Original Article

Harmful Effect of Semicarbazide on the Ovary of Adult Albino Rats and the Possible Reversibility of the Induced Lesions (Biochemical and Histological Study)

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ABSTRACT

Introduction: Semicarbazide (SEM) has gained concerns about its existence, excessive exposure and possible hazards to the human health. Infants and children are at more risk of exposure to SEM because their high intake of food put in glass jars as jams, fruit juices, honey, sterilized vegetables, mayonnaise, mustard, sauces and ketchups.

Aim of Work: To estimate the effects of semicarbazide on histological ovarian structure of adult albino rats and the chance for recovery after its withdrawal.

Materials and Methods: Forty-five adult female albino rats were divided into three groups. Control, semicarbazide treated (40 mg/kg body weight / day for four weeks) and withdrawal group (given SEM for four weeks as the previous group, then SEM was withdrawn for 2 weeks). Samples were gathered and examined by light and transmission electron microscopes. Changes in body weight, Gonadotrophin releasing hormone (GnRH), Luteinizing hormone (LH), Follicle stimulating hormone (FSH) and Estradiol (E2) levels were estimated.

Results: SEM enhanced significant drop in body weight, GnRH, LH, FSH and E2 hormone levels as well as histopathological alterations of ovarian tissues. Ovarian alterations appeared as degenerated follicles with exfoliated cells and hyaline material in their lumen. Granulosa cells were widly separated with ill-defined corona radiata. Various ultrastructural changes in follicular cells with irregularity and thickening in basement membranes surrounding them. Many lipid droplets in the cytoplasm of granulosa lutein cells were also demonstrated. These alterations were partially reversed on withdrawal of SEM.

Conclusion: Semicarbazide induced dangerous changes in rat ovarian structure. Parts of these changes were reversed after withdrawal of this agent but others were still present which have negative impact on reproductive functions.

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Key Words: Food contamination, ovaries, rat, semicarbazide.

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INTRODUCTION

Semicarbazide (SEM) is a chemical compound by-product of azodicarbonamide (ADC) widely used in many drugs such as the antibacterial nitrofurazone[1]. It is also used in industry to prepare photochromic dyes and medicine intermediates[2].

It can gain access to food through thermal breakdown of ADC when used as a blowing factor in plastic gaskets of food jars to prevent leak or microbial infection. It also gains access through food processing of breads made from ADC treated flour for improving baking properties. In addition, semicarbazide could be detected in some dried food[3].

Infants and children are at more risk of exposure to SEM because their high intake of food put in glass jars as jams, fruit juices, honey, sterilized vegetables, mayonnaise, mustard, sauces and ketchups. These jars hold higher proportion of gasket area relative to the amount of food in these small pack sizes[3,4]. Existence of SEM in aquatic environment in some places was also detected. Therefore, longstanding consumption of their aquatic products will be harmful. The polysaccharide Carrageenan taken from red seaweeds produces SEM under some circumstances. Carrageenan is utilized in food industries for thickening processes of food[5].

Excess exposure to SEM from different sources makes toxicity from it is very possible. It can induce bone[6] and vascular damages by affecting collagen and elastin cross-linking reactions[7]. Aortic aneurysms also have been documented[8]. In *vitro* changes of lymphocytes exposed to SEM pointed to its toxicological affection on human immunity[9]. In addition, SEM reduces mRNA expression levels of GnRH receptors, gonadotropins and testosterone hormone and disrupting reproductive function in male[10,11.]

Although many records about the hazardous outcomes of semicarbazide were developed, Europian Food Safety Authority documented, through European Commission, low amounts in foods from not detectable up to 20 µg/kg of food[3]. However, the usage of ADC as blowing factor in plastics is prohibited. Semicarbazide is still applied

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in food packing in some countries[12] that might increase the threat of exposure to its toxicity. So, many researches are needed to assess its effect on tissues of different body organs. Accordingly, the aim of this work was to estimate the effects of semicarbazide on histological ovarian structure of adult albino rats. The chance for recovery after its withdrawal was also assessed.

MATERIALS AND METHODS

Chemicals

The white powder semicarbazide hydrochloride, CAS Number: 563-41-, was bought from Sigma-Aldrich Chemicals, Cairo, Egypt. Its 99%.

Experimental animals

Forty-five adult female albino rats, weighing 180–200 g, 8 weeks old, were gained and kept at the Breading Animal House of Faculty of Medicine, Zagazig University in cages with suitable temperature and humidity. The female rats reach the sexual maturity and their estrus cycle begins at 8 weeks[13,14]. The rats were chosen at 8 weeks old and kept for accommodation time 2 weeks before the beginning of the experiment at the age of 10 weeks.

The experiment was done in adherence with the institutional guidelines for using experimental animals and agreed by the Medical Research Ethics Committee of Zagazig University, Egypt.

Experimental design

The rats were randomly separated into three equal groups.

Group I (control): kept without treatment for four weeks.

Group II (Semicarbazide treated): they were given semicarbazide hydrochloride (SEM) dissolved in distilled water orally one time a day by a gastric tube. the dose was 40 mg/kg body weight for four weeks[15].

Group III (withdrawal group): they were given SEM for 4 weeks as group II, then SEM was withdrawn for two weeks[16].

After ending of the experiment (group I and II were sacrificed after four weeks and group III after six weeks), rats were weighted, anesthetized with thiopental (50 mg/kg) intraperitoneally[17], then sacrificed. Capillary tubes were used to collect blood from retro-orbital plexuses for biochemical studies, centrifuged after clotting and the sera were stored at -80[18]. The ovaries were cut out & processed for light and transmission electron microscopic examinations.

Biochemical study

Serum levels of GnRH, FSH and E2 were detected using Enzyme linked immunoassay (ELISA) kits (CusaBio Biotech Co., Ltd., Wuhan, China; GnRH, cat. no. CSB-E08037r; FSH, cat. no. CSB-E06869r and E2, cat. no. CSB-E05110r respectively). Serum LH were assessed by Elisa kits (Cas no. MBS729873 from MY biosource company), according to manufacturers' protocols.

Histopathological study

Haematoxylin and eosin (H&E) stain: Specimens were placed in saline formalin and managed to develop 5-µm-thick paraffin sections[19].

Ultrastructural study

Specimens were immediately fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.4), post fixed in osmium tetroxide in the same buffer at 4°C, dehydrated and embedded in epoxy resin to get ultrathin sections (Leica ultra-cut UCT). after staining with uranyl acetate and lead citrate[20], sections were photographed (JEOL JEM 1010 transmission electron microscope; Jeol Ltd, Tokyo, Japan) in Electron Microscope Research Center belonging to the Faculty of Agriculture, El Mansoura University, Egypt.

Toluidine blue stain: Semi-thin sections (Leica ultra-cut UCT) stained with toluidine blue were examined by light microscope.

Statistical analysis

Mean ± standard deviation (X ± SD) for data (body weight and serum levels of GnRH, FSH, LH and E2) was calculated. SPSS program version 21 (Chicago, USA) was used. Statistically, a significant difference was settled by one-way analysis of variance (ANOVA), followed by the LSD and post-hoc test for several comparisons between different groups. The *P* less than 0.05 was considered significant and highly significant when the *P values* were less than 0.001.

RESULTS

General toxicity and body weight

No observed general toxicity on rats with no death rates. A significant reduction in the body weight of SEM treated rats was recorded when compared with control and withdrawal groups (Table 1)

Biochemical study

Significant decrease in the mean values of GnRH, LH, FSH and E2 were detected in SEM and withdrawal groups when compared with control group. On the other hand a significant improvement in the serum levels of GnRH, LH, FSH and E2 were registered in withdrawal group when compared with SEM group ($p<0.01$) (Table 1)

Histopathological results

Light microscope results

H&E results

Examining H&E stained sections belonging to the control group showed that ovarian tissues were covered by surface epithelia and possessed many follicles at different phases of development; primordial, primary and secondary

follicles. The follicles were separated by stromal cells. Graffian follicles appeared with oocytes, corona radiata, cumulus oophorus, zona pellucida and antral spaces. Many corpora lutea were also seen (Figure 1A,B). Ovarian cortex of semicarbazide treated group contained many degenerated follicles. Some of them contained hyaline material in their lumen, others contained dark nuclei in their granulosa cells which were widely separated and some were exfoliated in the lumen. Mature graffian follicles were shown with ill-defined corona radiata. Congested dilated blood vessels were also seen between the follicles (Figure 1C,D). Nearly normal ovarian tissue in the withdrawal group (Figure 1E).

Toluidine blue results

Semithin sections in the ovary stained by toluidine blue of the control group showed mature follicles with polyhedral granulosa cells that have round vesicular euchromatic nuclei with one or two nucleoli. Regular basement membrane separated them from elongated theca cells that were differentiated into theca interna and theca externa (Figure 2A). Semicarbazide treated group showed granulosa cells of degenerated follicle appeared with vacuolated cytoplasm, wide separations and hyaline materials between them. Theca externa cells were filled with lipid droplets. Dilated blood vessels were also seen. Poly morphnuclear neutrophils were seen in ovarian tissues of this group (Figure 2B,C). Near normal withdrawal group tissues with granulosa and theca cells separated by basement membranes (Figure 2D).

Electron microscope results

Transmission electron micrograph of the control ovary showed the contents of primary follicles where oocytes surrounded by simple cuboidal layer of granulosa cells. Thin regular basement membranes separated the granulosa cells from the stromal cells of the ovary (Figure 3A).

The granulosa cells of mature follicles were surrounded by elongated theca cells and were separated by basement membranes (Figure 3B).

The oocytes of mature follicles were bounded by thick layers of zona pellucida. The oolema were thrown into microvilli projecting into the zona pellucida. The ooplasms contained mitochondria and abundant cytoplasmic annulate lamellae (Figure 3C).

Corpora lutea had granulosa lutein cells with euchromatic nuclei and few lipid droplets. They were surrounded by regular basement membrane separating them from elongated theca lutein cells (Figure 3D).

The ultrathin sections of the semicarbazide treated group showed some forms of degenerations in the form of cytoplasmic vacuoles in granulosa cells, degenerated mitochondria, dilated rough and smooth endoplasmic reticula. Some cells were observed with electron dense nuclei and others with irregular nuclei and dilated nuclear envelopes. Many wide spaces separated granulosa cells from each other. Thick basement membranes with collagen fibers separated granulosa and theca cells (Figure 4A,B,C).

Corpora lutea of the treated group showed granulosa lutein cells with peripheral clumping of chromatin in their nuclei and many lipid droplets in their cytoplasm (Figure 4D).

Granulosa lutein cells also had destroyed microvilli, mitochondria with destroyed cristae and irregular nuclei with dilated nuclear envelopes (Figure 5A,B,C).

Irregular basement membranes with numerous collagen fibers were seen surrounding granulosa lutein cells (Figure 5D). Huge lipid droplets appeared in some granulosa lutein cells (Figure 5E).

Ultrathin sections of the withdrawal group of the ovary revealed mature follicles with granulosa cells that are enclosed by elongated theca cells. Granulosa and theca cells were separated by basement membranes. Most of graulosa cells had euchromatic nuclei but few cells appeared with electron dense nuclei. Also, some separations appeared between few cells (Figure 6A,B).

Corpora lutea of the withdrawal group showed normal granulosa lutein cells with regular euchromatic nuclei and some lipid droplets in their cytoplasm (Figure 6C).

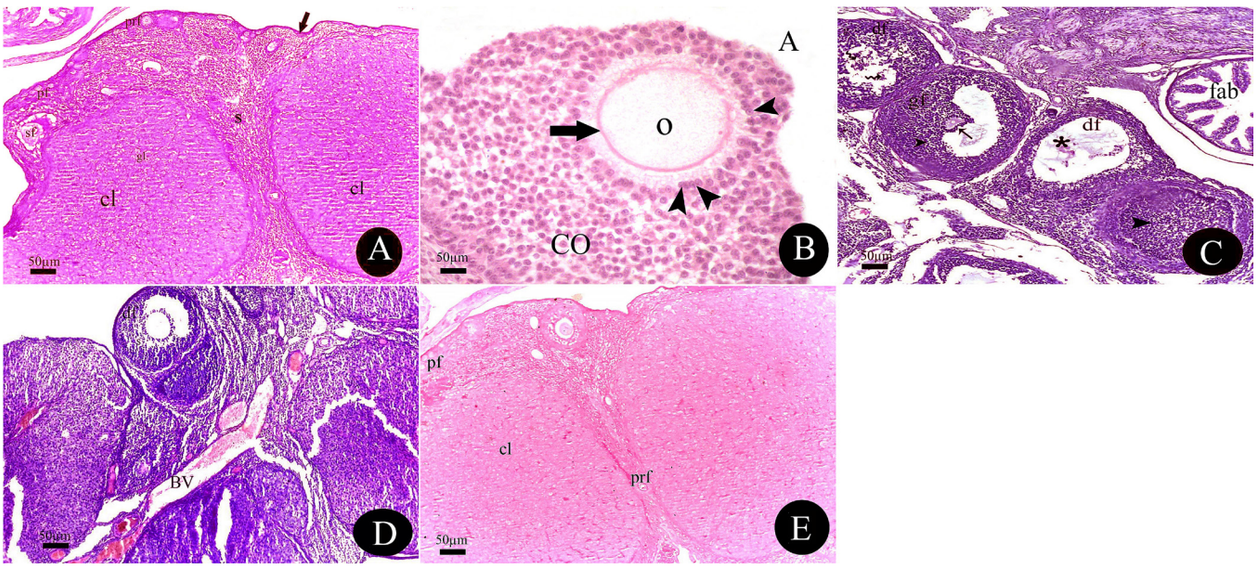

Fig. 1: Photomicrograph of H&E stained sections showing: A – Ovarian cortex of control group showing many follicles at several phases of development, primordial follicles (pf), primary follicles (prf), and secondary follicles (sf). The follicles are separated by stromal cells (s). Corpora lutea (cl) having granulosa lutein cells (gl) are also seen. The covering surface epithelium (thick arrow) is observed. B – Part of mature graffian follicle of control group having oocyte (O), corona radiate (arrowhead), cumulus oophorus (CO), zona pellucida (thick arrow) and antral space (A). C,D – Ovarian cortex of semicarbazide treated group showing degenerated follicles (df). Some of them contain hyaline substance in the lumen (star), others are seen with granulosa cells (g) having dark nuclei (arrowhead), wide spaces between them (S) and some cells are exfoliated in the lumen (zigzag arrow). Mature graffian follicle (gf) with dark nuclei of granulosa cells (arrowhead), ill-defined corona radiata (arrow) are noticed. Congested dilated blood vessels (BV) are seen between the follicles. Notice, presence of part of fallopian tube (fab). E – withdrawal group showing primordial follicles (pf), primary follicles (prf), and corpora lutea (cl).

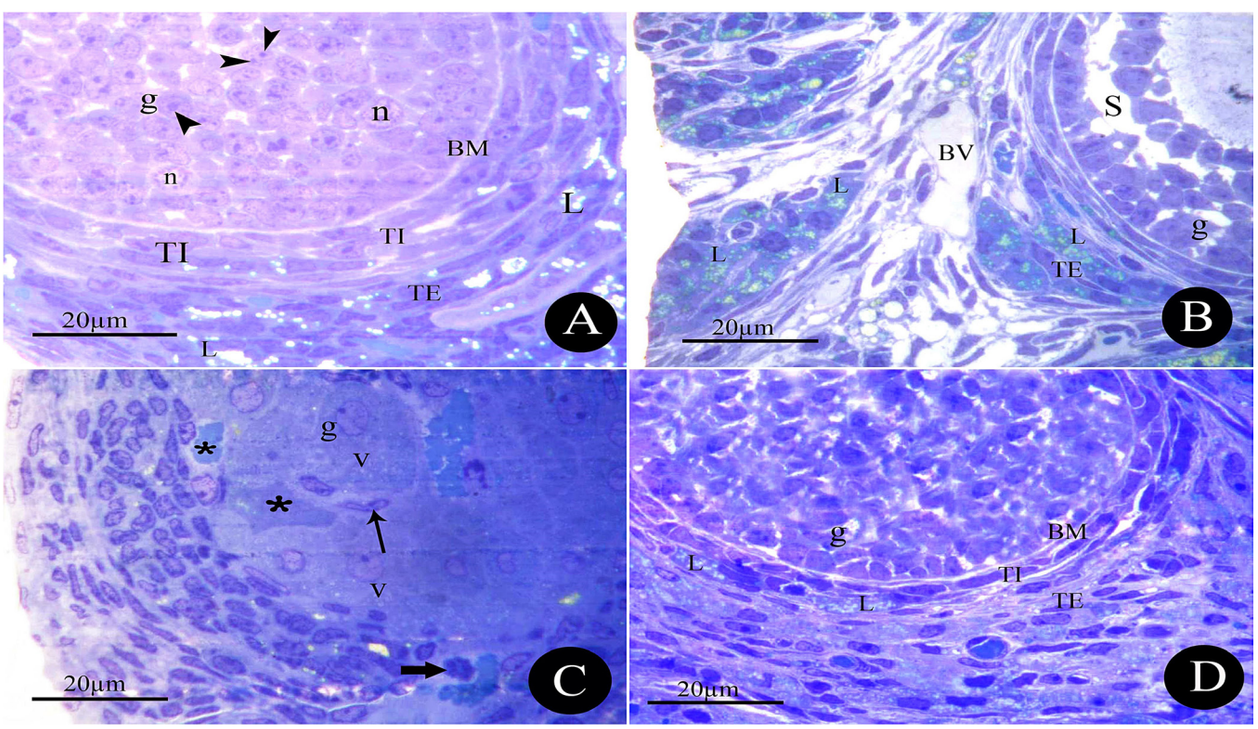

Fig. 2: Toluidine blue semithin sections. A – Part of mature follicle with polyhedral granulosa cells (g) having round vesicular euchromatic nuclei (n) with one or two nucleoli (arrowhead). Regular basement membrane (BM) separates them from theca cells that are differentiated into polyhedral theca interna (TI) and flat theca externa (TE) with lipid droplets appear in their cytoplasm (L). B - semicarbazide treated group showing wide separation (S) between granulosa cells (g) and theca externa cells (TE) filled with lipid droplets (L). Dilated blood vessel (BV) can also be seen. C – granulosa cells (g) of degenerated follicle with vacuolation in their cytoplasm (v). Hyaline materials (star) are seen between the cells. Some cells with irregular nuclei (arrow) are embedded between granulosa cells. A poly morphnuclear neutrophil is seen (thick arrow). D- withdrawal group showing polyhedral granulosa cells with vesicular nuclei (g). Regular basement membrane (BM) separates them from theca cells that are differentiated into polyhedral theca interna (TI) and flat theca externa (TE) with lipid droplets appear in their cytoplasm (L).

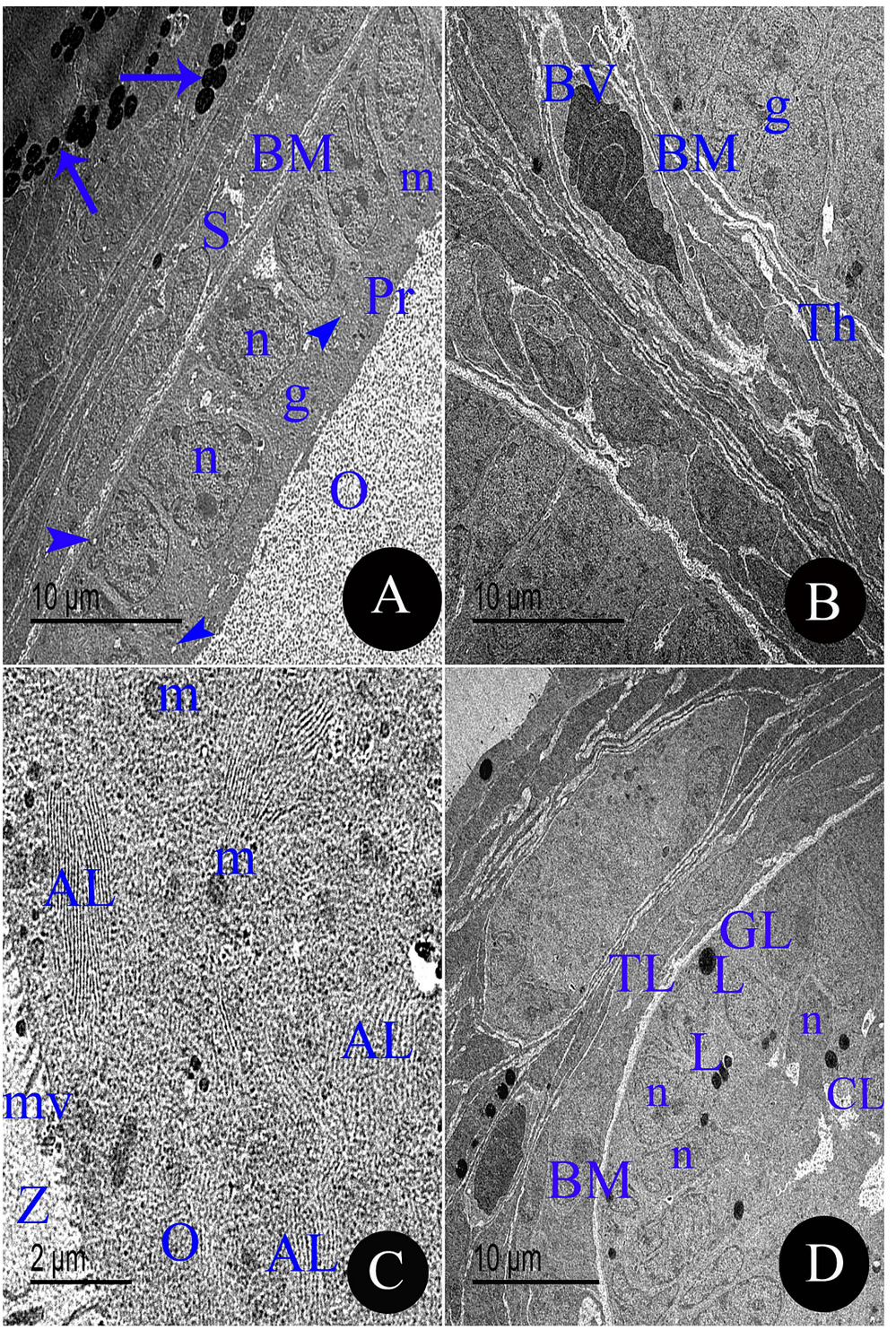

Fig. 3: An electron micrograph of the control ovary: A – Primary follicle (Pr) contains an oocyte (O) surrounded by simple cuboidal sheet of granulosa cells (g) having nuclei (n), mitochondria (m) and smooth endoplasmic reticulum (arrowhead). A basement membrane (BM) separates the granulosa cells from the stromal cells of the ovary (S). Notice, the presence of lipid droplets in the stromal cells (arrow). B – Parts of two follicles showing granulosa cells (g) surrounded by elongated Theca cells (Th). Granulosa and Theca cells are separated by basement membrane (BM). Blood vessel (BV) is seen in the stroma between the two follicles. C – The oocyte (O) is bounded by a thick layer of zona pellucida (Z). The oolema is thrown into microvilli (mv) projecting into the zona pellucida. The ooplasm contains mitochondria (m) and abundant cytoplasmic stacks of annulate lamellae (AL). (D) Corpus luteum (CL) with granulosa lutein cells (GL) containing euchromatic nuclei (n) and few lipid droplets (L). They are surrounded by regular basement membrane (BM) separating them from elongated theca lutein cells (TL).

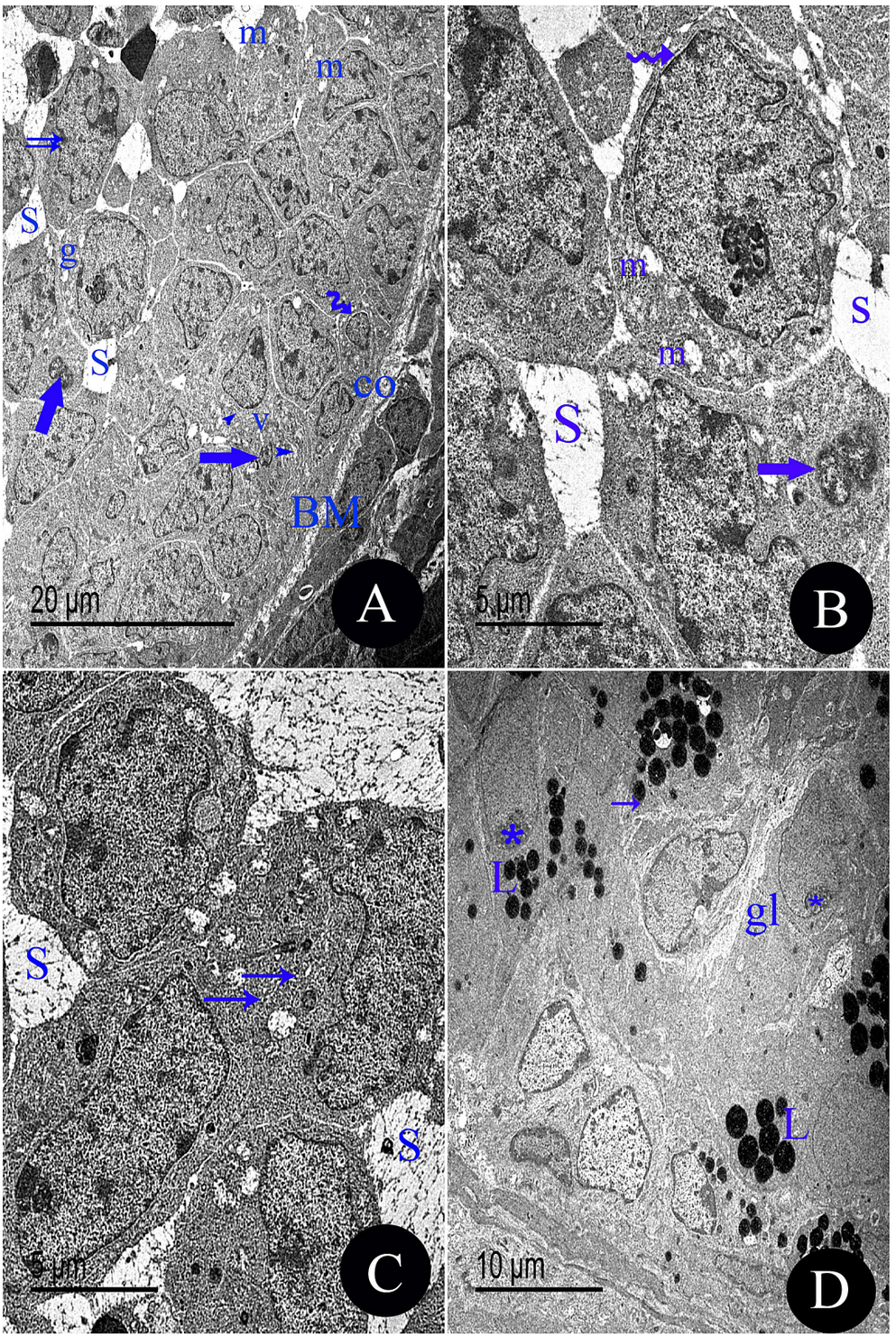

Fig. 4: An electron micrograph of the semicarbazide treated group of the ovary: A - Parts of ovarian follicle showing granulosa cells (g) with vacuolations in their cytoplasm (v), degenerated mitochondria (m), dilated rough endoplasmic reticulum (arrow head). Some cells appear with electron dense pyknotic nuclei (thick arrow), others appear with irregular nuclei (double arrows) and dilated nuclear envelope (zigzag arrow). Many wide spaces separate granulosa cells from each other (S). Thick basement membrane (BM) with collagen fibers (co) separates granulosa and theca cells. B – Higher magnification of A showing dilated nuclear envelope (zigzag arrow), pyknotic nuclei (thick arrow), degenerated mitochondria (m) and wide spaces separate granulosa cells from each other (S). C – granulosa cells of the follicles with dilated smooth endoplasmic reticulum (arrow) and wide spaces between the cells (S). D - Corpus luteum of treated group shows granulosa lutein cells (gl) with peripheral clumping of chromatin in their nuclei (ŝtar), many lipid droplets (L) and dilated smooth endoplasmic reticulum (arrow) in their cytoplasm.

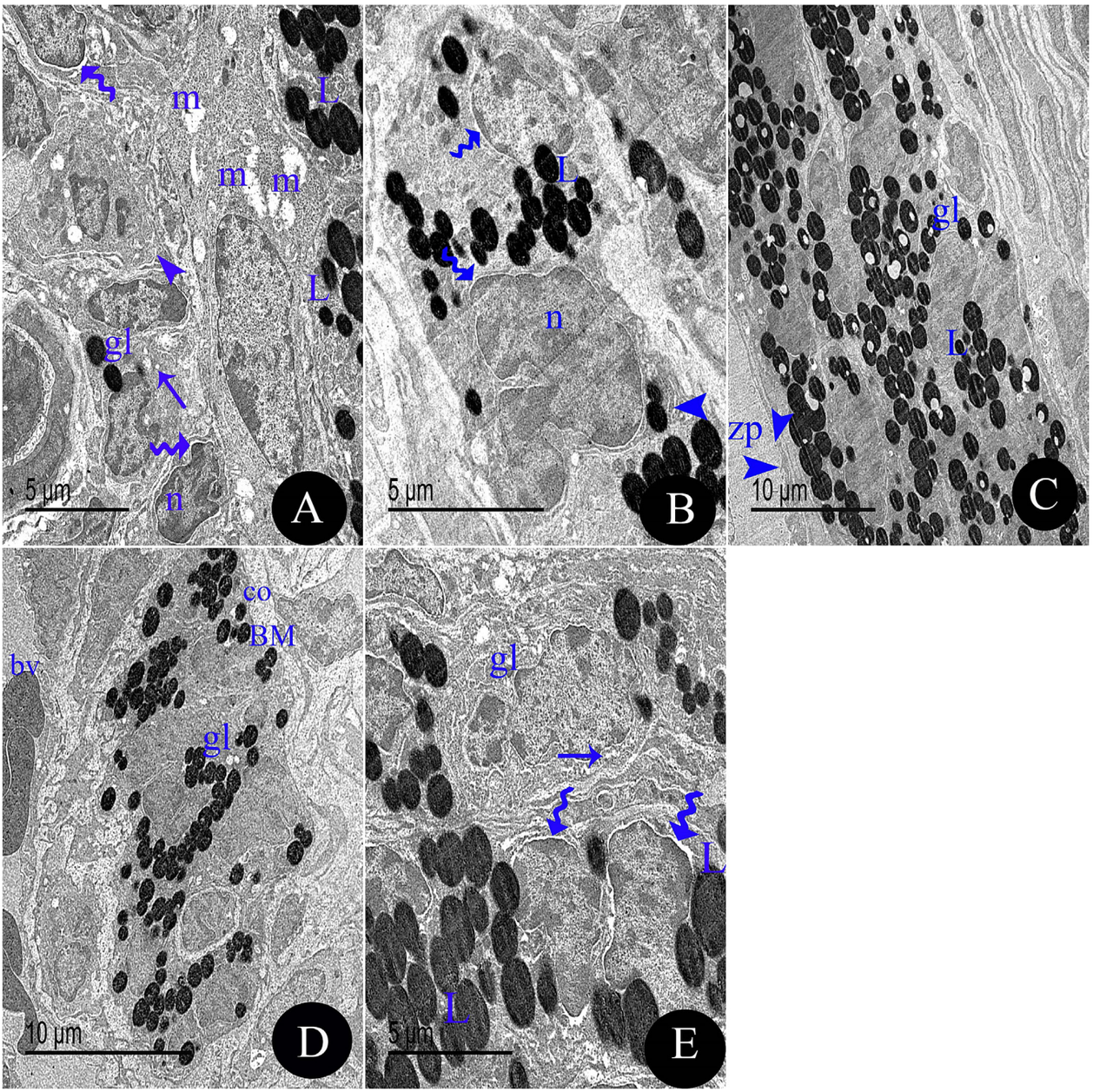

Fig. 5: An electron micrograph of the semicarbazide treated group of the ovary: A, B - granulosa lutein cells (gl) having many lipid droplets (L), mitochondria with destroyed cristae (m), rough endoplasmic reticulum (arrowhead) and smooth endoplasmic reticulum (arrow). Irregular nuclei (n) with widened nuclear envelope (zigzag arrow) are observed. C - granulosa lutein cells (gl) having many lipid droplets (L) and destroyed microvilli (arrow head) facing zona pellucida (zp). D – Irregular basement membrane (BM) is seen with many collagen fibers (co) surrounding granulosa lutein cells (gl) and congested dilated blood vessel (bv). E- Huge lipid droplets (L), dilated smooth endoplasmic reticulum (arrow) in the cytoplasm of granulosa lutein cells (gl). Also, dilated nuclear envelope (zigzag arrow) also appear.

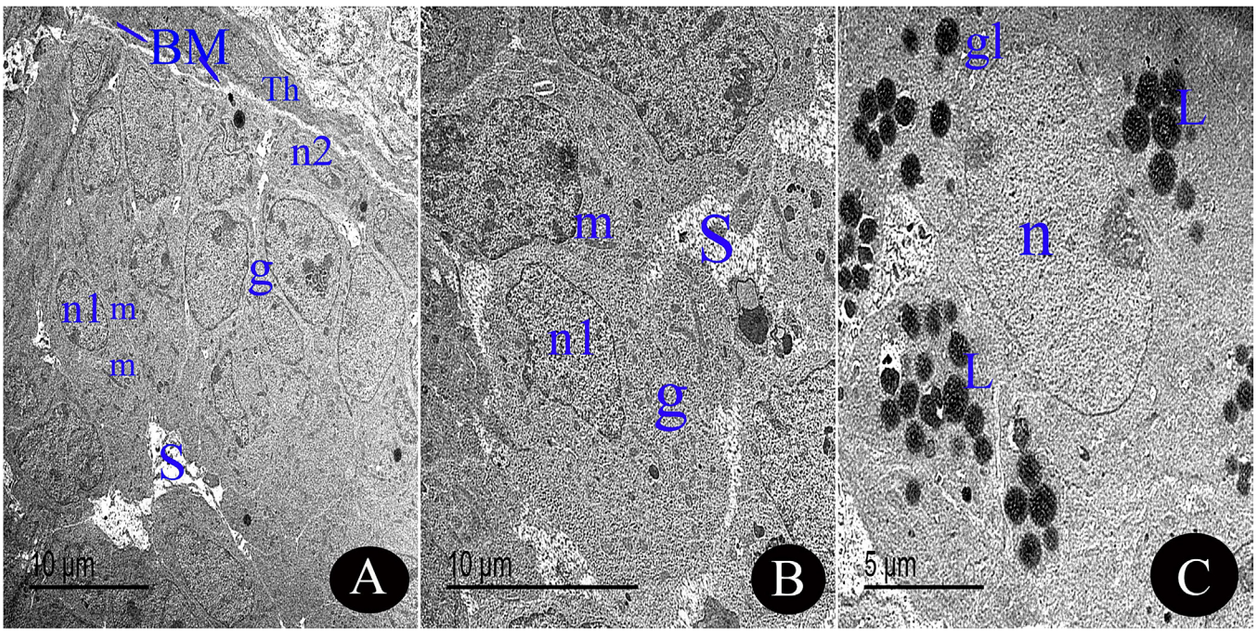

Fig. 6: An electron micrograph of the withdrawal group of the ovary: A, B – a Part of mature follicle showing granulosa cells (g) surrounded by elongated Theca cells (Th). Granulosa and Theca cells are separated by regular basement membrane (BM). granulosa cells have euchromatic nuclei (n1) and mitochondria appeared in their cytoplasm (m). Few cells appear with electron dense nuclei (n2). Some separations (S) appear between few cells. C - Corpus luteum shows granulosa lutein cells (gl) with regular euchromatic nuclei (n) and lipid droplets (L) in their cytoplasm.

Table 1: Statistical comparison among control, semicarbazide and withdrawal groups regarding body weight, serum levels of GnRH, LH, FSH and E2 using ANOVA and post-hoc tests

Parameters \ Groups	Group I (control)	Group II (semicarbazide treated)	Group III (withdrawal group)	*P value*
	N=15	N=15	N=15	
		Mean±SD		
Body weight (g)	190.80±7.75	153.86±10.16[a]	171.73±6.95[a,b]	<0.05*
GnRH(pg/ml)	1264.53±90.28	628.06±79.80[a]	1072.73±135.76[a,b]	<0.05*
LH (ng/ml)	2.04±0.02	0.72±0.13[a]	1.47±0.22[a,b]	<0.05*
FSH(mIU/ml)	16.27±1.37	7.94±0.98[a]	11.54±1.34[a,b]	<0.05*
E2(pg/ml)	121.2 ± 15.25	82.2 ± 10.31[a]	103.7± 10.87[a,b]	<0.05*

SD : standard deviation *p<0.5 : significant p <0.01 : significant by post hoc test a:vs control group ,b: vs SEM group

DISCUSSION

Daily consumption of food submitted in glass jars carries the risk of exposure to semicarbazide emerging toxicity. In our study, female rats received semicarbazide 40 mg/kg daily for four weeks had significant decrease in their weight relative to control group. Similar results were seen with other studies who found increased leptin transcription after exposure to semicarbazide. Increased leptin affects food consumption, fat deposition, steroidogenesis and so cause weight reduction[21].

The ovarian tissues of SEM group showed degenerated follicles, dark nuclei of granulosa cell, vacuolated cytoplasm and exfoliated cells in follicular lumena. Similar results were seen with other studies[21]. Hirakawa *et al*.[22] attributed degenerative changes of semicarbazide to DNA damage through induction of reactive oxygen radicals. Toxins cause cellular apoptosis and cytoplasmic vacuolations through disturbing their metabolic process as mentioned by Manivannan *et al*.[23].

The ultrastructural findings of granulosa cells were electron dense nuclei and peripheral clumping of chromatin. In the same context, F. Maranghi *et al*.[24] found chromatin condensation in oocytes treated with semicarbazide and uterine epithelial changes similar to the effects of anti-estrogen administrations that affect female reproductive function. These findings run with our biochemical results. We found significant drop in the estrogen, GnrH, LH and FSH levels in SEM treated group with dose of 40mg/kg. This suggests that the effect of SEM on the sex hormone is due to central effect even at low doses. Francesca Maranghi *et al*. and Yu *et al*.[10,21] proved decreased serum E2 levels with semicarbazide treatment with dose dependent effect.

The mechanism of this endocrine disruption of female hormones is due to SEM affection on the neurotransmitter systems (antagonism of the N-methyl-D-aspartate receptor [NMDAR])[25]. In addition, SEM inhibits glutamic acid decarboxylase (GAD) enzyme causing decrease gamma-aminobutyric acid synthesis[26,27] Accordingly, it inhibits GnRHs, FSH and LH[21,26] sharing in follicle degeneration and histological changes as mitochondrial degeneration, dilatation of rough and smooth endoplasmic reticulum seen in this study. In addition, mentioned that the degenerative changes of mitochondria make it unable to convert cholesterol to pregnenolone causing reduction in estrogen production[28].

Maintaining of granulosa cells depends mainly on estrogen hormone. So, inhibition of secretion of this hormone results in degeneration of granulosa cells[29]. This clarifies the wide spaces and destroyed microvilli seen in the current research.

Semicarbazide causes impaired aromatase enzyme formation and expression. This leads to accumulation of androgen hormone in SER leading to dilatation of cisternae as seen in this study[21,30,31]. The imbalance between steroids with SEM exposure may be other cause of various toxicological insults 10. Synchronizing with male testis; LH must attach to its receptors on Leydig cells to stimulate cAMP and synthesis of testosterone from cholesterol[32]. Accordingly, reduction in LH with SEM as seen with Yu *et al*.[11] leads to dilatation of SER due to accumulation of cholesterol and failure of testosterone synthesis.

Granulosa lutein cells of SEM group showed large amount of accumulated lipid droplets and some of them appeared huge. Olzmann *et al*.[33] stated that lipid droplets (LDs) are plants for synthesis of steroid hormones. The smaller LDs can easily break and offer cholesterol for steroid hormone synthesis[34]. From this point, we suggest that the large sized LDs seen in this work contribute to decrease estrogen hormone level. De Araújo *et al*.[35] recorded increased CYP11A1 (cytochrome p 450 family 11 subfamily A member 1) and lipin-1 which are regulators of steroid biosynthesis in rats exposed to Tributyltin and aid in excess lipid deposition.

Vascular changes in the form of dilated blood vessels and thick basement membrane in SEM group in this research go hand in hand with Mercier *et al*.[7] who related these changes to impaired cross-linking reactions of extracellular matrix (ECM) proteins as collagen and elastin. This occur by binding of semicarbazide to lysyl oxidase enzyme which is known to stabilize ECM[16,36].

In investigating the same group, we found showed acidophilic hyaline substance in the interstitial spaces associated with multiple vacuoles. Increased vessels permeability may explain this acidophilic hyalinization[37].

After the withdrawal period, there were different responses in ovarian tissue sections in this study. Most of granulosa cells of the follicles had euchromatic nuclei but few cells possessed electron dense nuclei and others were widely separated. The injuries severity were also decreased with in tibia and femur exposed to SEM but not in aorta[16]. They attributed this conflict to the differences in tissues and the growth stage of the organs. They added that lesions in elastic lamina in aorta are irreversible especially if they occurred during the time of maturation. In addition, Mohamed & Mohamed[38] mentioned that the ovarian tissue has the capability of recovery after cessation of the injurious insult such as tramadol.

CONCLUSIONS

The present results provoked that excess administration of semicarbazide induced dangerous changes in rat ovarian structure and function. Parts of these changes reversed after withdrawal of this agent but others were still present which have negative impact on reproductive functions.

RECOMMENDATIONS

As far as avoid excess consumption of food products sold in glass jars and using fresh products better for healthy life.

CONFLICT OF INTERESTS

There are no conflicts of interests.

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الملخص العربى

التأثير الضار للسيميكاربازايد على مبيض الجرذان البيضاء البالغه وإمكانية تراجع الاصابات المستحثه (دراسة كيميائية حيوية و هستولوجيه)

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المقدمة: اكتسب السيميكاربازايد مخاوف بشأن وجوده والتعرض المفرط له والمخاطر المحتملة على صحة الإنسان كما أن الرضع والأطفال أكثر عرضة لخطر التعرض له بسبب تناول كميات كبيرة من الطعام في برطمانات زجاجية.
هدف العمل: لمعرفه آثار السيميكاربازايد على التركيب النسيجى للمبيض فى الجرذان البيضاء البالغة وفرصة الشفاء بعد سحبها.
المواد والطرق: تم تقسيم ٤٥ أنثى بالغة من الجرذان البيضاء إلى ثلاث مجموعات. المجموعه الضابطه و المجموعه المعالجه ب السيميكاربازايد (٤٠ مجم / كجم من وزن الجسم يوميا لمدة أربعة أسابيع) ومجموعة الانسحاب (أعطيت السيميكاربازايد لمدة أربعة أسابيع مثل المجموعة السابقة ، ثم تم سحبه لمدة أسبوعين). تم جمع العينات وفحصها تشريحيا. تم تقدير مستويات هرمونات الغدد التناسلية (GnRH) والهرمون (LH) والهرمون (FSH) ومستويات الاستراديول (E٢).
النتائج: عزز السيميكاربازايد انخفاضًا كبيرًا في مستويات هرمون GnRH و LH و FSH و E٢ بالإضافة إلى التغيرات النسيجية المرضية لأنسجة المبيض مثل الجريبات المتحلله مع الخلايا المقشرة والمواد الهياليئية في تجويفها والتغييرات النسيجيه الدقيقه المختلفة في الخلايا الجرابية مع سماكة الأغشية القاعديّة المحيطة بها. تم تحسن هذه التعديلات جزئيًا عند سحب السيميكاربازايد.
الخلاصة: تسبب السيميكاربازايد في تغييرات خطيرة في التركيب النسيجى لمبيض الجرذان. تحسنت بعض من هذه التغييرات بعد سحب هذا العامل مع بقاء تغيرات أخري والتي لها تأثير سلبي على القدرة الانجابية.