Original Article

The Effect of Semicarbazide on the Thyroid Gland of Juvenile Male Albino Rat and Possibility of Recovery: Histological and Immunohistochemical Study

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ABSTRACT

Background: Semicarbazide (SEM) is a byproduct of azodicarbonamide widely employed in the industry of plastic gaskets used in sealing glass jars, especially baby foods jars.

Aim of the Study: To determine the effects and possibility of recovery of semicarbazide on thyroid histology of male juvenile albino rats.

Materials and Methods: Thirty juvenile male albino rats (3 weeks old) were Split into 3 equal groups; group I (control), group II (treated with SEM 40 mg/kg oral daily for 4 weeks), group III (recovery group allowed to recover for 4 weeks after stoppage of SEM treatment). Different histological and immunohistochemical techniques were used on thyroid specimens.

Results: In comparison to the control group, group treated with SEM revealed distortion and degeneration of the thyroid follicles, some follicles appeared markedly distended and lined by flat cells with flat nuclei, desquamated follicular cells were located in the lumen, peripheral vacuolation of colloid, empty follicles, wide interstitial space and dilated congested blood vessels in between follicles. Collagen fibers amount in the interstitial space has increased significantly "$P < 0.05$". The mean epithelial height and colloid area percentage have both decreased significantly. Activated caspase-3 immunohistochemical expression was significantly increased. Contrary, in the recovery group, most of the Follicles appeared to be normal except for minimal changes in some follicles.

Conclusion: Semicarbazide causes significant histological structural changes in the juvenile thyroid gland and its stoppage caused marked improvement in the thyroid gland histology. It is advisable to replace semicarbazide as a sealing agent for glass jars with other natural products.

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Key Words: Caspase-3; recovery; semicarbazide; thyroid follicles.

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INTRODUCTION

Semicarbazide (SEM) is a hydrazine derivative that is mainly derived from the decomposition of nitrofurazone (banned veterinary antibiotics) and thermal breakdown of azodicarbonamide (a blowing agent used in food processing by hypochlorite treatment)[1,2,3]. Semicarbazide is present in the form of a white crystalline powder that is highly water-soluble[4]. Semicarbazide has been used in making the plastic gaskets that seal the jar lids of multiple products, such as baby foods, fruit juices, jams, pickles, mayonnaise, ketchup and mustard, with the aim of prevention of leakage and microbial contamination of the jar content. Semicarbazide is usually released during the heating of packaged food to ensure a tight seal[5,6]. Moreover, semicarbazide is used in certain countries as a flour treatment agent due to its dough improvement effect[7].

Intake of SEM has been reported to be higher in infants than in adults due to the high intake of food in glass jars by infants and the infants' small body weight. Previous study revealed that the intake of SEM-HCl per kilogram of body weight in young animals was about twice that in adult rats. Additionally, young animals received SEM-HCl at a dose of 500 ppm appeared with more severe lesions when compared with adults received SEM-HCl at a dose of 1,000 ppm. Therefore, young rats are more susceptible to SEM-HCl than adults[8]. The toxic effect of SEM on many body organs has been investigated. It was proved that SEM can induce severe alterations in the histological structure of epiphysial cartilage with deformation of articular cartilage[9]. Additionally, many teratogenic effects have been observed as a complication of SEM intake such as cleft palate and aortic aneurysms[10]. Moreover, the toxicity of SEM during the peripubertal period on the testes was reported in many studies[11,12,13]. Studies reported that SEM appeared to act as an endocrine disrupter in both sexes by alteration in the onset of puberty and in the levels of sex steroid hormones[14].

The thyroid gland is an endocrine gland that secretes groups of hormones called thyroid hormones. Thyroid hormones are necessary for almost all the functions of body organs. They have a crucial role in growth and development especially brain development. Additionally,

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they are involved in the regulation of protein, carbohydrates and lipid metabolism[15,16]. A previous study was done to evaluate the thyroid disruption induced by semicarbazide in Japanese flounder (Paralichthys olivaceus)[3]. This study's purpose was to assess the effect of semicarbazide on male albino rats' thyroid glands morphology, and whether they could recover after stopping the medication.

MATERIAL AND METHODS

Materials

1. Semicarbazide was purchased from Aldrich Chemical Company Inc. United States
2. Rabbit polyclonal anti-rat activated caspase-3 antibody ab2302, an apoptotic marker, Abcam, Cambridge, Massachusetts, USA.

Animals and experimental architecture

In this study thirty young, 40-50 grams weight male albino rats (3 weeks of age, recently weaned) were used. They were maintained during the 12 hours of light/12 hours of darkness before and during the trial. Animals have been kept in well ventilated clean cages with unlimited access to a well-balanced laboratory diet and plenty of water. The Local Ethics Committee of Tanta University's Faculty of Medicine gave their approval to the experiment. The animals were divided into three equal groups at random:

Group I (control group): Ten animals were divided equally into two equal subgroups; subgroup (Ia) was left without treatment for eight weeks, and subgroup (Ib) received 0.5 ml distilled water orally by gavage once daily for 4 weeks and then left without treatment for another 4 weeks.

Group II (Semicarbazide (SEM) group): Ten animals received semicarbazide by oral gavage once daily at a dose of 40 mg/kg body weight dissolved in 0.5 ml distilled water for 4 weeks[6].

Group III (Recovery group): Ten animals was given 40 mg/kg body weight semicarbazide dissolved in 0.5 ml distilled water by gastric gavage once a day for 4 weeks before being left untreated for another 4 weeks.

Specimens processing and staining

Animals were euthanized at the end of the experiment by administering pentobarbital (40 mg/kg) intraperitoneally[17]. For light-microscopic examination, thyroid glands have been dissected and processed.

For the histological study, Specimens of thyroid gland had been fixed for 24 hours in a neutral-buffered formalin of 10 per cent, processed in flat molds and subsequently embedded in paraffin, by routine procedures. Finally, hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) reactions, as well as Masson's trichrome stain, were used to stain 5 µm sections[18,19].

For the immunohistochemical study, sections of 5 µm thickness were derived from the formalin-fixed, paraffin-embedded specimens, and incubated with the primary antibody (activated caspase-3 antibody) in a humid chamber overnight. The sections were then rinsed three times in PBS before being incubated for one hour at room temperature with the corresponding biotinylated secondary antibody. After that, the streptavidin peroxidase was applied for 10 minutes and rinsed three times in PBS. The chromogen 3,3'diaminobenzidine (DAB)-hydrogen peroxide was used to visualize immunoreactivity. Mayer's haematoxylin was used to counterstain the sections. By omitting the primary antibodies, negative control sections were developed[20].

Morphometric study

The image analysis system (Leica Q 500 MC programme) at Tanta University's Faculty of Medicine was used to test the following measurements:

1. The height of the follicular epithelium in H&E-stained slides.
2. The area percentage of colloid in PAS-stained slides.
3. The area percentage of collagen fibers in Masson's trichrome stained-slides.
4. The color intensity of caspase-3 positive immunoreaction.

Ten different microscopic fields from each specimen in every experimental group were examined for the above-mentioned parameters at a magnification of X400.

Statistical analysis

The analysis of data was done using single-way variance analysis (ANOVA), followed by a group-to-group comparison test using statistical package for social sciences statistical analysis software (IBM SPSS Statistics for Windows, IBM Corp, Version 22.0. Armonk, NY, USA). The mean and standard deviation were used to express all of the results. If the probability value $p < 0.05$, the differences were considered significant[21].

RESULTS

Histological results

Regarding H&E stained sections, the control group showed that the thyroid gland was divided into numerous lobules by thin connective tissue septa containing blood vessels. The lobules were mostly made up of variable-sized follicles with simple cuboidal epithelial cells lining and central lumen, which was filled with homogeneous acidophilic colloid (Figures 1,2).

Thyroid sections from SEM treated group revealed distortion of the normal architecture of the thyroid follicles, some follicles appeared markedly distended and were lined by flat cells with flat nuclei. In the lumen of some follicles, desquamated follicular cells were located (Figure 3). Additionally, peripheral vacuolation of colloid was noticed in many follicles (Figures 3,4). Empty follicles

and wide interstitial space were observed in some sections (Figure 4). Other sections showed disruption of follicular basement membrane and fusion of the follicles (Figures 3,5). Follicles with hyperplasic wall and disorganized irregular follicles were also present (Figure 5). Furthermore, some follicular cells had vacuolated cytoplasm and nuclei that were deeply stained (Figure 6). In between thyroid follicles, dilated congested blood vessels were also observed (Figure 6).

On examination of the recovery group, four weeks after stopping the intake of SEM, most of the Follicles appeared to be similar to the control group, with simple cuboidal epithelium lining them and homogeneous acidophilic colloid filling their lumens. Few follicles were observed with peripherally vacuolated colloid. Moreover, dilated congested blood vessels appeared also in some sections (Figure 7).

In PAS stained sections of the control group, follicular cells basal laminas showed moderate PAS reaction, and a strong reaction appeared in the colloid (Figure 8). Regarding SEM- treated group, follicular cells basal laminas appeared with a weak PAS reaction, besides, the colloid exhibited a moderate PAS reaction (Figure 9). However, in the recovery group, moderate PAS reaction appeared in basal laminas with strong PAS reaction in the colloid (Figure 10).

Regarding sections stained with Masson's trichrome, the control group had thin collagen fibers that separated the gland lobules as well as the follicles (Figure 11). In contrast, the SEM-treated group revealed an excessive amount of collagen fibers separating the gland lobules and in between the follicles (Figure 12). As regard the recovery group, a few amount of collagen fibers in-between gland lobules and in between the follicles were noticed (Figure 13).

Immunohistochemical results (Caspase-3 protein)

The control group showed a weak positive reaction that appeared in the nuclei and/or the cytoplasm of few follicular cells (Figure 14). Contrary, the SEM-treated group showed strong immunohistochemical expression of caspase-3 protein in nuclei and cytoplasm of many follicular cells (Figure 15). Considering the recovery group, weak positive caspase-3 immunoreactivity was observed in the nuclei and/or the cytoplasm of few follicular cells (Figure 16).

Morphometric and statistical results (Table 1, Histogram 1)

- The mean height of the follicular epithelium in the SEM-treated group was significantly decreased compared to the control, while the recovery group showed a non-significant decrease compared to the control group.
- As compared to the control group, the mean area percentage of colloid in the SEM-treated group was significantly decreased, while the recovery group showed a non-significant decrease in the mean area percentage of colloid compared with the control group.
- The mean area percentage of collagen fiber content in the SEM-treated group showed a significant "$P < 0.05$" increase compared to the control group. On the contrary, the recovery group revealed a non-significant increase in the mean area percentage of collagen fiber compared to the control group.
- The mean color intensity of activated caspase-3 positive immuno-reactivity in SEM-treated group showed a significant "$P < 0.05$". increase compared to the control group, while recovery group showed a non-significant increase compared to control group.

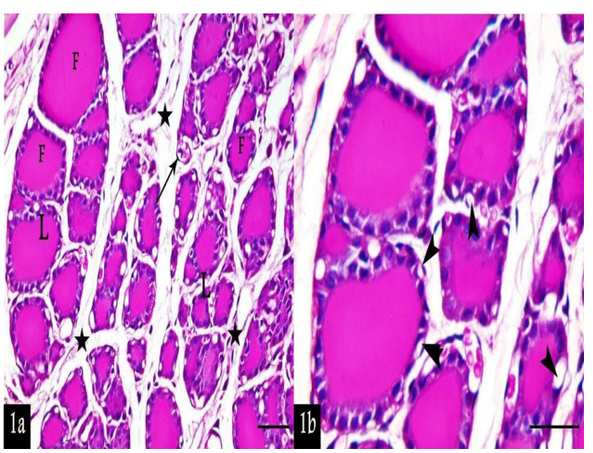


Fig. 1(a,b): A photomicrograph of a thyroid gland section of the control group showing numerous irregular lobules (L) containing follicles of various sizes (F) separated by CT septa (stars) containing blood vessels (arrow). Notice the presence of blood capillaries around the thyroid follicles (arrows head). (a: H&E. x400; scale bar= 50µm. b: H&E. X1000; Scale bar = 20µm)

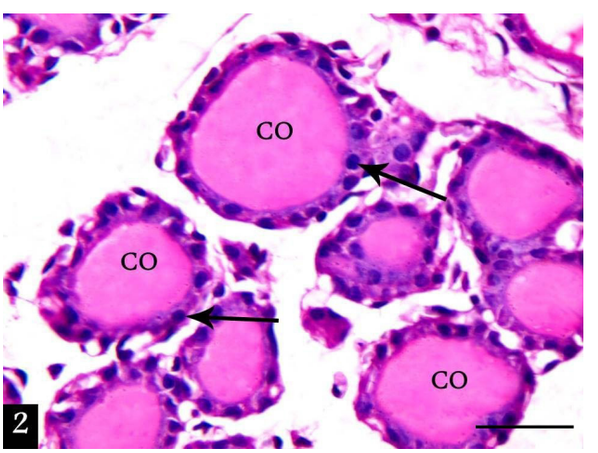


Fig. 2: A photomicrograph of a thyroid gland section from the control group showing thyroid follicles lined with follicular cuboidal epithelial cells with rounded nuclei (arrows) and filled with homogenous acidophilic colloid (CO). (H&E. X1000; Scale bar = 20µm)

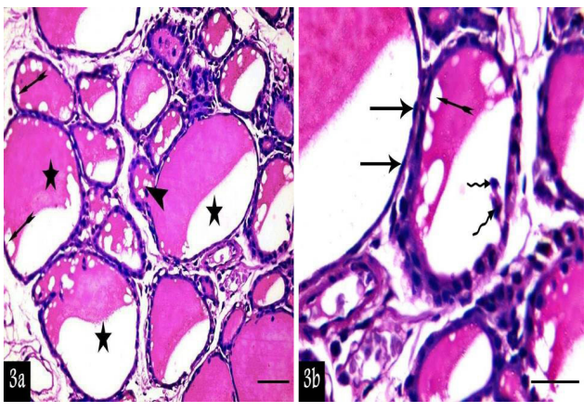


Fig. 3 (a,b): Photomicrographs of a thyroid gland section of semicarbazid-treated group showing disorganized thyroid follicles. Some follicles appear markedly distended (stars) and lined by flat cells with flat nuclei (arrows), peripheral vacuolation of colloid (notched arrows) are present in some follicles. Note the disruption of follicular basement membrane with fusion of follicles (arrow head) and desquamated cells in the follicular lumen (wavy arrow). (a: H&E. x400; scale bar= 50µm. b: H&E. X1000; Scale bar = 20µm)

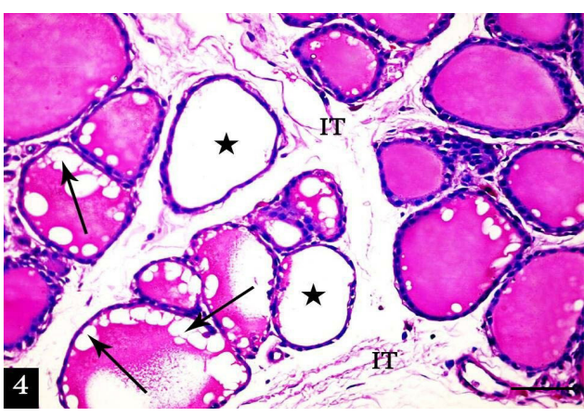


Fig. 4: A photomicrograph of a thyroid gland section of semicarbazide-treated group showing some follicles with peripheral vacuolated colloid (arrows) and other follicles appear empty (stars). Notice the wide interstitial space (IT). (H&E. x400; scale bar= 50µm)

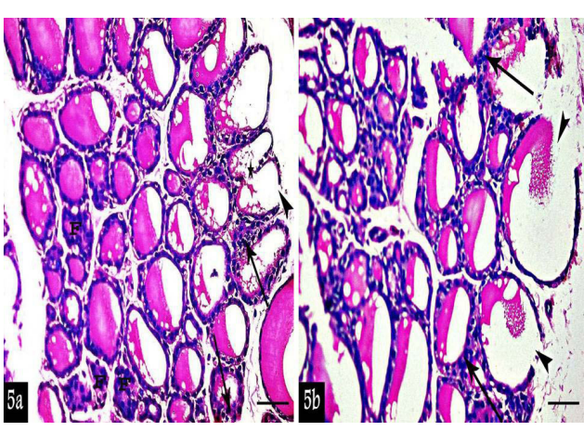


Fig. 5 (a,b): Photomicrographs of thyroid gland sections of semicarbazide-treated group showing follicles with hyperplastic wall (arrows), disruption of the basement membrane of some follicles (arrows heads) and fused follicles (star). Notice the presence of disorganized irregular follicles (F). (H&E. x400; scale bar= 50µm)

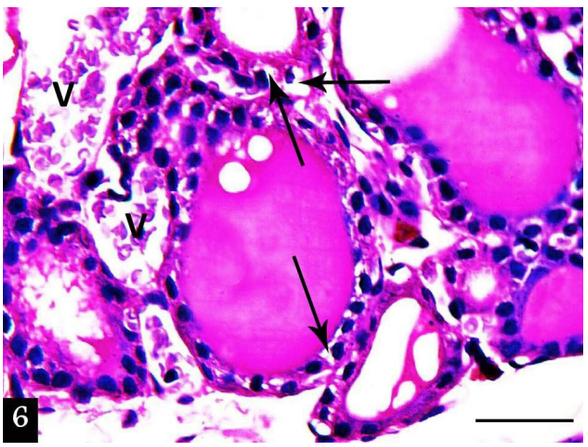


Fig. 6: A photomicrograph of a thyroid gland section of semicarbazide-treated group showing vacuolated cytoplasm and deeply stained nuclei in some follicular cells (arrows). Notice congested dilated blood vessels (V) in between follicles. (H&E. X1000; Scale bar = 20µm)

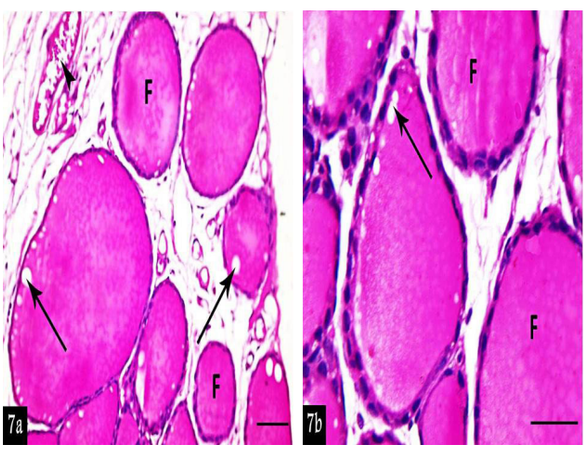


Fig. 7 (a,b): Photomicrographs of thyroid gland sections from the recovery group showing many apparent normal follicles (F). Some follicles appear with peripheral vacuolated colloid (arrows). Note congested blood vessel (arrow head). (H&E. X400; Scale bar = 50µm)

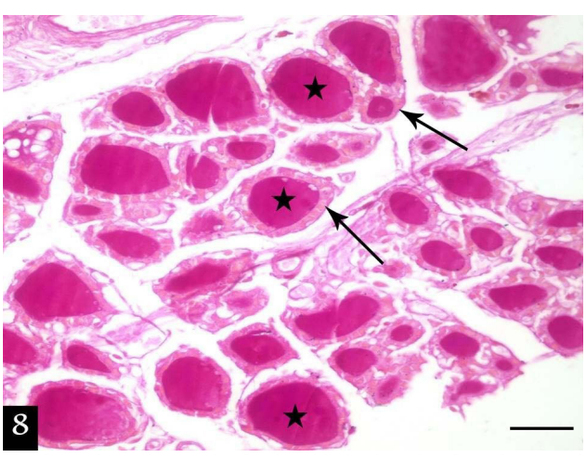


Fig. 8: A photomicrograph of a thyroid gland section from the control group showing strong PAS reaction (stars) in the colloid of the follicles and moderate reaction (arrows) in the basal lamina of the follicular epithelium. (PAS. X400; Scale bar = 50µm)

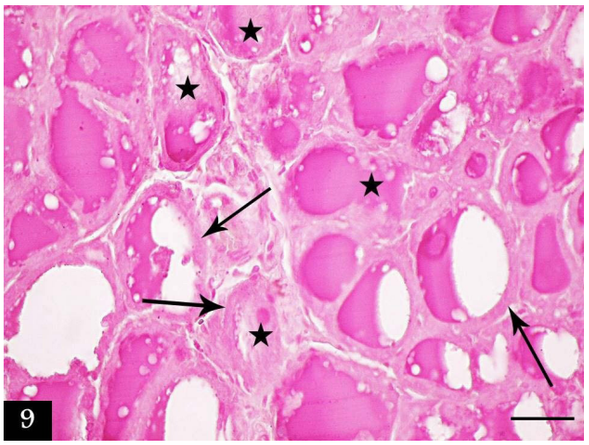


Fig. 9: A photomicrograph of a thyroid gland section from semicarbazide-treated group showing moderate PAS reaction (stars) in the colloid of the follicles with weak reaction (arrows) in the basal lamina of the follicular epithelium. (PAS. X400; Scale bar = 50µm)

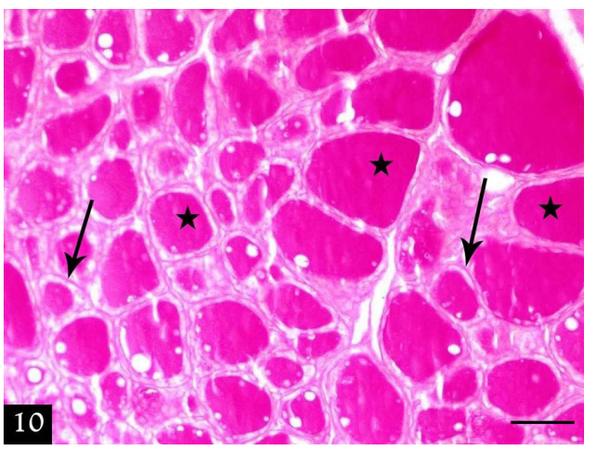


Fig. 10: A photomicrograph of thyroid gland section from recovery group showing strong PAS reaction (stars) in the colloid of the follicles and moderate PAS reaction in the basal lamina of the follicular epithelium (arrows). (PAS. X400; Scale bar = 50µm)

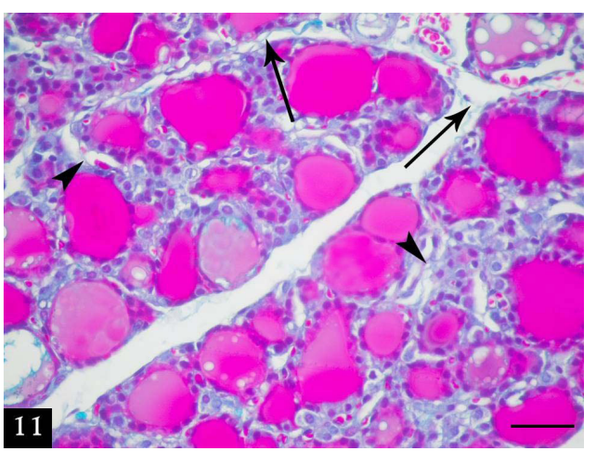


Fig. 11: A photomicrograph of thyroid gland section from control group showing thin collagen fibers (arrows) separating the gland lobules and present also in between the follicles (arrows head). (Masson's trichrome X400; Scale bar = 50µm)

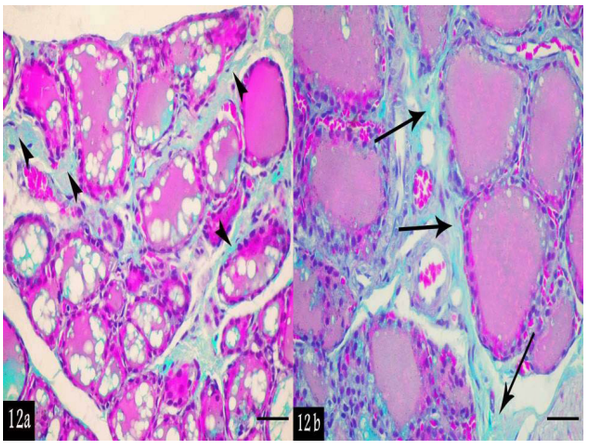


Fig. 12 (a,b): Photomicrographs of thyroid gland sections from semicarbazide-treated group showing excessive amount of collagen fibers separating the gland lobules (arrows) and excessive amount of collagen fibers in between the follicles (arrows head). (Masson's trichrome. a, b X400; Scale bar = 50µm)

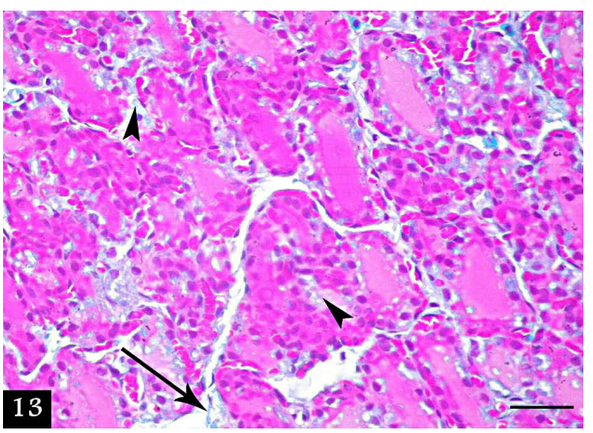


Fig. 13: A photomicrograph of a thyroid gland section from recovery group showing few collagen fibers separating the gland lobules (arrows) and also in between the follicles (arrows head). (Masson's trichrome. X400; Scale bar = 50µm)

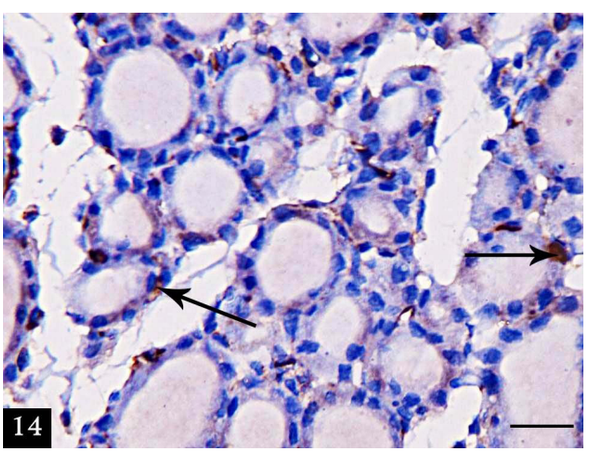


Fig. 14: a Photomicrograph of a thyroid gland section from the control group showing weak positive nuclear and/or cytoplasmic immunoreactivity (arrows) for caspase-3 in few follicular cells. (Caspase-3 immunostaining, X400; Scale bar = 50µm)

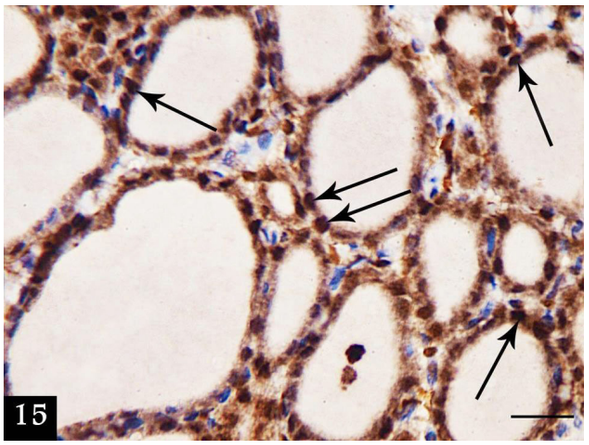


Fig. 15: Photomicrographs of thyroid gland sections from the semicarbazide-treated group showing strong positive nuclear and/or cytoplasmic immunoreactivity (arrows) for caspase-3 in many follicular cells. (Caspase-3 immunostaining, X400; Scale bar = 50µm)

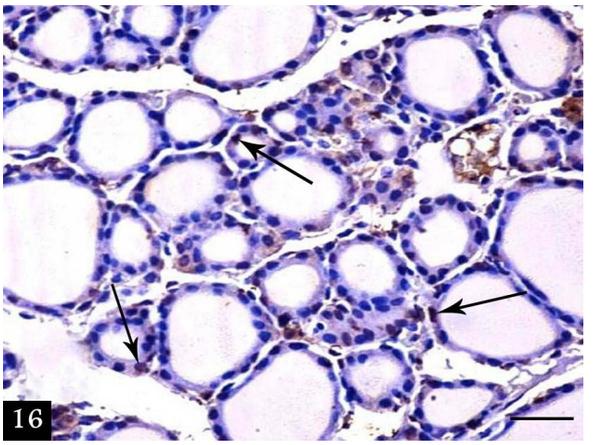


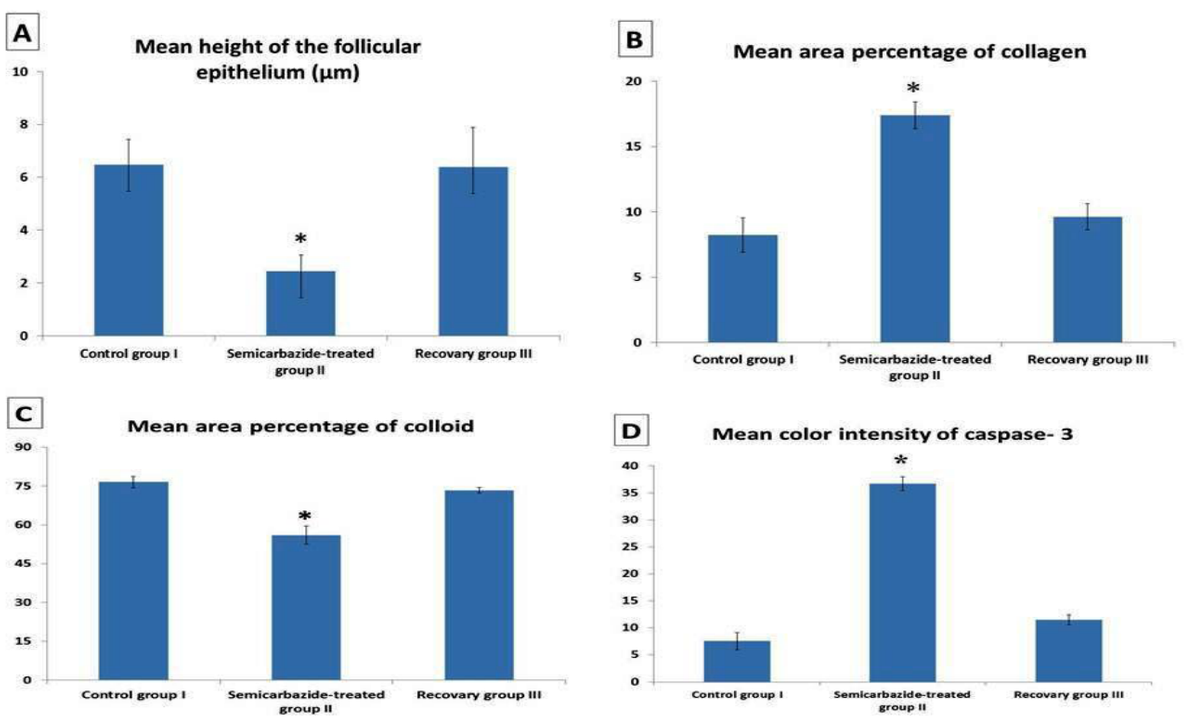
Fig. 16: A photomicrograph of a thyroid gland section from the recovery group showing weak positive nuclear and/or cytoplasmic immunoreactivity (arrows) for caspase-3 in few follicular cells. (Caspase-3 immunostaining, X400; Scale bar = 50µm)

Table 1: Morphometric analysis of the thyroid gland specimens in different groups.

Parameters	Group I	Group II	Group III
Mean height of the follicular epithelium (µm)	6.475±0.96	2.453±0.605*	6.395±1.491
Mean area percentage of collagen	8.231± 1.34	17.406±1.031*	9.619±1.008
Mean area percentage of colloid	76.536±2.21	56.0304±3.44*	73.3546±1.17
Mean color intensity of caspase-3	7.547±1.579	36.733±1.303*	11.508±0.944

Data are expressed as mean ± standard deviation

*$P < 0.05$ is significant versus control.



Histogram 1: Morphometrical and statistical analysis of the different study groups; A] Mean height of the follicular epithelium (µm), B] Mean area percentage of collagen, C] Mean area percentage of colloid, D] Mean color intensity of caspase-3. * indicates significant vs control.

DISCUSSION

Semicarbazide (SEM) is a by-product of azodicarbonamide processing. European Food Safety Authority reported that SEM is found in certain foods, including mainly baby foods, which are packed in glass jars with plastic gaskets around the metal lids[22]. The discovery of the high incidence of baby foods contamination with semicarbazide as a result of packaging has paid the attention to the toxicity of this substance[23].

The thyroid gland is an essential endocrine gland that secretes hormones such as thyroglobulin, triiodothyronine and thyroxin. All reactions necessary for the synthesis of T3 and T4 are affected and controlled by thyroid-stimulating hormone (TSH) of the pituitary gland. Thyroid hormones are important for the normal growth, development and maintenance of functions of different tissues and organs. The functions of the thyroid gland can be influenced by various factors in experimental animals and humans with subsequent major effects on growth, hearing, mental acuity, and reproductive system development and function[24,25]. The current work was carried out to evaluate the effects of SEM on thyroid gland morphology of juvenile albino rats with evaluation of the probability of recovery following its withdrawal.

The examination of the SEM-treated group revealed distortion of the thyroid follicles' normal architecture. Follicles appeared markedly distended with peripherally vacuolated colloid. Vacuolated cytoplasm and deeply stained nuclei were found in several follicular cells. Other changes were observed in the form of many empty follicles, desquamated cells, congested dilated blood vessels, wide interstitial space and hyperplastic follicles.

Previous researches were in accordance with our results. They reported that the administration of SEM during the juvenile period caused a dose–dependent obvious alteration in the architecture of thyroid tissue in the form of exfoliation of necrotic follicular epithelial cells into the lumen and colloidal fluid with subsequent impairment of thyroid functions in adults[6]. The increased susceptibility of the juvenile period to the toxic effects of semicarbazide in comparison to adulthood may be due to the immaturity of the elimination systems such as the immature metabolizing enzymes and renal functions[26].

The degenerative changes caused by SEM were assumed to be a result of the release of SEM-derived free radicals and reactive species[27]. Elevation of the levels of ROS as hydrogen peroxide (H_2O_2), hydroxyl radicals, and superoxide radical ions causes a subsequent increase in lipid peroxidation, DNA damage and finally cell death[28,29]. Previous studies proved that the release of ROS resulted in DNA fragmentation, increased the release of cytochrome C from the mitochondria to the cytosol and upregulated pro-apoptotic markers with an increase in the incidence of apoptosis. This is in accordance with our results and can explain the strong immunohistochemical expression of the caspase-3 protein in follicular cells nuclei and cytoplasm of SEM- treated rats[30]. The increase in the release of ROS and lipid peroxidation could also explain the excessive deposition of collagen fibers in-between the gland lobules and follicles in this SEM-treated group[31].

Moreover, most of the structural changes that occurred in the thyroid follicles might be a response to TSH hypersecretion in order to compensate for declining T3 and T4 levels. The decreased level of thyroid hormones resulted from high levels of ROS which caused damage of thyroid follicular cells (the main sites of thyroid hormones synthesis)[32]. TSH is considered a major growth factor for the thyroid gland, it can exert its action on the follicular cells through a group of molecules as neuropeptides and peptides which are secreted by Para follicular cells[33]. The high levels of TSH can explain the hyperplastic wall that appeared in some follicles because TSH is responsible for thyroid follicles morphological appearance[34]. Additionally, high TSH level can explain the vacuolation of the colloid observed in most follicles. It was reported that during the high demand for thyroid hormones pseudopods may extend from the follicular cells into the follicular lumen to envelop and absorb the colloid with subsequent appearance of colloid vacuolation[35].

Examination of the SEM-treated group revealed a significant "$P < 0.05$" decrease in the height of the follicular epithelium and the mean area percentage of colloid in comparison with the control group. These changes could be assumed to be the results of gland degeneration. The reduction of the height of the follicular epithelium could be explained by the thyroid hypofunction or unstimulated follicular epithelial cells due to reduction of the thyroid hormones that can follow the state of oxidative stress[36].

Other changes were observed in the SEM-treated group including irregular disorganized follicles, empty follicles, desquamated cells in the lumen of some follicles. These changes might be explained by accumulation of colloid in follicular cells that caused disruption and collapse of the cells which resulted in collapse of the follicles[37]. Wide interstitial spaces noticed in this study might be a sequence of disruption of the connective tissue because SEM impairs cross-linking reactions of collagen and elastin, which are essential for maturation of connective tissues,[8,38].

Dilated congested blood vessels were found in between follicles. A previous study attributed this change to the effect of SEM which caused tissue ischemia followed by reperfusion state which was accompanied by infiltration of the inter-tubular spaces by different types of cells as macrophage and leukocytes, the cytokines secreted by macrophages caused vascular congestion[12]. Additionally, mast cells that might be located around blood vessels secreted some mediators or growth factors that could induce angiogenesis[39]. Other researchers attributed this vascular congestion to the high TSH level[40].

In this work, the group of animals allowed to recover for four weeks after cessation of SEM treatment. Specimens of this group showed restoration of the normal

histological architecture of the gland to some extent. Most of the follicles appeared normal with their simple cuboidal epithelial lining and filled with colloid except for peripheral vacuolation of colloid in some follicles. The amount of collagen fibers and caspase-3 immunoreactivity were decreased. While the height of the follicular epithelium and the mean area percentages of colloid is significantly increased. The mechanism of SEM toxic effects was suggested by researchers to be due to its inhibitory action on superoxide dismutase and glutathione peroxidase, the major two enzymes responsible for scavenging harmful reactive oxygen species (ROS)[41]. we can suppose that stoppage of the treatment with SEM led to decrease of its inhibitory effect of these enzymes with subsequent relieve of the state of oxidative stress and improvement in the morphology of the thyroid gland after recovery.

In addition, a recent study proved the probability of rapid recovery and regeneration of thyroid gland after its damage by diphtheria toxin and tamoxifen, 8 weeks after their cessation via the resident stem cells[42]. It is well known that resident stem cells in tissues are maintained in a quiescent state and are awakened from their cell cycle arrested state to an active proliferative state as a response to injury[43]. Some recent researches stated the presence of thyroid "cancer stem cells" within the thyroid or after partial resection of the thyroid gland[44]. Thus, restoration of the normal histological structure of thyroid gland can be explained by the surge in stem cell activity after SEM injury[42].

CONCLUSION

It can be concluded that semicarbazide exerted toxic effects on the thyroid gland of juvenile rats. Stoppage of the use of semicarbazide caused marked improvement in the thyroid gland histology. So we recommend stopping usage of any food products contained in glass jars especially for babies and replace them with fresh foods. Moreover, factory owners must replace semicarbazide as a sealing agent for glass jars with other natural products.

CONFLICT OF INTERESTS

There are no conflicts of interest

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الملخص العربى

تأثير السيميكاربازيد على الغدة الدرقية لذكر الفأر الأبيض في سن الحداثة وإمكانية التعافي: دراسة هستولوجية وكيميائية مناعية

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مقدمة: يعد السيميكاربازيد منتجا ثانويا لمادة الأزودايكربوناميد ويُستخدم على نطاق واسع في صناعة الجوانات البلاستيكية التى تستخدم للحام أغطية الأطعمة المعبأة في أواني زجاجية ، وخاصة أطعمة الأطفال.
الهدف من العمل: تقييم تأثير السيميكاربازيد على التركيب الهستولوجى للغدة الدرقية لذكر الفأر الأبيض النامي وإمكانية التعافي.
مواد و طرق البحث: تم استخدام ثلاثين من ذكور الجرذان البيضاء في الدراسة, بلغ عمرهم ٣ أسابيع. وتم تقسيمها بالتساوي إلى ٣ مجموعات تجريبية. المجموعة الأولى (الضابطة) ، المجموعة الثانية التي تم علاجها بواسطة السيميكاربازيد (٤٠ ملجم / كجم) عن طريق الفم لمدة ٤ أسابيع ، المجموعة الثالثة المسموح لها بالتعافي لمدة ٤ أسابيع بعد توقف علاج السيميكاربازيد. وقد تم تجهيز عينات الغدة الدرقية للدراسة الهستولوجية والهستوكيميائية المناعية وقد تم عمل دراسة قياسية شكلية وتحليلها إحصائيا للمجموعات المختلفة.
النتائج: بالمقارنة مع المجموعة الضابطة ، أظهرت المجموعة التي عولجت بالسيميكاربازيد تشوية وتنكس في كريات الغدة الدرقية مع زيادة في كمية ألياف الكولاجين بين الكريات. كما لوحظ نقص كبير في كل من ارتفاع النسيج الطلائي المبطن للكريات والنسبة المئوية لمساحة المنطقة الغروانية. كما وجد زيادة في التفاعل المناعي للكاسبيز-٣. وقد أظهرت مجموعة التعافي استعادة البنية النسيجية الطبيعية للغدة الدرقية الى حد كبير.
الإستنتاج: يمكن استنتاج ان استخدام السيميكاربازيد يسبب تغيرات هيكلية كبيرة في التركيب الهستولوجى للغدة الدرقية لصغار الفئران البيضاء ويؤدى توقف استخدامه الى تحسن ملحوظ في هستولوجيا الغدة الدرقية ولذلك يُوصى باستبدال السيميكاربازيد بمنتجات طبيعية أخرى كعامل مانع للتسرب في الأواني الزجاجية.