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# Modulatory role of celery *(Apium graveolens)* leaves extract against mercuric chloride-induced gonadal dysfunction in female rats and their pups

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## Abstract

***Background:*** Mercury (Hg) is a toxic heavy metal that has been recognized as one of the strongest environmental pollutants. Exposure to Hg even in low doses can induce adverse effects on the structure and functions of the different body organs including gonads. Celery (*Apium graveolens*) leaves are rich with antioxidant compounds like apiin, apigenin, and quercetin, and vitamins like E and C. ***Aim:*** This work aimed to evaluate the possible modulatory role of celery leaves extract against gonadal toxicity induced by $HgCl_2$ in female rats and their pups during gestation and lactation periods. ***Material &methods:*** Twenty-four female Wistar rats (180-200g) were used in this experiment. They were mated with males. After confirmation of the gestation test, the pregnant rats were divided into four groups (six for each) as follows: control, celery extract (CE) supplement (200 mg/kg b.wt), $HgCl_2$ (2mg/kg b.wt) and $HgCl_2$ co-supplemented with CE groups. The experiment extended from the $4^{th}$ day of gestation till the end of the weaning period. At the end of weaning, the female rats and their pups were weighed and sacrificed to collect the blood and remove the gonads for estimation of biochemical and histopathological changes. ***Results:*** The obtained results revealed that exposure of female rats to $HgCl_2$ resulted in a significant decrease in the activities of antioxidant enzymes (SOD, CAT, and GPx) and sexual hormones (FSH, LH, estrogen, progesterone, and testosterone) in both mother rats and their pups if compared with control. Further, a light microscopic investigation revealed that exposure to HgCl2 during gestation and lactation periods induced deleterious histopathological changes in the ovaries of mothers as well as in the ovaries and testes of their offspring. Additionally, the ovarian sections from mothers and their offspring displayed weak to negative expression for calretinin antibody, however, the testicular sections from offspring appeared strongly stained with COX-2 antibody (inflammatory marker) and weakly stained with Ki67 antibody (proliferative marker). Further study by flow cytometric technique revealed a highly significant increase in the mean % value of apoptotic cells in both ovaries and testes. On the other hand, co-supplementation of CE to $HgCl_2$ treated group successfully alleviated the altered antioxidants and hormones as well as the histological changes near to the normal as control. ***Conclusion***: Celery extract has a powerful ameliorative role against $HgCl_2$-induced deleterious biochemical and histopathological changes in the gonads of female rats and their pups.

**Keywords:** $HgCl_2$, Celery, Gestation, Ovaries, Testes, Histopathology, apoptosis.

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## 1. Introduction

Exposure to environmental contaminants during gestation may extend negative impacts in early childhood and later life (Gluckman et al. 2008). Although the placenta may act as a selective transporter that prevents the passage of potentially toxic substances to the developing fetus, some environmental contaminants can freely or partially cross the placental barrier (Needham et al. 2011). Some heavy metals like mercury, arsenic, cadmium, and lead could extend the health risk to the fetus even at a low level through trans-placental circulation (Chen et al. 2014)**.** The toxicological effects of heavy metals could alter the physiological changes during pregnancy, the critical phase of fetal cell division and differentiation (Gundacker and Hengstschlager 2012). As an example, prenatal cadmium exposure could impair steroidogenesis which leads to suboptimal fetal growth and development (Stasenko et al. 2010)**.** Lead exposure could interfere with calcium deposition in the bone, resulting in decreased fetal bone growth (Stasenko et al. 2010). Arsenic exposure during pregnancy may also contribute to placental insufficiencies, which could lead to intra-uterine growth retardation through inducing oxidative stress (Vahter 2007). Mercury can impair the brain, nerves, kidneys, muscles of adults, and the developing fetus (Alina et al. 2012; Tsuji et al. 2019)**.** Bronchitis, asthma, and temporary respiratory problems have been occurring due to inhalation or exposure to mercury vapors (Bernhoft 2012). Additionally, exposure to mercury vapors leads to infertility in both men and women and erectile dysfunction (Dickman et al. 1998)**.** The mercury-mediated disruption of reproductive functions might be attributed to its inhibitory effect on the antioxidant defense system (Boujbiha et al. 2009)**.** Mercury can accumulate in ovaries leading to changes in reproductive behavior, infertility, and ovarian failure (Al-Saleh and Di Renzo 2009; Bjorklund et al. 2019). Increased doses of mercury may induce infertility, stillbirth, congenital malformations, and spontaneous abortion (Schuurs 1999) as well as increased incidence of hormonal disorders and increased rates of adverse reproductive outcomes (Henriques et al. 2019). Exposure of females to mercury can cause an inhibitory effect on the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary (Chen et al. 2006a)**.** This can alter the levels of estrogen and progesterone, and consequently ovarian dysfunctions. Female mice exposed to 0.25–1.00 mg/kg/day of methylmercury showed reduced fertility and induction of many pathological lesions in the ovaries (Khan and Khatoon 2004)**.** Additionally, ovaries exposed to mercury had various histo-morphometric alterations like reduction in the ovarian size, total number of primordial, primary, and Graafian follicles and pronounced increase of atretic follicles (Altunkaynak et al. 2016). Mercury exposure in male rats can disrupt spermatogenesis, sperm motility, and induce testicular pathological changes (Martinez et al. 2014; Da Silva et al. 2011). reduced testicular weight, degenerated germinal epithelium, hypertrophy of seminiferous tubules, and irregular vacuolized basement membrane (Adelakun et al. 2020; Almeer et al. 2020). Exposure of mice to a very low dose of mercury caused degenerative lesions in the testes (Orisakwe et al. 2001)**.** Also, a significant reduction was noticed in the numbers of Sertoli cells, spermatogonia, spermatocytes, and spermatids (Altunkaynak et al. 2015). Recently, the extracts from medicinal plants are used as an alternative approach for treatment of several diseases instead of traditional drugs. Celery (*Apium graveolens L.*) is a herbal plant that belongs to the *apiaceae* family and is one of the annual or perennial plants that grow throughout Europe and the tropical and subtropical regions of Africa and Asia (Gauri et al. 2015)**.** *Apium graveolens* was cultivated in Egypt almost 3, 000 years ago (El-Shinnawy 2015).The juice of celery leaves possesses effective biochemical parameters such as reduced glutathione content catalase xanthine oxidase, glutathione peroxidase, and peroxidase activities which affect

the intensity of lipid peroxidation in homogenized liver and blood (Kolarovic et al. 2009)**.** Also, this herb is rich in antioxidant compounds such as flavonoids (like apiin, apigenin, and quercetin), as well as vitamins E and C (Fazal et al. 2012). In experimental studies, celery has been documented as an antifungal (Momin and Nair 2002), anti-hyperlipidemic (Aburjai et al. 2009), and anti-inflammatory (Mencherini et al. 2009)**.** Moreover, celery has been widely used as a food for treatment of arthritis (Kulthanan et al. 2016), anemia (Mansouri et al. 2015)**,** renal diseases (Wu et al. 2014), neurologic and mental disorders (Saki et al. 2014), coronary heart diseases (Mansouri et al. 2015), diabetes (Kooti et al. 2016), and cancer (Asadi-Samani et al. 2016)**.** Antioxidant compounds of celery were found to enhance sperm function and improve fertility (Kooti et al. 2014), as well as in scavenging of free radicals (Li et al. 2014; Shittu et al. 2008; Morovvati et al. 2013) found that celery quercetin improves the thickness of the uterine endometrium as well as the number and density of cells and glands in the endometrium in the ovariectomized rats. Accordingly, this work aimed to evaluate the potential protective role of celery leaves extract against ovarian toxicity induced by mercuric chloride in mother rats during gestation and lactation periods and the complications on the gonads of offspring.

## 2. Materials and methods

### 1. Mercuric chloride ($HgCl_2$)

$HgCl_2$ was purchased in powder from El-Gomhouria Company for Trading Chemicals and Medical Appliances, Alexandria. The pregnant rats received $HgCl_2$ in an oral dose of 2 mg/kg body weight each other day from gestation day 4 till the end of lactation (weaning) (Taha et al. 2010).

### 2. Celery *(Apium graveolens)* extract (CE)

Fresh cached Celery was obtained from the local vegetable market, in Damanhour city and the leaves were immediately separated from the stem, then dried in the shade and milled, and powders were stored in a refrigerator (4 °C) until extraction. 200 g of the dry leaves extract was mixed in 800 ml of distilled water in a Soxhlet apparatus for 24 hr. Then, the mixture was filtered with filter paper and the filtrate was concentrated in a rotary evaporator. In the end, the concentrated extract was stored at 4 °C until use.

### 3. Experimental animals

For this study, twenty-four female rats were mated with eight males (3 female: 1 male) in special cages overnight. After 3-4 days and ensuring pregnancy via observation of a vaginal plug and using the vaginal smear method, pregnant females were separated from males. On the $4^{th}$ day of gestation, the pregnant rats (24 total) were divided into 4 groups, six for each group (n=6) as follows.

**Group 1** (control): The pregnant rats were fed on usual food and distilled water.

**Group 2 (**Celery extract): They were fed on usual food with an oral dose of 200mg/kg body weight of CE each other day (Ramezani et al. 2009).

**Group 3** ($HgCl_2$): They received $HgCl_2$ in an oral dose of 2 mg/kg body weight each other day (Taha et al. 2010).

**Group 4 (**$HgCl_2$+CE): They received $HgCl_2$ each other day alternatively with CE by the same doses in groups2&3.

Treatment with CE and $HgCl_2$ was performed from gestation day 4 till the end of lactation (21 days postnatal)

### 4. Sample collection and tissue preparation

At the end of the experimental period ($21^{st}$ days postnatal), the mother's rats and their offspring (at $21^{st}$ days old, six offspring for each group) were sacrificed by decapitation and the blood was collected in glass tubes. The serum was separated by centrifugation at 3000 rpm for 10 min and stored at −80°C for biochemical analysis. The animals were dissected and the whole ovaries of mothers and the gonads of their offspring were removed immediately, washed in normal saline, and processed for histological, immunohistochemical, and flow cytometry studies.

**5. Investigated parameters.**

**5.1 Body weight**

The mother's rats and their offspring were weighed at postnatal day (PND) 1, 7, 14, and 21 (end of weaning).

**5.2 Serum analysis**

***5.2.1 Determination of Follicular stimulating hormone (FSH), Luteinizing (LH), Estrogen, progesterone, and testosterone hormones***

Serum FSH and LH concentrations were measured in the serum of female rats and their pups (both males and females) by duplicate DELFIA IFMA (Wagner et al. 2011) using reagents obtained from Wallac (Turku, Finland). Estrogen and progesterone levels were measured in female rats and their female pups at PND21. Estrogen (estradiol) levels were measured by ELISA (Assay Designs), according to the manufacturer's protocol (Westwood 2008). The levels of serum progesterone were determined by the radioimmuno method according to the procedures of **AOAC** provided in the kits (Feldsine et al. 2003). The level of serum testosterone in male offspring was determined using a solid phase enzyme-linked immunosorbent assay (ALPCO Diagnostics, Cat No. 55-TESMS-E01, USA) based on the principle of competitive binding (Darney et al. 1996).

***5.2.2 Estimation of serum antioxidants (SOD, CAT, and GPx) and MDA***

Catalase (CAT) activity was determined spectrophotometrically by the method of Koroliuk et al. (Koroliuk et al. 1988). Briefly, 10 µL of the sample was incubated with 100 µmol/mL of $H_2O_2$ in 0.05 mmol/L Tris-HCl buffer pH = 7 for 10 min. The reaction was terminated by rapidly adding 50 µL of 4% ammonium molybdate. The yellow complex of ammonium molybdate and $H_2O_2$ was measured at 410 nm. One unit of catalase activity was defined as the amount of enzyme required to decompose 1 µmol $H_2O_2$ per min. The determination of the Superoxide Dismutase (SOD) activity was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazon dye (Bahrami et al. 2016). Glutathione peroxidase activity was determined according to the previously reported method by Paglia &Valentine (1976). An enzyme unit was defined as the amount of enzyme that catalyzes the release of µmol NADPH/min at 37 °C. Specific activity was in terms of units per gram of hemoglobin. The amount of hemoglobin was determined by the Merck test cat. No. 3317. Malondialdehyde (MDA) levels in serum were measured using the thiobarbituric acid reaction method of Placer et al. (Placer et al. 1966).

**5.3 Histological investigation.**

The gonads (ovaries from mothers, and the ovaries and testes from offspring) were fixed in 10% neutral buffered formalin. After fixation for 4 days, the specimens of gonads were dehydrated with ascending grades of ethanol, cleared with xylene, and embedded in paraffin. Five micrometer-thin sections were cut using an ordinary microtome. The sections were proceeded and finally stained with Mayer`s hematoxylin and eosin (Bancroft and Gamble 2008). The sections were investigated under a bright field light microscope and photographed to evaluate the histological structures of gonads.

**5.4 Immunohistochemical staining of calretinin, Ki67, and COX-2**

***5.4.1 Immunohistochemical labeling of calretinin.***

The immunohistochemical study for calretinin was focused on the ovarian sections of mothers and their offspring of 21 days old. Briefly, five-µm sections from the ovaries were mounted on lysine-coated slides (Surgipath, Richmond, IL). The sections were deparaffinized by xylene, graded ethanol, and water, then digested with a mixture of proteinase K (DAKO Corporation, Carpinteria, CA) and protease (0.1%, Sigma, St. Louis, MO) for 10 minutes. The sections were then incubated with a polyclonal rabbit anti-calretinin antibody (1: 50, ZYMED Laboratories Inc, San Francisco, CA) at room temperature for 30 minutes. After that the sections were washed with

PBS, followed by incubation in the secondary antibody (biotinylated goat anti-mouse/rabbit IgG, DAKO) for 20 minutes and then immersed in peroxidase-conjugated avidin (DAKO) at 25 $^0$C for 20 min. The reaction product was detected with 3, 3-diaminobenzidine chromagen (DAKO). The sections were counterstained with 0.1% hematoxylin (Cao et al. 2001).

### *5.4.2 Immunohistochemical labeling of Ki67 in the testicular tissues of offspring.*

5µm thin sections from the testes of 21-day-old offspring were deparaffinized and rehydrated in descending grades of ethanol, incubated in 3% hydrogen peroxide to block endogenous peroxidase activity, and treated with primary antibody of Ki67 at 4 ℃ overnight. After 3 washes in PBS, the sections were incubated with poly-HRP anti-Rabbit IgG, and the sections were stained with 3-3'diaminobenzidine tetrahydrochloride (DAB). The sections were counterstained with 0.1% hematoxylin.

### *5.4.3 Immunohistochemical labeling of cyclooxygenase-2(COX-2) in the testicular tissue of offspring*

Immunohistochemical staining for COX-2 was performed as reported previously (Tada et al. 2004)**.** Briefly, 5µm thin sections from the testes were placed in a biotin-blocking system (X0590; DAKO Corp., Kyoto, Japan) to block the endogenous biotin within the sections. Next, the sections were immersed in 0.3% $H_2O_2$ in methanol to inhibit endogenous peroxidase and then precoated with 1% nonfat milk in phosphate-buffered saline to block nonspecific binding. Polyclonal rabbit anti-COX-2 antibody (160116, Cayman Chemical, Ann Arbor, MI, USA) (1: 200–500 dilution) was used as the primary antibody, and a biotinylated polyclonal goat anti-rabbit antibody (1: 500 dilution) was applied as the second antibody. Immunoreaction was performed with a Vectastain ABC-Elite kit (Vector Laboratories, Burlingame, CA, USA) and visualized with 0.02% diaminobenzidine tetrahydrochloride as a substrate, and then lightly counterstaining with hematoxylin.

The immunohistochemically stained sections were counterstained with Mayer's hematoxylin, mounted, and photographed by phase contrast light microscopy (Olympus® digital camera installed on Olympus® microscope). Incidences of cellular accumulations of calretinin, Ki67, and COX-2 proteins were determined for each group. Additionally, the images were analyzed on Intel® Core I7® based computer using Video Test Morphology® software (Russia) with a specific built-in routine for the area, % area, measurement, object counting, and contact Angle.

### 5.5 Flow cytometry detection of Activated Caspase-3 and annexin-v

Flow cytometric analysis was performed on FACS can (Becton Dickinson) using standard setting: fluorescence 1 (FL1), 4 decades (logarithmic), detector 648 V, log amplifier, compensation 1.1%; fluorescence 2 (FL2), 4 decades (logarithmic), detector 496 V, log amplifier, compensation 22.8%. Data analysis was performed using lysis software (Becton Dickinson).

Cell suspensions from the ovaries of mothers and gonads of offspring were prepared with Tris-EDTA buffer (pH 7.4) (Sigma-Aldrich Co.). The cell suspension was fixed in ice-cold 96-100 % ethanol (Sigma) at 4 ºC overnight, centrifuged at 1, 500 rpm for 10 min, and then re-suspended in PBS containing 50 µg/mL propidium iodide (PI) (Sigma-Aldrich Co.). For each sample, since the analysis was based on the measurement of 10000 cells. Single-cell suspensions were prepared from gonads from at least six rats of each group, and 1.5- 3 X106 cells were stained for expression of the designated lineage markers.

Flow cytometric detection of caspase-3 was done to check the number of apoptotic cells in the ovarian tissues of mother rats and their pups. This technique is applicable where the fluorochrome is directly linked to the primary antibody (PE and FITC conjugate). The cells were prepared appropriately. The cell suspension was adjusted to a concentration of $1 \times 10^6$ cells/ml with PBS/BSA buffer (phosphate-buffered saline and 1% BSA). An Aliquot of 100µ L of cell suspension was put into test tubes as required. The antibody

(FITC rabbit anti-active caspase-3, solid as, material No.559341, catalog No. 554714, from BD Pharmingen) was added at the recommended dilution (10µ L for each sample), mixed well, and incubated at room for 30 min. After that, the cells were washed with 2 ml of PBS/BSA then centrifuged at 1500 rpm for 5 min, and discard the resulting supernatant. The cells were re-suspended in 0.2 ml of PBS/BSA or with 0.2 ml of 0.5% Para formaldehyde in PBS/BSA if required.

Testicular cell apoptosis and necrosis were assessed with flow cytometry using an annexin V FITC/PI staining kit (Pharmingen, Becton Dickinson Co., San Diego, CA, USA). After 48 h of transfection, the harvested cells were washed twice in PBS (sodium chloride NaCl 40.0 g, potassium chloride KCl 1.0 g, potassium dihydrogen phosphate anhydrous $KH_2PO_4$ 1.0 g, disodium hydrogen phosphate anhydrous $Na_2HPO_4$ 4.6 g, and distilled water to make up to 51 mL; 4 °C). The cells were re-suspended in the binding buffer (10 mm HEPES/ NaOH pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$), stained with fluorescein isothiocyanate-conjugated annexin V (annexin V-FITC).

The data were acquired by flow cytometry. This analysis was performed in the Mansoura University Hospital using FACS (flow activated cell sorter) Calibur Flow Cytometer (Becton Dickin-son, Sunnyvale, CA, USA) equipped with a compact air-cooled low power 15 mW Argon ion laser beam (488 nm).

**Statistical Analysis**

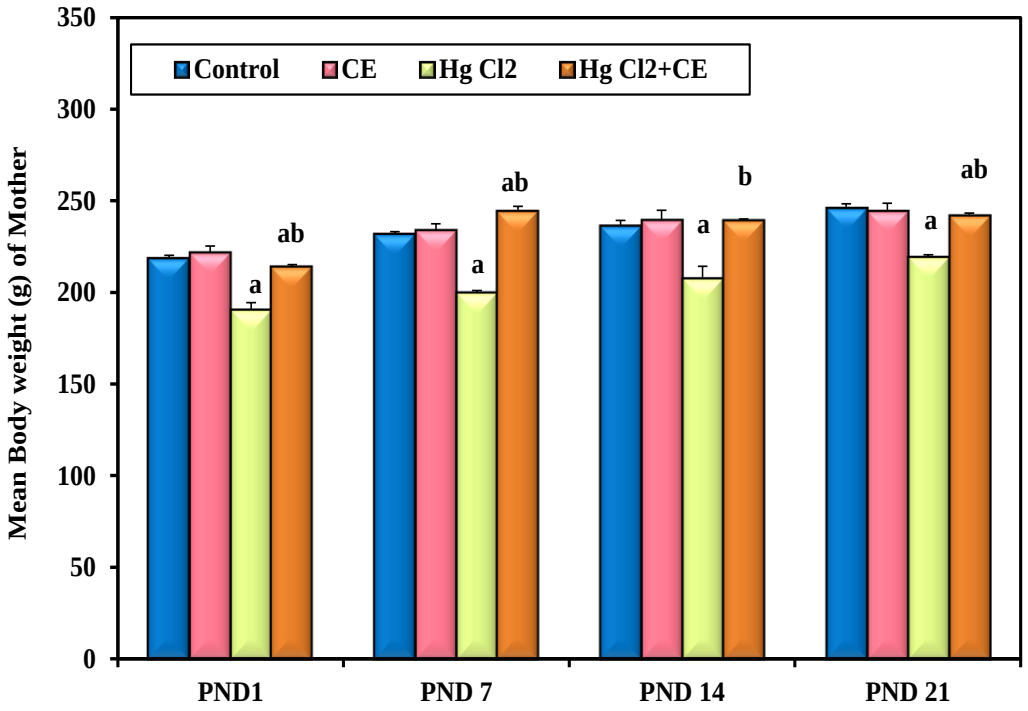
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using mean, standard deviation, median and. The significance of the obtained results was judged at the 5% level. The used tests were: 1 - F-test (ANOVA) for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons.

## 3. Results

### 1. Changes in the body weights of mother's rats and their pups

The mean body weights of CE supplemented mother's rats at PND1, 7, 14 and 21day appeared with non-significant change ($P>0.001$) if compared with control, however, in $HgCl_2$ treated mothers rats the mean body weights appeared significantly lowered ($P<0.001$) than control. On the other hand, supplementation of CE to $HgCl_2$ treated mothers' rats successfully restored the body weight near to the control values (Figure 1)

As shown in figure (2), the mean body weights of PNDs 1, 7, 14, and 21old offspring maternally supplemented with CE alone appeared with no significant change with control; however, the mean body weights of $HgCl_2$ maternally induced offspring at PNDs 1, 7, 14 and 21 appeared significantly lowered ($P<0.001$) than control offspring. On the other hand, the offspring maternally treated with $HgCl_2$ and CE showed remarkable restoring in their body weights near to the normal control but still significantly lower at PNDs 7&14 if compared with control.




**Fg.1:** The mean body weights among the different studied groups of *mother* rats at PND1, 7, 14, and 21.

*Data were expressed using Mean ± SD. p: p-value for one-way ANOVA test using Post Hoc Test (Tukey) for comparing between Control and CE. $p_1$: p-value for one-way ANOVA test using Post Hoc Test (Tukey) for comparing between Control and $HgCl_2$. $p_2$: p-value for one-way ANOVA test using Post Hoc Test (Tukey) for comparing between $HgCl_2$ and $HgCl_2$ + CE. $p_3$: p-value for one-way ANOVA test using Post Hoc Test (Tukey) for comparing between Control and $HgCl_2$ + CE *: Statistically significant at $p \leq 0.05$.*

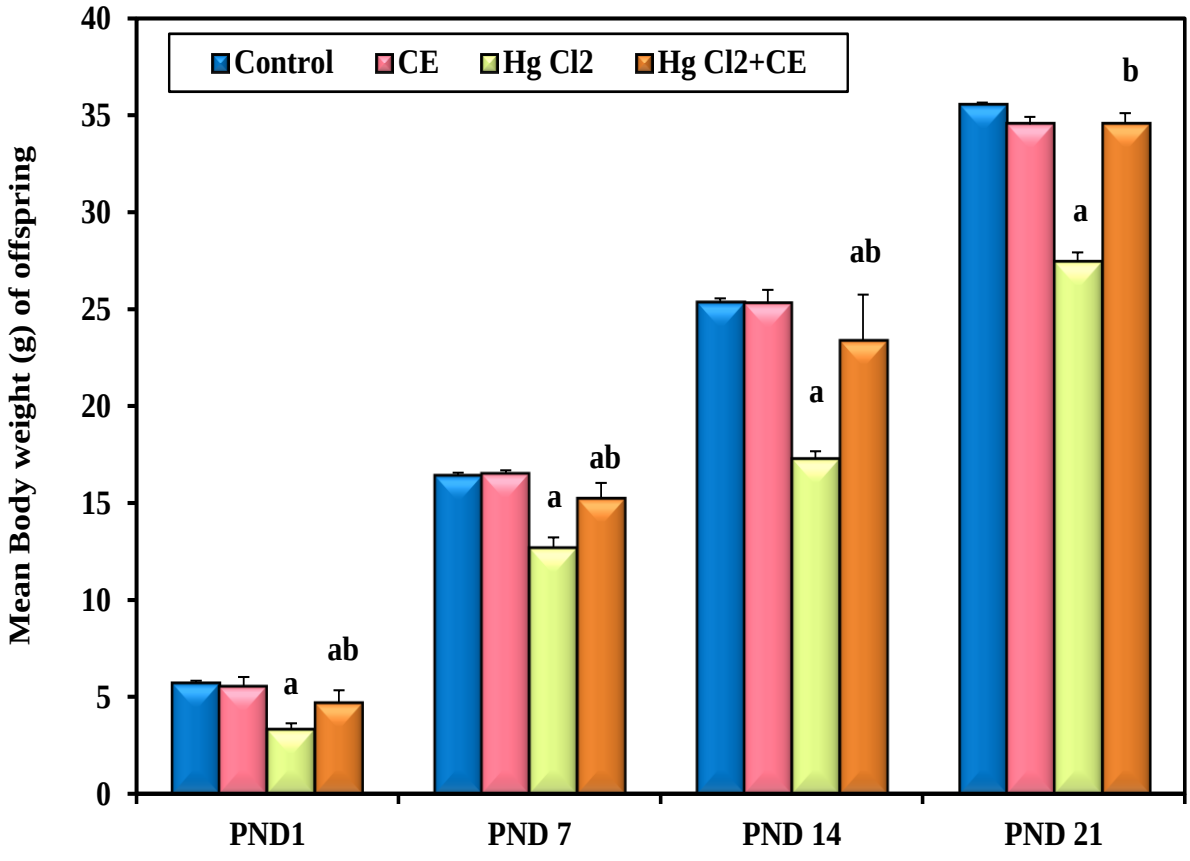



**Fig. 2:** The mean body weights among the different studied groups of rats' offspring at PND1, 7, 14, and 21.

**2. Changes in serum antioxidants (SOD, CAT, and GPx) and MDA**

As shown in figure (3), the levels of serum antioxidants (CAT, SOD, and GPx) and MDA in CE-supplemented mothers rats appeared in the normal standard range as control, however in $HgCl_2$ the levels of serum antioxidants appeared significantly lowered ($P<0.001$) while the level of MDA appeared significantly higher ($P<0.001$) than control. Supplementation of CE to $HgCl_2$-treated mother rats successfully restored the levels of antioxidants near the control values.

In maternally CE-supplemented offspring, the levels of serum CAT, SOD, GPx, and MDA showed non-significant change with control. However, in $HgCl_2$ maternally exposed offspring, the levels of serum antioxidants appeared significantly lowered ($P<0.001$) while the level of MDA appeared significantly higher ($P<0.001$) than control. In maternally $HgCl_2$ and CE-treated offspring, the levels of antioxidants appeared with non-significant change ($P>0.001$) if compared with control while the level of MDA appeared significantly lowered if compared with $HgCl_2$ treated offspring alone but still significantly higher than control ($P<0.001$) (Figure 4).

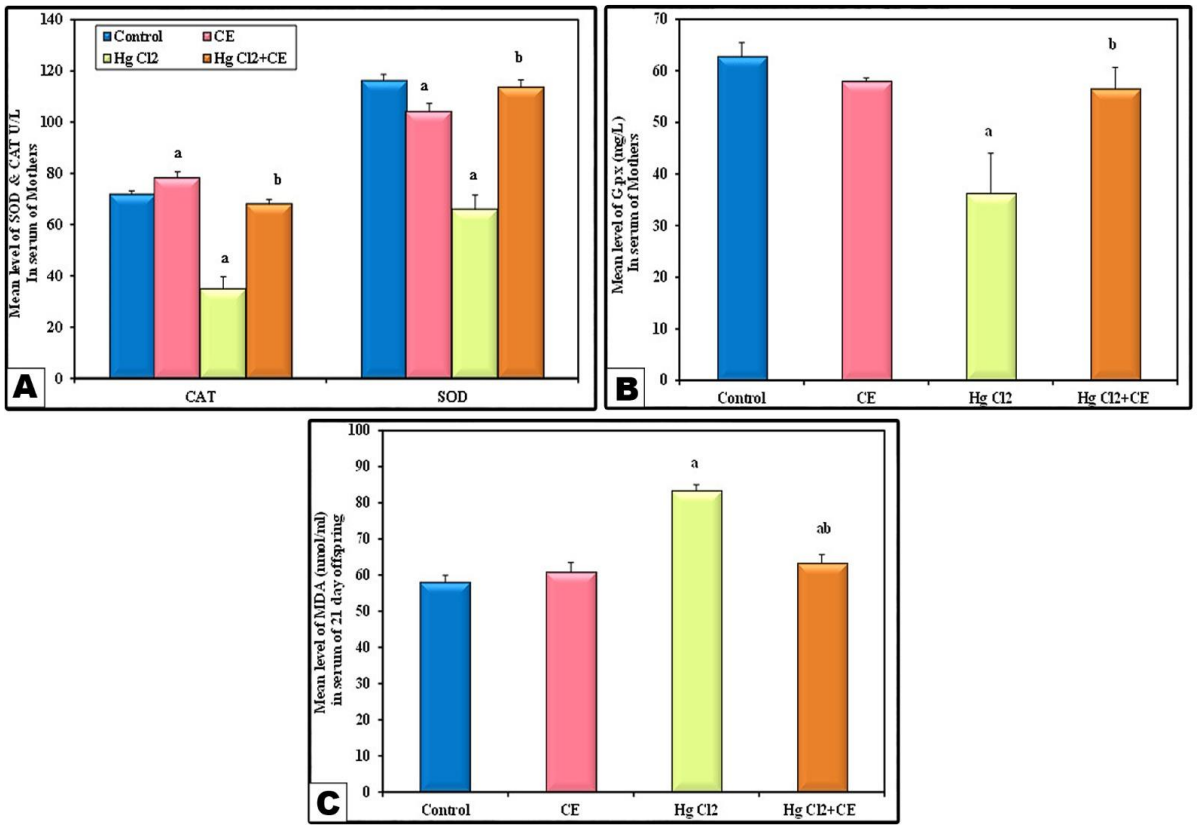



**Fig. 3:** The mean levels of serum CAT and SOD (U/L), GPx (mg/L), and MDA **(nmol/ml)** among the different studied groups of mothers' rats.

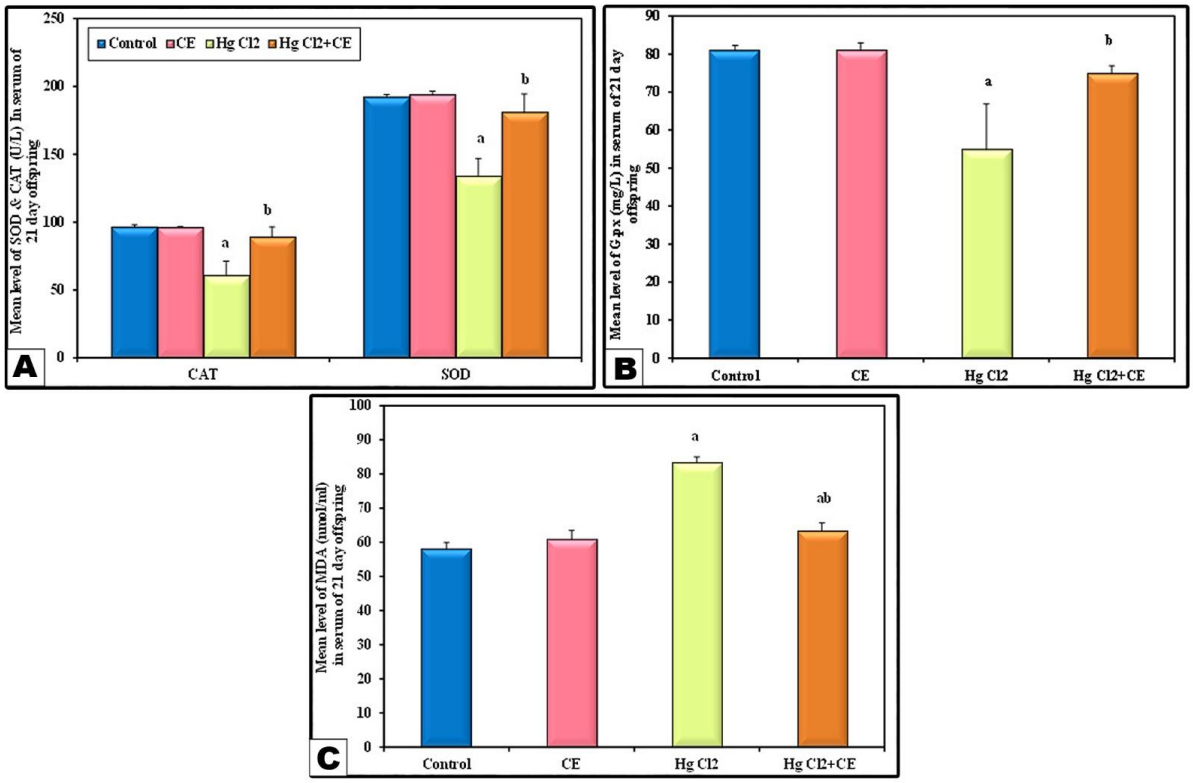



**Fig. 4:** The mean levels of serum CAT and SOD (U/L), GPx (mg/L), and MDA (nmol/ml) among the different studied groups of 21st-day-old offspring of rats.

### 3. Changes in the levels of serum FSH, LH, estrogen (E2), progesterone, and testosterone

As shown in figure (5), the mean levels of serum FSH and LH in CE-supplemented mothers rats appeared slightly lower than in control while the levels of estrogen and progesterone hormones appeared with non-significant change compared with control. On the other hand, a highly significant decrease in the levels of serum FSH, LH, estrogen, and progesterone was recorded in $HgCl_2$-treated mother rats. Co-supplementation of CE to $HgCl_2$-treated mothers' rats significantly increased the levels of four hormones if compared to the $HgCl_2$-treated mothers alone but still significantly lower than the control.

In 21st-day-old offspring maternally supplemented with CE, the levels of serum FSH, LH, and progesterone appeared significantly higher ($P<0.001$) than in control while the level of estrogen showed non-significant change ($P>0.001$) with control. In $HgCl_2$ maternally induced offspring the levels of the four hormones appeared significantly lowered ($P<0.001$) than control. On the other hand, the $HgCl_2$ and CE maternally treated offspring showed a highly significant increase in the levels of FSH, LH, and progesterone hormones that exceeded the normal value of control while the level of estrogen still appeared less than the normal value of control (Figure 6). The levels of serum testosterone among the different studied groups of male offspring are indicated in Figure (6). The results showed a highly significant decrease in the level of testosterone among the offspring maternally treated with $HgCl_2$ if compared with control however co-supplementation of CE with $HgCl_2$ successfully restored the level of testosterone near to the normal control.

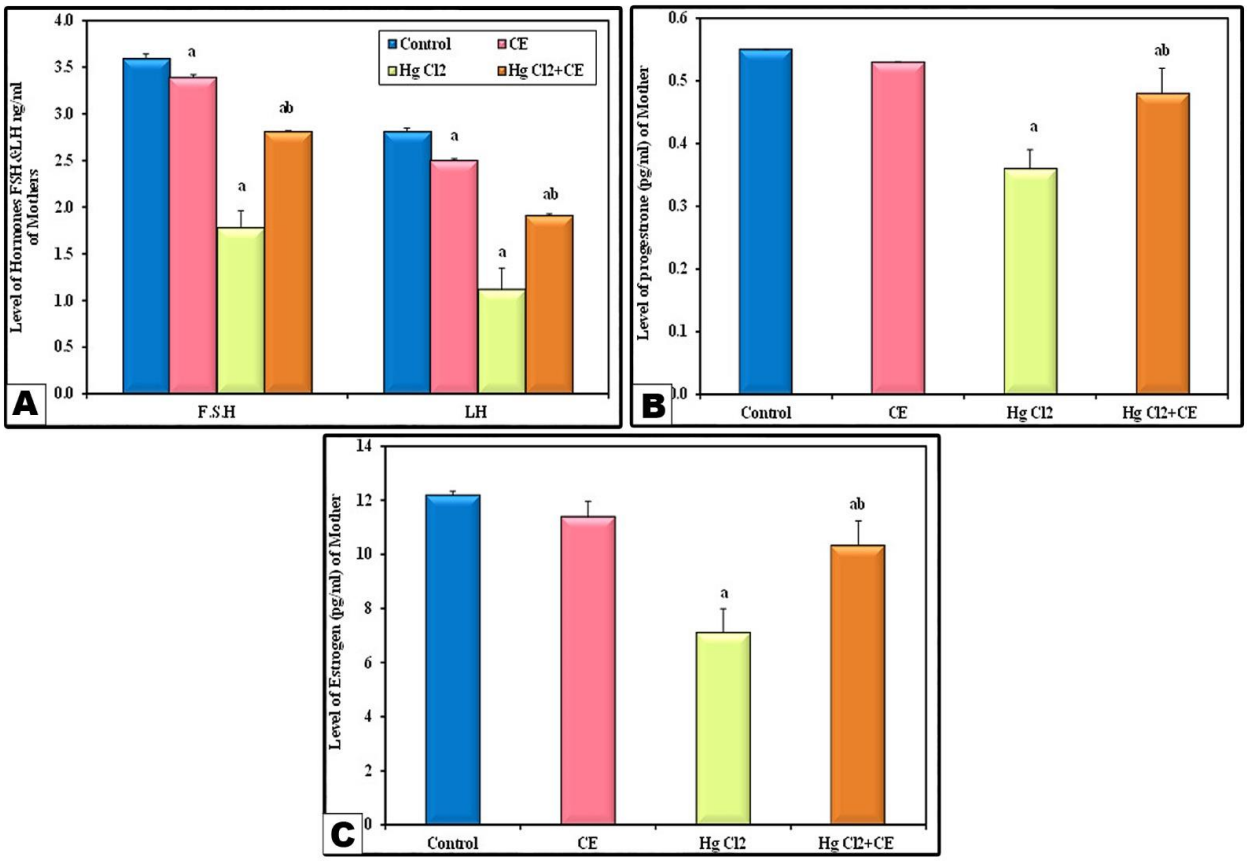



**Fig. 5:** The mean levels of serum FSH, LH (ng/ml), progesterone, and estrogen (pg/ml) among the different studied groups of mother rats.

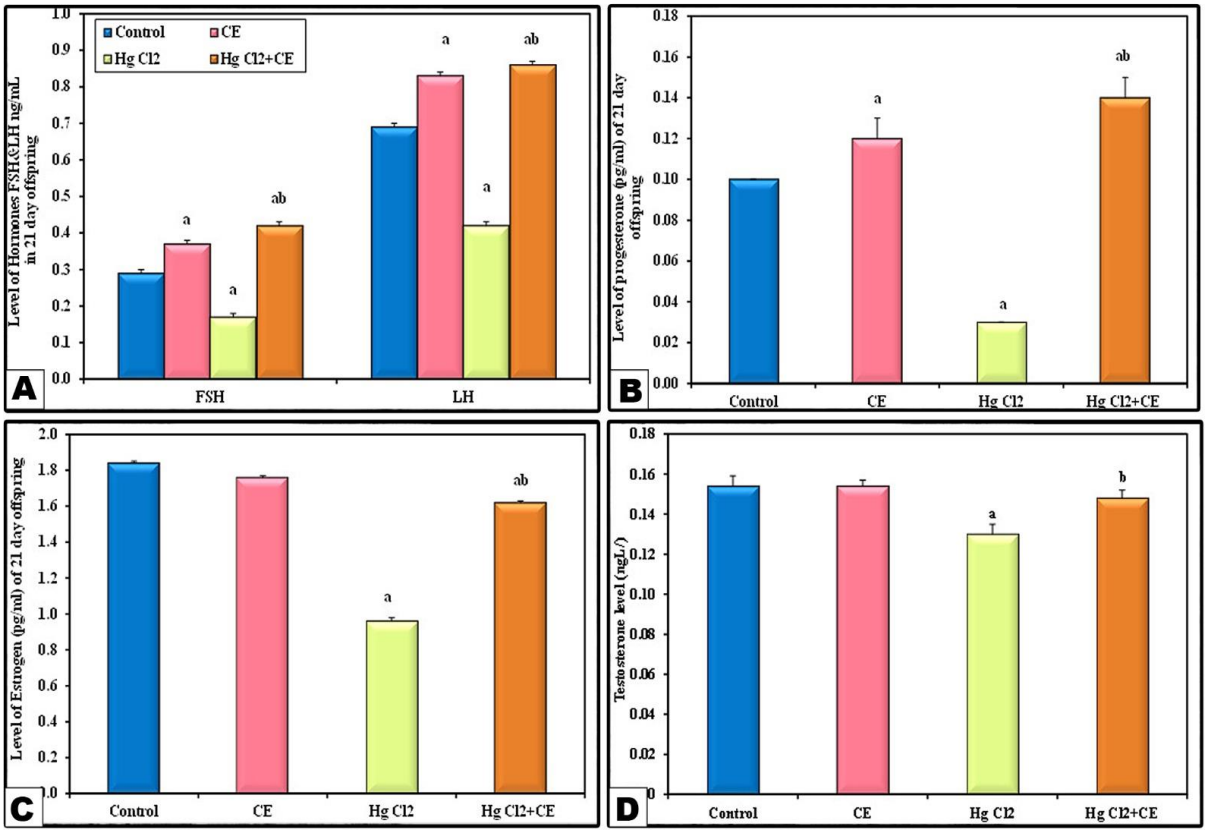



**Fig. 6:** The mean levels of serum FSH, LH (ng/ml), progesterone and estrogen (pg/ml), and testosterone (g/ml) among the different studied groups of 21st-day-old offspring of rats.

## 4. Histological changes

### *4.1 Changes in the ovaries of mothers' rats and their offspring*

The histological sections of the ovaries from control and celery extract (CE) supplemented mothers' rats appeared with normal histological architecture including inner stroma, peripheral follicles, and corpora lutea in different stages. The stroma consisted of connective tissue and scattered blood capillaries. The follicles are represented by intact primary and mature Graffian follicles. The Graffian follicles consisted of the outer layer of theca externa and inner theca interna (follicular cells) that enclosed centrally located antrum and peripheral oocyte. The corpus lutea appeared as sacs that were filled with remnants of antral liquids (Figure 7A-D). Investigation of ovarian sections from $HgCl_2$-treated mother's rats showed deleterious histopathological signs including degenerated ovarian stroma with pronounced congested capillaries. The ovarian follicles appeared atretic with lysed oocytes (Figure 7E-H). On the other hand, the ovarian sections from $HgCl_2$-treated rats co-supplemented with CE displayed remarkable recovery of the ovarian follicles and stroma, despite little congested capillaries and atretic follicles still present in some areas of the sections (Figure7 I-J).

In control and CE-supplemented 21days old offspring of rats, the ovarian sections appeared filled with intact follicles at different stages including primordial, primary, and pre-mature follicles. Additionally, the ovarian stroma appeared compact (Figure 8A-D). In $HgCl_2$- maternally induced offspring, the ovarian sections displayed little follicles some of which appeared degenerated and lost their oocytes. In addition, the ovarian stroma appeared with obvious vacuolation, and atretic follicles with little hemorrhage (Figure 8E & F). Supplementation of CE to $HgCl_2$-induced offspring successfully restored the histopathological features of the ovary induced by $HgCl_2$ whereas; a high density of intact follicles appeared scattered in the sections (Figure 8G & H).

### *4.2 Changes in the testicular tissues of 21-day-old offspring of rats*

The testicular sections from control and CE maternally supplemented 21-day-old offspring showed a high density of densely packed and -preserved seminiferous tubules. Most of the seminiferous tubules (ST) had rounded or oval contours with regular basal membranes and were filled with a high density of germinal epithelium and spermatogenic cells that separated from each other by spindle-shaped Sertoli cells. The ST separated from each other by inter-tubular connective tissues in which the Leydig (interstitial) cells are inserted. (Figure 9 A-D). In maternally $HgCl_2$-induced offspring the testicular sections displayed severe histopathological signs including degenerated ST, wide inter-tubular spaces, lost interstitial cells, obvious vacuolation in the spermatogenic cells, and scattered hemorrhage spots (Figure 9 E-H). Co-supplementation of CE to $HgCl_2$-treated offspring successfully alleviated the deleterious histopathological signs caused by $HgCl_2$ whereas the histological architecture of seminiferous tubules appeared like control (Figure 9 I&J).

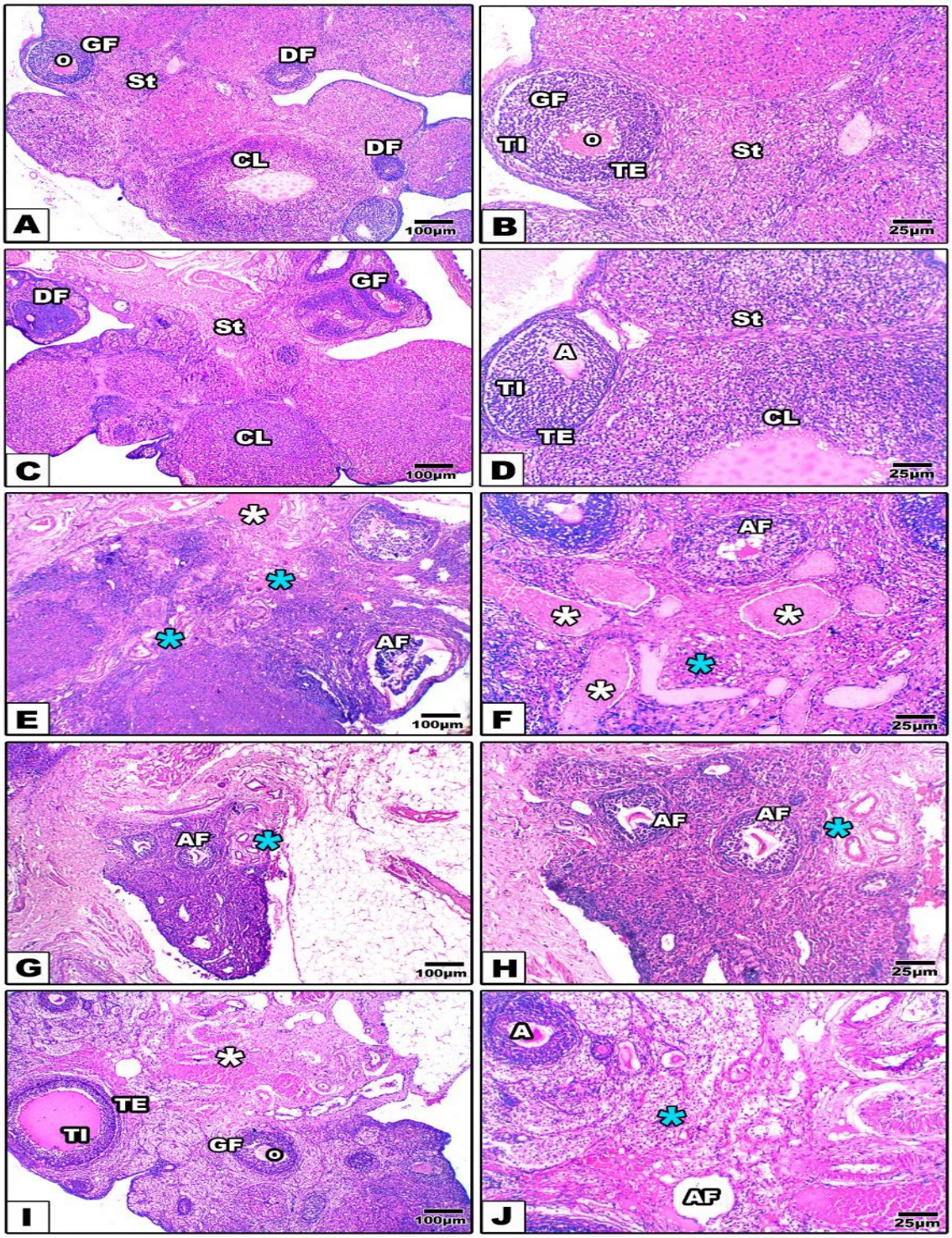



**Fig. 7:** Photomicrograph of histological sections through the ovaries among the control (A&B), CE (C&D), $HgCl_2$ (E-H), and $HgCl_2$+CE (I&J) groups of mother rats. In $HgCl_2$ treated group, the ovarian sections showed atretic follicles (AF), degenerated and fibrotic stroma (blue asterisks), and congested capillaries (white asterisks)

**Abbreviations:** A; antrum, CL; corpus luteum; DF; developing follicle, GF; Graffian follicles, O; oocyte, S; troma; TE; theca externa, TI; theca interna, AF; atretic follicle. (*Hx&E, left panels ×=100, right panels ×=250)*

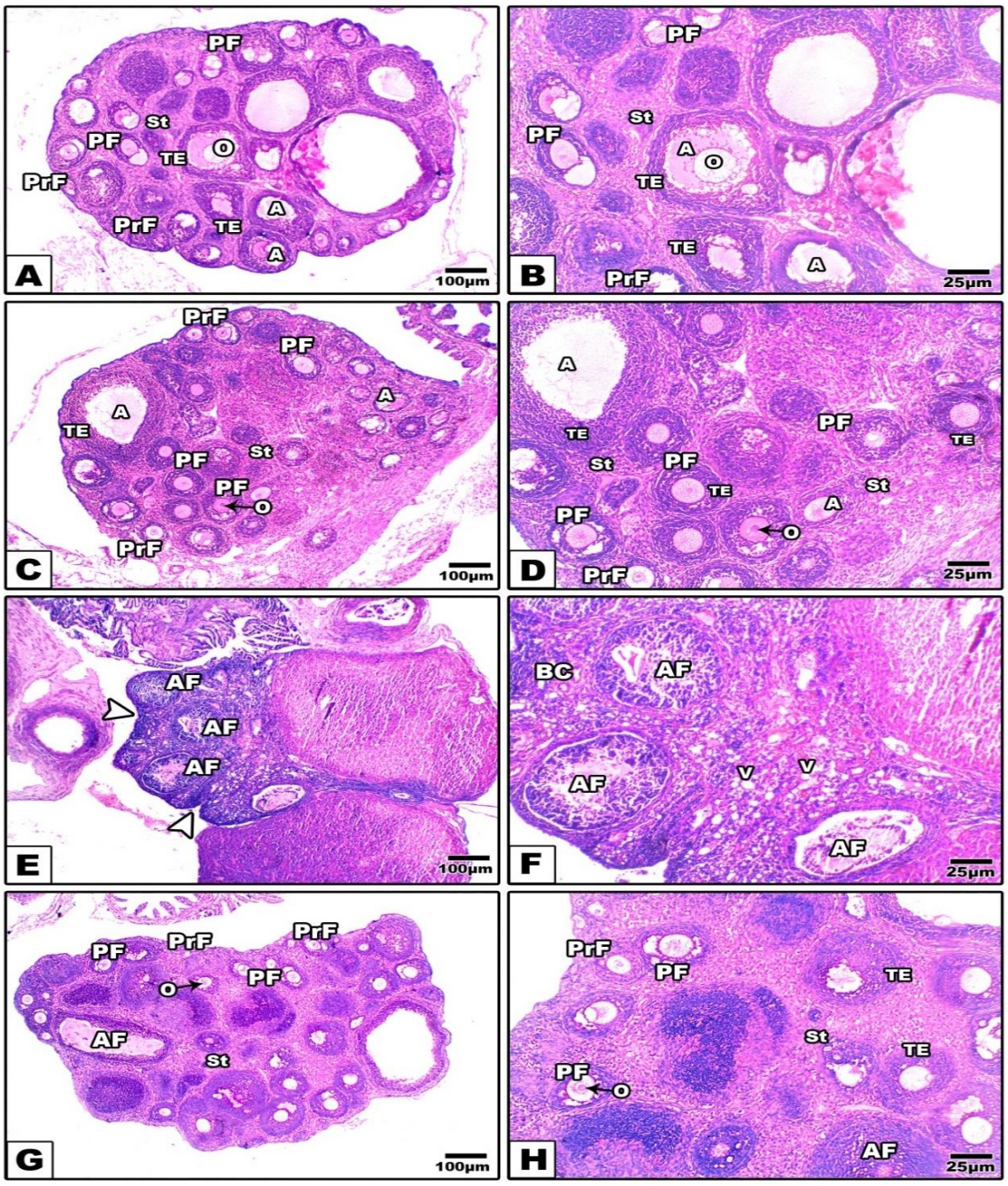



**Fig. 8:** Photomicrograph of histological sections through the ovaries among the different studied groups of 21-day-old offspring of rats (A&B: control, C&D: CE, E&F: $HgCl_2$ and G&H: $HgCl_2$ + CE). The ovarian sections from control and CE-supplemented offspring of rats appear with well-organized ovarian stroma and condensed follicles. In maternally $HgCl_2$ maternally treated offspring, the ovarian sections show shrinkage of the ovarian capsule (arrowheads), atretic follicles, and vacuolated stroma. In the $HgCl_2$+CE group of offspring, the ovarian strom as well as the developing follicles appear intact. **Abbreviations:** A: antrum, AF; atretic follicle, PF; primary follicles, PrF; primordial follicles; O; oocyte, St; stroma, V; vacuolation, TE; theca externa. (H&E, panels: A, C, E & G ×=100, panels: B, D, F&H ×=250).

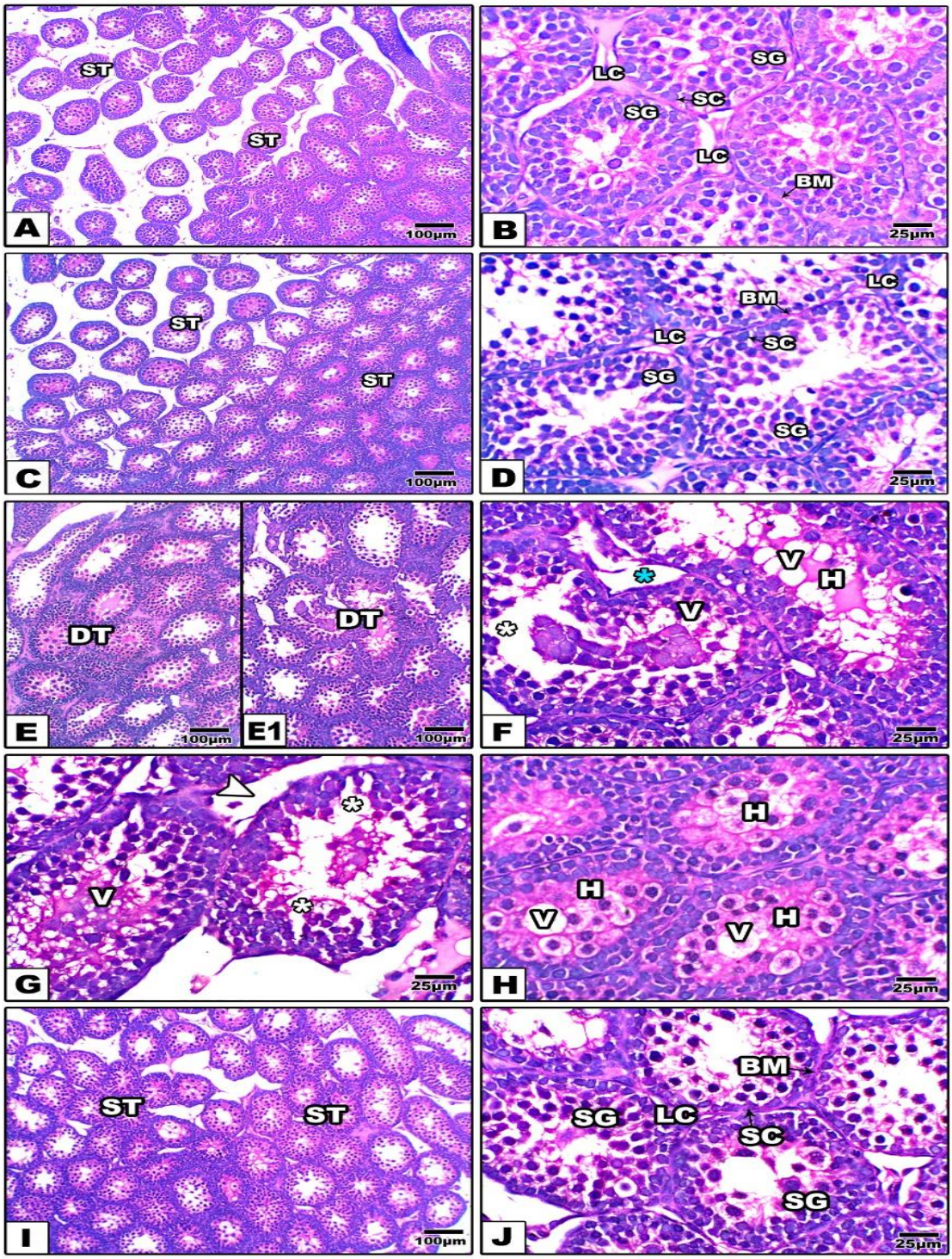



**Fig.9:** Photomicrograph of histological sections through the testes of 21st-day-old offspring (A&B: control, C&D: CE, E-H: $HgCl_2$, and I&J: $HgCl_2$ + CE). *(H & E, panels: A, A1, &E ×=100, panels, B, C, D, &F ×=250)*
**Abbreviations:** Seminiferous tubules (ST), Leydig cells (LC), Basal membrane (BM), Spermatogenic cells (SG), Sertoli cells (SC), Degenerated seminiferous tubules (DT), hemorrhage spots (H), vacuolated cells (V), lost germinal cells (white asterisks) fragmented basal membrane (arrowhead), wide inter-tubular spaces (blue asterisks). (H&E, left panels ×=100, right panels ×=250)

## 5. Immunohistochemical observations for calretinin (in the ovaries), COX2, and Ki67(in the testes)

### 5.1. Immunohistochemical localization of calretinin in the ovaries

In control and CE-supplemented mother rats, the ovarian sections displayed moderate to strong positive expression for calretinin protein. Such expression is more localized in follicular cells and has little area in the ovarian stroma (Figure 10A&B). In $HgCl_2$-treated mother rats, the degree of calretinin immuno-reactivity appeared to be very weak in the follicular cells of mature follicles and negative in the ovarian stroma (Figure 10C). In $HgCl_2$-treated rats co-supplemented with CE, the ovarian stroma displayed weak immune expression for calretinin; however, the follicular cells appeared moderately stained (Figure 10D).

The ovarian sections from control and CE maternally supplemented 21-day-old offspring displayed weak to moderate positive expression for calretinin, especially in the follicular antrum while the ovarian stroma appeared negatively stained (Figure 10A1 &B1). On the other hand, the ovarian sections from maternally $HgCl_2$-induced offspring displayed negative expression for calretinin except for the little area in the follicles (Figure 10C1). In 21-day-old offspring maternally treated with $HgCl_2$ and CE, the ovarian stroma showed negative expression for calretinin protein however the follicular antrum appeared weakly expressed (Figure 10D1).

### 5.2. Immunohistochemical localization of COX-2 and Ki67 in the testicular tissues of 21day old offspring

As shown in figure (11A-B), the germinal epithelium inside the seminiferous tubules for all studied groups of offspring displayed positive expression for COX-2 but this expression appeared strongly expressed in $HgCl_2$ maternally induced offspring if compared with weak expression for the other three studied groups.

In control and CE-supplemented offspring, the proliferative activity of lining germinal epithelium of seminiferous tubules appeared strongly reacted for Ki67 antibody, however, this reaction appeared weak in the lining epithelium of the seminiferous tubules of testicular sections from $HgCl_2$-maternally induced offspring. In the $HgCl_2$ and CE group of offspring, the immunoreactivity of Ki67 appeared moderately expressed in the germinal epithelium of seminiferous tubules (Figure 11A1-B1).

### 5.3. Image analysis for immunohistochemically stained sections

The ratio of calretinin positively stained the area of the ovarian sections among the four studied groups of mother's rats and their pups was indicated in Figure (12). The ratio of COX-2 and Ki67 positively stained area of the testicular sections among the four studied groups of rats' offspring was indicated in figure (13) using the image analysis technique.

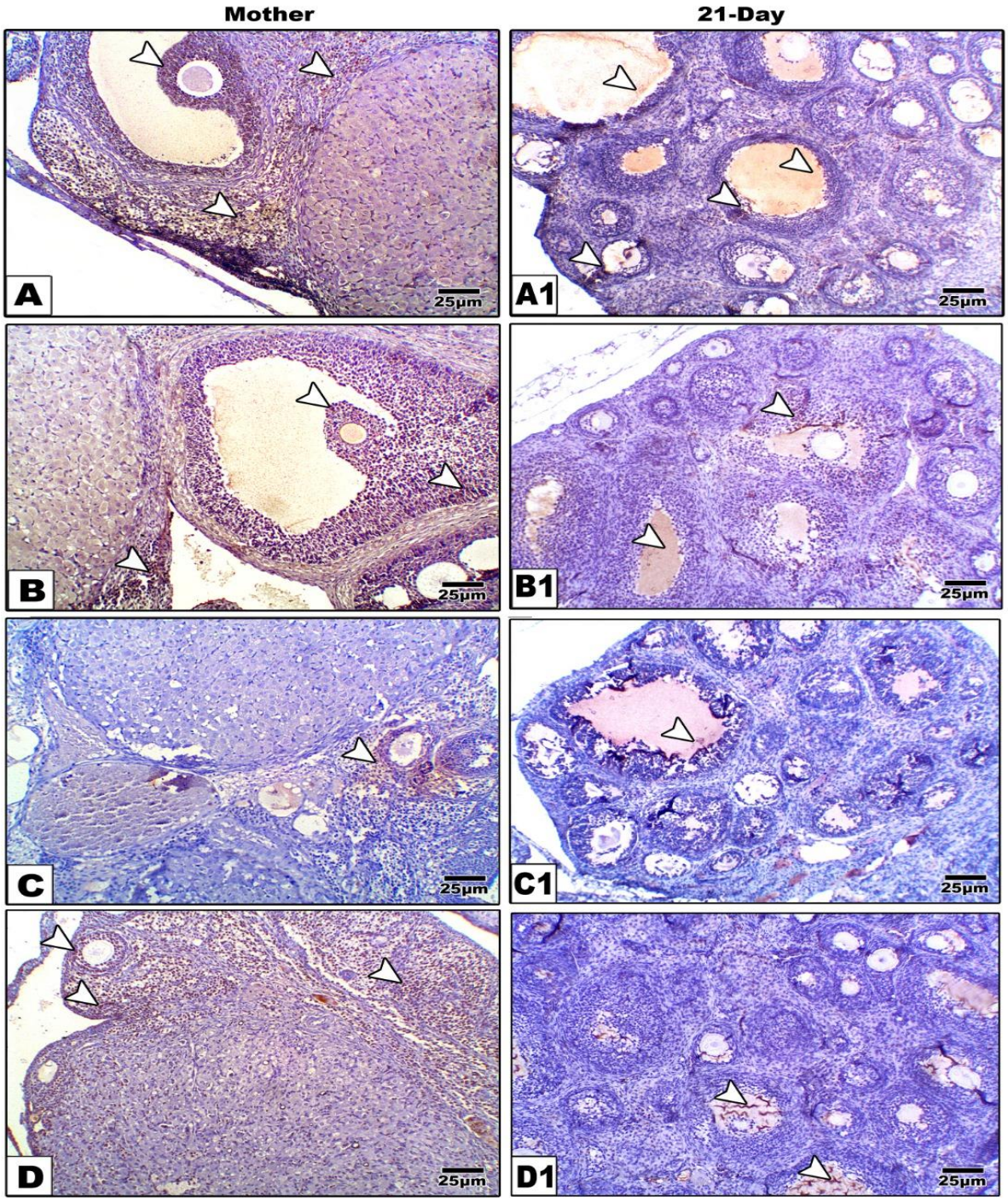



**Fig.10:** Photomicrograph of paraffin-embedded sections through the ovaries of the different studied groups of mothers' rats (panels A-D) and their offspring (panelsA1-D1) stained with calretinin antibody (A&A1: control, B&B1: CE, C&C1: $HgCl_2$ and D&D1: $HgCl_2$ + CE).

Note a moderate to strong expression of calretinin in the follicular cells and ovarian stroma in both the control and CE groups. In the $HgCl_2$-induced group, the immunoreactivity of calretinin appears very weak in the follicular cells and negative in the ovarian stroma. In $HgCl_2$-treated rats co-supplemented with CE, the ovarian follicles appear moderately stained while the ovarian stroma shows weak calretinin expression. The arrowheads refer to the immunoreactivity of calretinin positively stained cells. (Calretinin antibody, X: 250)

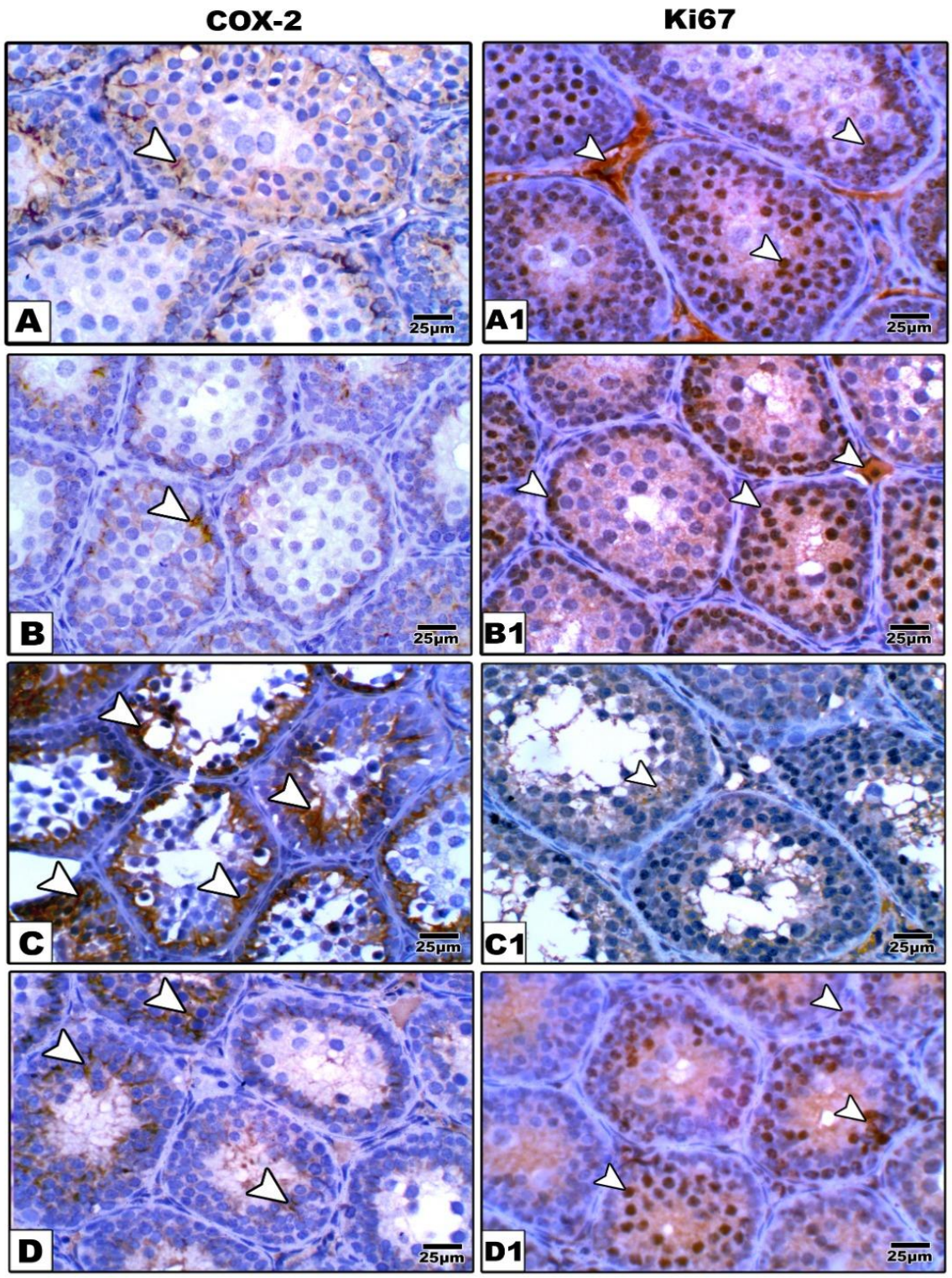



**Fig.11:** Photomicrograph of paraffin-embedded sections through the testes of the different studied groups of 21st-day-old offspring stained with Cox-2 antibody (panels A-D) and Ki-67 antibody (panelsA1-D1) (A&A1: control, B&B1: CE, C&C1: $HgCl_2$ and D&D1: $HgCl_2$ + CE).

Note a strong expression of COX-2 while a weak expression for KI-67 in the seminiferous tubules of offspring maternally treated with $HgCl_2$ in comparison with control. The arrowheads point to the immunoreactivity of calretinin positively stained cells. (X: 250)

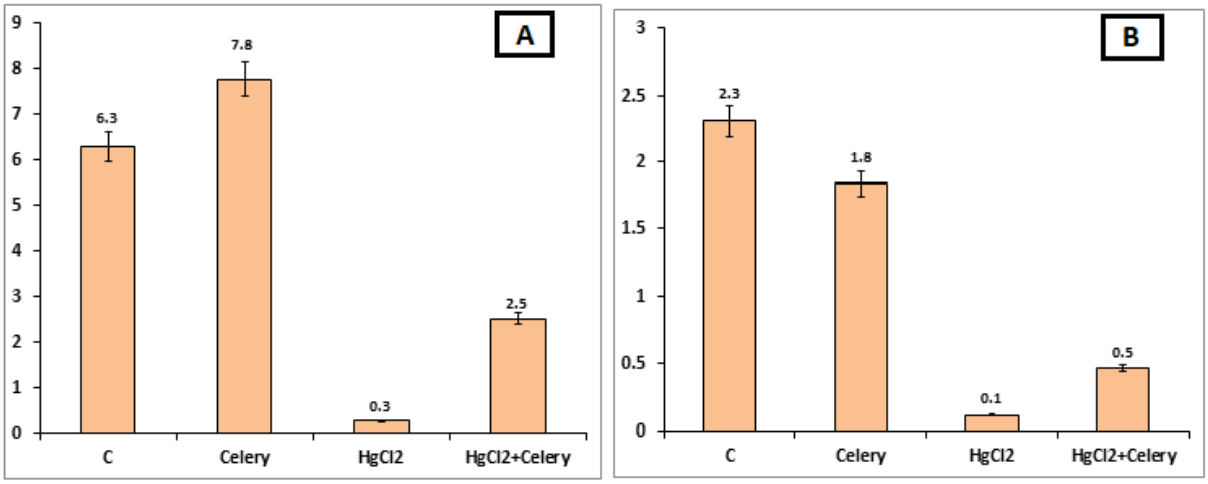



**Fig. 12:** The ratio of calretinin positively stained area of the ovarian sections among the four studied groups of mother's rats (panel A) and their offspring (panel B) using image analysis technique. The ratio of calretinin positively stained area in the $HgCl_2$ treated group appears significantly lower than control however, after supplementation with CE, this ratio is significantly elevated but still lower than control. The images were analyzed on Intel® Core I7® based computer using Video Test Morphology® software (Russia) with a specific built-in routine for the area, % area, measurement, object counting, and Contact Angle.

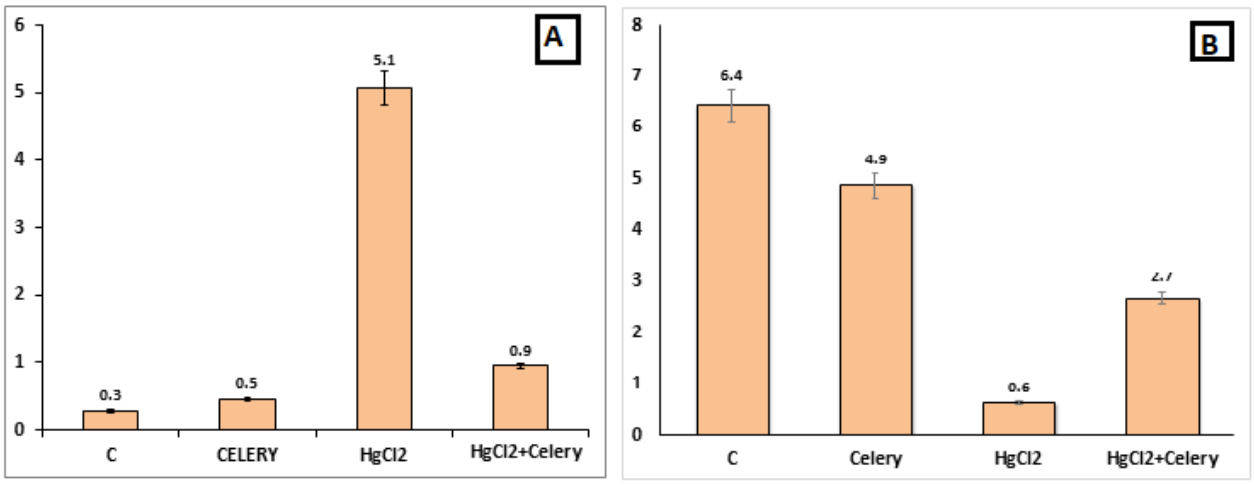



**Fig. 13:** The ratio of COX-2 (panel A) and Ki-67 (panel B) positively stained area of the testicular tissues among the different studied groups of male offspring using the image analysis technique. The images were analyzed on Intel® Core I7® based computer using Video Test Morphology® software (Russia) with a specific built-in routine for the area, % area, measurement, object counting, and Contact Angle.

## 6. Flow cytometry analysis for the activity of caspase-3 (in the ovaries) and annexin-v(in the testes)

### 6.1 Caspase-3

In control and celery extract groups of mother's rats, the ovarian tissues appeared with a normal standard percentage value of caspase-3 positively expressed cells (11.5% and 11.7% respectively). In $HgCl_2$-induced mother's rats, the mean percentage value of positively caspase-3 reacted cells of ovarian tissues appeared significantly higher if compared with the control (21.7%). In $HgCl_2$-treated mothers' rats co-supplemented with celery extract the mean % value of positively caspase-3 reacted cells of ovarian tissues appeared significantly lowered (13.2%) near to the normal value as control (Figure 14 left panel).

In control and maternally CE-supplemented offspring, the mean % value of positively caspase-3reacted cells of ovarian tissues appeared in the normal standard range (8.8% and 9% respectively). In offspring maternally induced with $HgCl_2$, the ovarian tissues appeared with a high %value of positively caspase-3 (26.9%) if compared with control. In offspring maternally induced with $HgCl_2$ and co-supplemented with celery extract, the ovarian tissues appeared with a low %value of positively caspase-3 expressed cells which were near to the control (12.2%) (Figure 14 right panel).

### 6.2 Annexin-V

In control and CE supplemented rats offspring, the mean % value of testicular viable cells (93% and 92.6% respectively; Annexin-negative/PI-negative), the mean % value of late apoptotic cells (3.6% &3.9% respectively, Annexin-positive/ PI-positive), the mean % value of early apoptotic cells (1.1%&1.3% respectively; Annexin-positive/PI-negative) and the % of necrotic cells (2.3% &2.2%; Annexin-negative/PI-positive) appeared in the normal range. On the other hand, the testicular cells from $HgCl_2$ maternally induced offspring revealed a highly significant decrease in the mean % value of viable cells (77%) and a highly significant increase in the late apoptotic cells (15%), early apoptotic cells (2.3%) and necrotic cells (5.7%) if compared with control. In $HgCl_2$ rat's offspring co-supplemented with CE, a highly significant increase was recorded in the % of viable cells (84.5%) and a highly significant decrease in the late apoptotic cells (8.9%), early apoptotic cells (1.8%) and necrotic cells (4.8%) if compared with $HgCl_2$ group but not reach to the standard value as control (Figure 15).

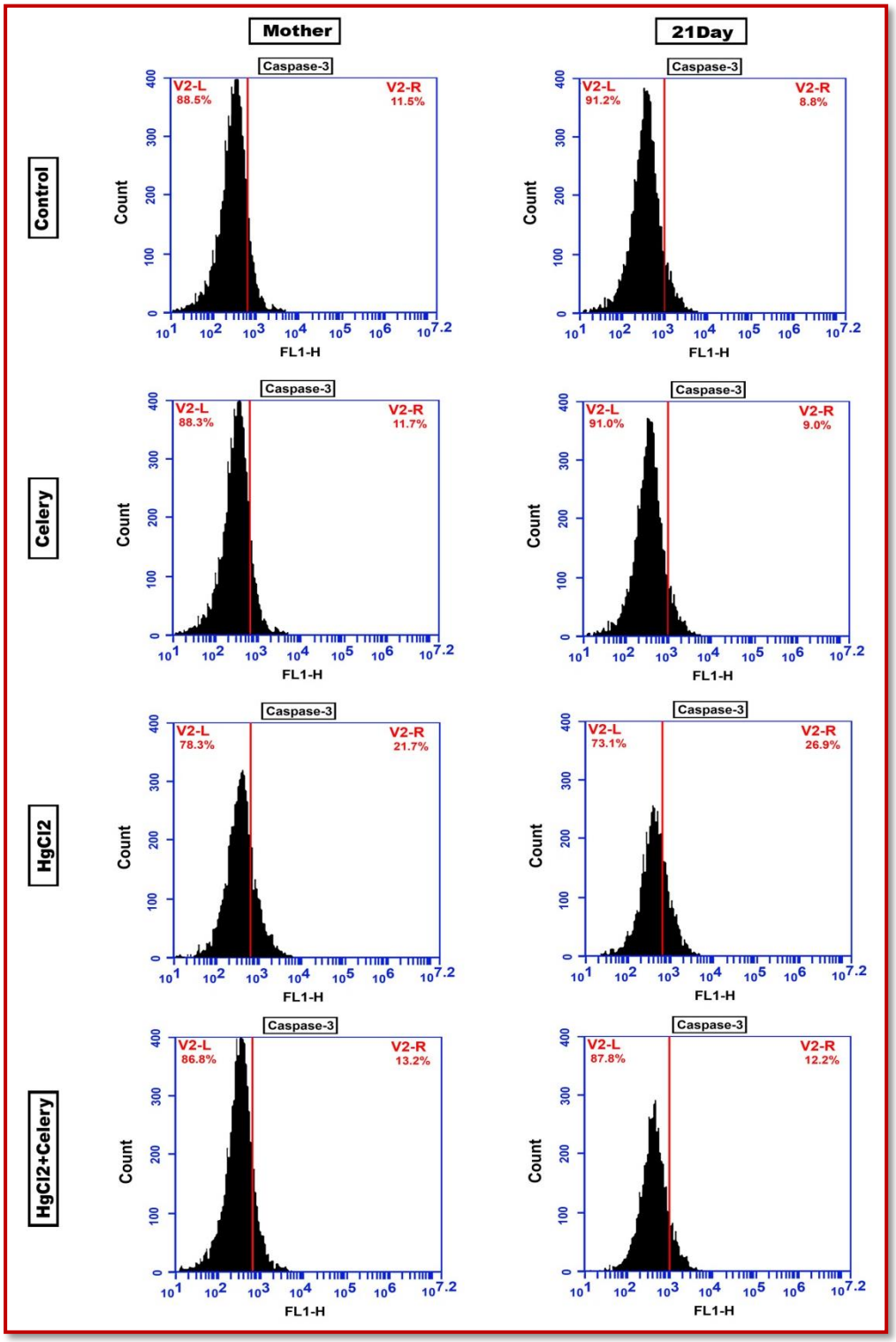



**Fig.14:** A flow cytometric chart illustrating the mean % of caspase-3 positively expressed cells by FAC Scan analysis via FL1 H in the ovarian tissues among the different studied groups of mother's rats (left panel) and their offspring(right panel). Note a high % value of caspase-3 positively expressed ovarian cells in $HgCl_2$ mother's rats and their offspring (21.7% and 26.9% respectively) if compared with control.

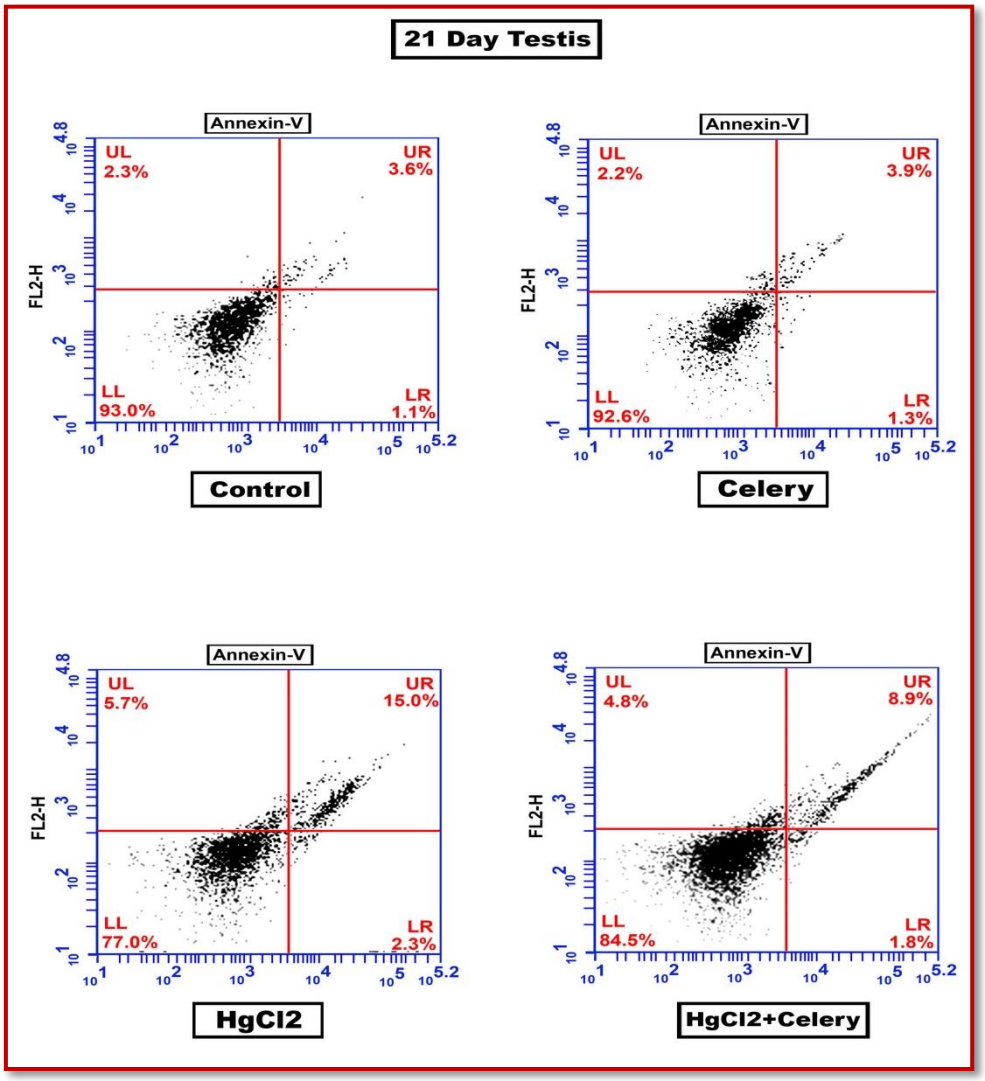



**Fig. 15:** A flow cytometric chart illustrating the mean % of apoptosis and necrosis by FAC Scan analysis via Annexin V-FL2- H/PI in the testicular tissues among the different studied groups of 21days old offspring of rats. Cells in the upper right quadrant indicate the % of Annexin-positive/ PI-positive (late apoptotic cells). Cells in the lower right (LR) quadrant indicate the % of Annexin-positive/PI-negative (early apoptotic cells). Cells in the upper left (UL) quadrant indicate the % of Annexin-negative/PI-positive (necrotic cells). Cells in the lower left (LL) quadrant indicate the % of Annexin-negative/PI-negative (viable cells). Note a high % value of late, early apoptotic, and necrotic testicular cells (15%, 2.3%, and 5.7% respectively) in $HgCl_2$ offspring rats if compared with control (3.6 %, 1.1%, and 2.3% respectively).

## 4. Discussion

Mercury (Hg) is a prominent environmental contaminant that causes detrimental effects on human health (Ung et al. 2010). Previous reports revealed that Hg exposure during gestation and lactation periods was found to induce toxicity in pregnant mothers and their developmental fetuses (Feng et al. 2004; Bose-O'Reilly et al. 2010). Other reports revealed that fetuses and infants are more sensitive to Hg exposure than adults (Axelrad et al. 2007; Marques et al. 2015). The high sensitivity of fetuses to mercury may be attributed to the immaturity of organs and their inability to resist toxic agents (JG et al. 1996).

Celery leaves contain vital compounds such as valerophenone (19.90%), 1-dodecanol (16.55%), 9-octadecanoic acid, and methyl ester (4.93%) (Nagella et al. 2012). Additionally, celery leaves contain important antioxidants, such as GSH content, CAT, xanthine oxidase, glutathione

peroxidase, and peroxidase activities which resist the intensity of lipid peroxidation in blood. Moreover, celery fatty acid contents are necessary for the proper function of spermatogenesis (Taati et al. 2011) and sperm parameters (Kerishchi et al. 2011). Fresh celery leaves have an appetite and libido stimulant, and it increases the secretion of breast milk. No research has been conducted on the potential role of celery leaf extract on the gonads of pregnant individuals and their pups, accordingly, the current work aimed to evaluate the possible protective effects of celery leaf extract against $HgCl_2$-induced gonadal damage in the mother rats and their offspring.

Body weight reduction is a primary important sign of disturbance in the individual general health (Uzunhisarcikli et al. 2016). The obtained results showed a significant decrease in the body weights of $HgCl_2$ treated mother's rats and their pups at PNDs1, 7, 14, and 21 if compared to control however after supplementation with CE, the body weights were successfully restored near to the normal. A recent study revealed that exposure of female rats to $HgCl_2$ in drinking water during gestation and lactation periods is implicated in the reduction of body weights of mams and their pups**.** The reduced body weight under the influence of $HgCl_2$ is mainly attributed to the fact that mercury can cause appetite loss resulting in decreased food consumption and water intake (Mahboob et al. 2001)**.** In contrast to our results, a remarkable increase in body weight was recorded in adult male mice that were administered with $HgCl_2$ for 45 days (Rao and Sharma 2001). This may be attributed to the dose of $HgCl_2$, the species resistance rate as well as the surrounding environmental conditions. Recovery of body weight in mother's rats and their offspring treated with $HgCl_2$ and CE go parallel with a similar finding reported by Abu-Taweel who found that CE can attenuate the decreased body weights of rats' offspring maternally induced by bacterial lipopolysaccharides (Abu-Taweel 2020). Additionally, it had been confirmed that fresh celery leaves have an appetite stimulant property that helps in maintaining body weight.

Durak et al. found that Hg exposure can elevate the production of intracellular ROS (Durak et al. 2010). Su et al. showed that MDA concentration elucidates the degree of damaged cells and tissues (Su et al. 2011). Other studies found a significant elevation in the levels of MDA in various tissues and plasma in response to $HgCl_2$ toxicity (Kalender et al. 2013; Aslanturk et al. 2014) It was revealed that increased MDA level is an indicator of tissue damage (Uzunhisarcikli et al. 2016)**.** Furthermore, Hg can cause oxidative stress by inducing the production of peroxides and superoxide anion radicals. This can lead to membrane lipid peroxidation, protein denaturation, DNA damage, and cellular injury. It was reported that the potentially harmful effects of free radicals generated by $HgCl_2$ are controlled by cellular antioxidants like SOD and CAT (Faix et al. 2003). SOD enzyme catalyzes the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen (Boujbiha et al. 2009)**.** CAT converts $H_2O_2$ to $H_2O$ and oxygen, protecting cells from oxidative damage (Renugadevi and Prabu 2010). Another related study revealed a significant reduction in the activities of CAT, SOD GSH, and glutathione content in $HgCl_2$-induced oxidative stress in hens (Ma et al. 2018)**.** In the current work, $HgCl_2$ exposure during gestations and lactation periods induced oxidative stress in both mother's rats and their offspring as indicated by significant elevations in the levels of serum lipid peroxidation product (MDA) and decreased serum SOD and CAT levels.

It had been reported that celery leaves are rich in essential antioxidant compounds like caffeic acid, *p*-coumaric acid, ferulic acid, apigenin, luteolin, tannin, saponin, and kaempferol (Yao et al. 2010). These antioxidant compounds can enhance the activities of SOD, CAT, and GSH (Yildiz et al. 2008; Kolarovic et al. 2010)**.** In the present work, CE successfully attenuated the oxidative stress induced by $HgCl_2$.

The protective effect of celery seems to be due to its antioxidant property (especially, its apigenin content) and its detoxification capacity (Hamza and Amin 2007). The celery effect might be related to the presence of flavonoids, especially apigenin (unpublished data). Apigenin is an antioxidant that is registered as one of the main active compounds in celery (Miean and Mohamed 2001)**.** It has also been reported that the cytochrome P450 activity was markedly reduced by celery juice in the liver of Syrian mice (Jakovljevic et al. 2002)

Mercury exposure has been associated with an increased incidence of hormonal disorders (Henriques et al. 2019)**.** In females, Hg has been shown to have an inhibitory effect on the secretion of LH and FSH from the anterior pituitary (Davis et al. 2001; Chen et al. 2006b). This can inhibit the secretory levels of estrogen and progesterone, causing ovarian dysfunctions. Moreover, the level of progesterone liberated was inhibited from the ovarian granulosa cells of pigs exposed to mercury. This confirms a direct effect of mercury on the secretory release of progesterone as well as interference of Hg in the pathways of steroidogenesis and apoptosis (Knazicka et al. 2013; Roychoudhury et al. 2015). In the current work, a highly significant decrease in the levels of serum FSH, LH, estrogen, and progesterone was recorded among $HgCl_2$-treated mother rats and their offspring. Kňažická et al. found that hormonal release from the pig ovarian granulosa is affected by different doses of mercury (Knazicka et al. 2013).

Heath et al. studied the effects of two doses of $HgCl_2$ on male rats for 30 consecutive days (1.0, or 2.0 mg/kg/day of $HgCl_2$) (Heath et al. 2012). The authors found a significant decrease in the levels of testosterone on exposure to either dose. This agrees with our obtained results that revealed a significant decrease in testosterone levels in $HgCl_2$ maternally induced offspring. Additionally, disruption of the pituitary-gonadal axis among $HgCl_2$-exposed mothers and their fetuses was evaluated by Lee et al. (Lee et al. 2019). The results reported that perinatal exposure of women to $HgCl_2$ can disrupt the pituitary-gonadal axis directly in women and via distribution in the placenta and lactating milk for fetuses.

Another study confirmed that ovaries exposed to organic or inorganic mercury had various histo-morphometric and histopathological changes, like a reduction in the total number of primordial, primary, and Graffian follicles as well as increased incidence of atretic follicles (Altunkaynak et al. 2016)**.** Our obtained results revealed similar histopathological signs in the ovaries of $HgCl_2$-treated female rats and their pups.

Previous reports revealed various histopathological signs in the testicular tissues of $HgCl_2$-treated rats. These included little numbers of seminiferous tubules, vacuolation of some spermatogenic cells, decreased spermatozoa, and irregular undulating basement membranes (Adelakun et al. 2020; Almeer et al. 2020). Additionally, mercury was also reported to induce swelling in the interstitial and inter-tubular areas, and necrosis of spermatogenic cells in male rats (Massanyi et al. 2007). This is in accordance with our obtained results showing severe histopathological alterations in the testicular tissues of 21-day-old offspring maternally induced with $HgCl_2$.

The ovarian and testicular histopathological alterations induced by $HgCl_2$ were mainly attributed to that mercury can induce oxidative stress in these organs indicated by significantly decreased levels of SOD, CAT, GPx, and elevated MDA (Kandemir et al. 2020).

It seems that celery improved sexual hormones in women and could be used as a safe, well-tolerated, and effective herbal medicine in women with sexual dysfunction (Madkour 2014) Madkour has revealed a significant elevation of FSH, LH, estrogen, and progesterone lowered by di (2-ethylhexyl) phthalate (Madkour 2014). In contrast, Kooti et al. observed a significant decrease in the levels of FSH, LH, and testosterone in male rats fed on hydro-alcoholic

extract of celery leaves (Kooti et al. 2014). This conflict of results may be attributed to the dose of CE as well as the type of solvent used for the preparation of the extract. Hardani et al. added that celery antioxidants can enhance the hypothalamic-pituitary-testicular axis thus increasing FSH, LH, and testosterone (Hardani et al. 2015). Furthermore, the antioxidants compounds of celery like flavonoids (apiein and apigenin), as well as vitamins E and C, were found to protect cell membranes against damage via the reduction of oxidative stress (Fazal et al. 2012; Kooti et al. 2014)**.** Another study revealed that exposure of rats to mercuric chloride (1 mg/kg bw) daily for 4 weeks resulted in severe histopathological alterations in the testicular tissues and remarkable increment in lipid peroxidation, and decreased SOD, CAT, GPx activities (Kalender et al. 2013; Kandemir et al. 2020). Additionally, apiin constituent from celery leaves had remarkable free radical–scavenging activities via inhibition of unwanted oxidation process (Nagella et al. 2012; Li et al. 2014).

In the present work, the immunohistochemical results revealed negative to very weak calretinin protein expression in the ovarian sections of $HgCl_2$-exposed female rats and their offspring if compared with their control however in treatment with CE, this expression was markedly increased.

Calretinin is a calcium-binding protein that regulates the $Ca^{2+}$ levels in most cells (Oladipo et al. 2015). It was first discovered in the nervous tissues and its function has been identified in cerebral tissues (Schwaller 2014). Lungi et al identified little calretinin expression in the granulosa cells of ovarian follicles and corpora lutea (Lugli et al. 2003). The authors suggested that calretinin is essential for maintaining androgen-producing cells of the ovarian follicles whereas cell calretinin deficiency results in disruption of ovarian follicles. This finding goes parallel with our obtained results including disrupted ovarian follicles of $HgCl_2$ treated mother's rats and their pups. The potential role of CE in the re-expression of calretinin in this study may be attributed to its antioxidant constituents that protect the follicular cells from $HgCl_2$ oxidative stress-induced cellular damage.

Cyclooxygenase2 (COX-2) is an inflammatory marker that participates in inflammation processes via the production of prostaglandins resulting in vasodilation (Gandhi et al. 2017)**.** A recent study by Kandemir et al. showed that exposure of male rats to a high dose of $HgCl_2$ (1.23 mg/kg/b.w) for 7 days increased the expression of COX-2 in the testicular tissues (Kandemir et al. 2020). CE has been confirmed to suppress carrageenan-induced edema in rats (Al-Hindawi et al. 1989). In mice, celery leaf extracts inhibited lipopolysaccharide (LPS)-induced miR-155 expression and lowered pro-inflammatory cytokine production (Che et al. 2020). Additionally, celery leaf extracts inhibited inducible NO synthase (iNOS) expression in LPS-activated J774.A1 macrophages (Mencherini et al. 2007). It had been shown that the serum level of COX-2 is elevated in rats treated with *Staphylococcus aureus* however on treatment with CE this level is significantly declined**.** All these along with our obtained results confirm that celery leaf extract may have an anti-inflammatory effect against $HgCl_2$-induced inflammation in the testicular tissues.

Oxidative stress caused by $HgCl_2$ is one of the factors linked with cell apoptosis**.** Further, $HgCl_2$ has a prominent affinity for thiol (-SH) of glutathione (GSH) and sulfhydryl proteins (Clarkson and Magos 2006), resulting in a decreased level of GSH and consequently the induction of oxidative stress (Ercal et al. 2001)**.** This is a major promoting factor of apoptosis (Leonard et al. 2004)**.**

The Ki-67 protein is a cellular proliferative protein marker (Scholzen and Gerdes 2000). The Ki-67 antigen can be detected within the cell nucleus, and on the surface of the chromosomes (Cuylen et al. 2016)**.** Low expression of Ki-67 protein in the cells is one of the indicative factors for apoptosis. In the current work, low Ki67 expression was recorded among the testicular tissues of $HgCl_2$ maternally induced offspring if compared with control. The obtained result goes parallel with the finding of

Massányi et al. who reported that exposure of male rats to two high doses of $HgCl_2$ (5 and 10 mg/kg b.wt) for 48 hours is implicated in the induction of cellular apoptosis in both testes and kidneys (Massanyi et al. 2007). The apoptotic effect of mercury is fundamentally attributed to its direct effect on the release of steroid hormones and interference with the pathways of steroidogenesis resulting in apoptosis (Roychoudhury et al. 2015). Further confirmation for apoptotic effects of $HgCl_2$ was done using flow cytometric detection of annexin-v marker. Annexin-v is a good marker used to discriminate the incidence of cellular apoptosis and necrosis. If compared with control, our flow cytometric result revealed a significant increase in the mean % value of pro-apoptotic, late apoptotic, and necrotic cells of testes from $HgCl_2$ maternally induced offspring if compared with control. It had been reported that prolonged exposure to $HgCl_2$ could induce oxidative stress on the testicular cells resulting in decreased antioxidants and liberation of free radicals leading to acceleration of apoptotic and necrotic pathways (Park and Park 2007). It was reported that exposure of rats to $HgCl_2$ led to necrotic and apoptotic effects on the cells of the brain, liver, kidneys, and testes (Abd Elghani et al. 2020).

Caspases have a key role in the induction of apoptosis and are usually found as inactive heterodimers known as pro-caspases (Fan et al. 2005)**.** There are 14 subtypes of caspases; the major one that activates apoptosis is caspase-3 (Indran et al. 2011)**.** In the current work, $HgCl_2$ exposure during gestation and lactation periods induced a significant elevation in the mean percentage value of caspase-3 positively expressed ovarian cells from mothers' rats and their pups if compared with the control. This finding confirms the apoptotic effects of mercury. As previously mentioned above the apoptotic effects of mercury are mainly caused by induction of oxidative stress (Zhang et al. 2016)**.** A similar study showed remarkable apoptosis in $HgCl_2$-exposed lung cell carcinoma in humans indicated by increased caspase-3 activity (Zhou et al. 2019).

In the current work, celery leaf extract alleviated the apoptotic effects of $HgCl_2$-treated rats and their pups. Similar reports evaluated the anti-apoptotic effects of celery leaves (Pandey et al. 2012; Shukla et al. 2014). All opinions agreed that the anti-apoptotic effect of celery leaves is mainly attributed to flavone compounds. Flavones like apigenin, chrysin, luteolin, baicalein, tangertin, and acacetin were found to inhibit apoptosis in different studies (Khoo et al. 2010).

**Conclusion:** Exposure of female rats to mercuric chloride during gestation and lactation periods caused remarkable deleterious biochemical, apoptotic, and histopathological changes in the gonads of both female rats and their pups. Celery leaf extract successfully alleviated most of these changes. The potential protective effect against deleterious changes induced by $HgCl_2$ is fundamentally attributed to their flavone compounds.

## 5. References

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