد إبراهيم و عصام محمود إبراهيم لعج و عبد المنعم فريد زمزم

يعد سيميكاربازيد منتجاً ثانوياً لمادة الأزودايكربوناميد الذي يستخدم لتحسين خصائص الأختام البلاستيكية المستخدمة في صنع أغطية الأطعمة المعلبة متل أطعمة الأطفال. و قد كان الهدف من هذه الدراسة هوتقييم تأثيرمادة السيميكاربازيد على التركيب الدقيق للأنابيب المنوية في خصية الجرذان البيضاء في سن الحداثة. وقد أجريت هذه الدراسة على ٢٠ من ذكور الجرذان البيضاء الذين يبلغ عمرهم ٤ أسابيع و قد تم تقسيم الحيوانات إلى مجموعتين رئيستين: المجموعة الأولى (الضابطة) و المجموعة الثانية (المعالجة بالسيميكاربازيد) التي تلقت سيمكاربازيدعن طريق الفم بواسطة أنبوب المعدة مرة واحدة يومياً بجرعة ٤٠ ملجم / كجم من وزن الجسم. و قد أخذت العينات من الخصيتين وتم إجراء دراسات هستولوجية مختلفة و دراسة هستوكيميائية بإستخدام صبغة شيف الحامضية و دراسة مناعية بإستخدام الأجسام المضادة ل BCL-2 بالإضافة إلى إجراء تحليل قياسى شكلى و إحصائي للنتائج.

وقد أظهرت نتائج هذه الدراسة فى المجموعة الثانية أن بها بعض التجاويف في السيتوبلازم فى بعض الخلايا المبطنة لجدار الأنابيب المنوية و كذلك فى خلايا سيرتولى و كذلك إنقطاعاً للنسيج المبطن لبعض الانابيب المنوية و قد وجدت خلايا مقشرة فى تجاويف بعض الأنابيب المنوية بالإضافة إلى فقد موضعى للغشاء القاعدى. كما أظهرت الدراسة إنخفاضاً ذا دلالة إحصائية في كل من متوسط إرتفاع الظهارة الجرثومية والنسبة المئوية لمساحة الظهارة في المجموعة الثانية كما أظهر إستخدام صبغة شيف الحامضية نقصاً موضعياً فى التفاعل مع صبغة شيف، كما أظهرت الصبغة المناعية BCL-2 إنخفاضاً ذا دلالة إحصائية فى الصبغة المناعية الموجودة فى سيتوبلازم خلايا النبيبات المنوية للمجموعة الثانية. وقد كشف الفحص المجهرى الإلكترونى للمجموعة الثانية تورم الميتوكوندريا، و توسع في الشبكة الإندوبلازمية الخام، و تجاويف و فقدان للسيتوبلازم في بعض مناطق من الخلايا المنوية و خلايا سيرتولى. كما ظهرت أنوية بعض الحيوانات المنوية الأولية وخلايا أرومة النطفة داكنة أو أصغر من حجمها الطبيعى أو مجزأة. علاوة على ذلك وجدت زيادة في سمك الغشاء القاعدى مع ترسب للكولاجين فيه. و يمكن إستنتاج أن مادة السيميكاربازيد تسبب تغييرات هيكلية ملحوظة فى التركيب الدقيق للأنابيب المنوية فى خصية ذكور الجرذان البيضاء فى سن الحداثة. كما يوصى باستخدام مواد بديلة فى عملية الحبك لأغطية الأطعمة المعلبة.

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