African J. Biol. Sci., 3 (3): 1 – 19 (2007)
E.mail, aasdjournal@yahoo.com

ISSN 1687-4870
Zoology

Histological and ultrastructural studies on the impact of antioxidants against the toxicity of certain heavy metals in the ovarian chicken

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ABSTRACT

The objective of this study was designed to determine the effects of mixture of two heavy metals (lead and cadmium) as lead and cadmium acetate (0.17 mg / l and 0.06 mg / l) respectively on the ovarian structure of the adult Lohaman chickens. Also, the possible ameliorative impacts of 250 mg/kg of diet, vitamin C and 200 mg/kg of diet vitamin E., separately and in combination as antioxidants in treating the heavy metal toxicity.

The chicks were classified into 6 groups. The light and ultrastructural microscopic studies showed marked histopathological changes in the lead and cadmium-treated groups (GII, GIII, and GIV) in comparison with G I, GV and GVI.

These changes were represented by increase in number of the growing ova, presence of edematous and fibrotic areas in the interstitial tissue, undifferentiation of the thecal layer, degeneration of the thecal glands and vacuolization of the follicular cells.

The administration of vitamin C alone with lead and cadmium diet (GIII), improved the constituents of the ooplasmic organelles which were represented by great numbers of elongated and healthy mitochondria and scattered transosomes. However, the administration of vitamin E alone with lead and cadmium diet (GIV), improved the cell membranes of many cells and their membranous structures such as RER and mitochondria either in thecal or follicular cells, and the fibers become more obvious.

The combination of vitamins C and E in diet containing lead and cadmium (GV) were more effective than the addition of each of them alone in preventing the degeneration of the thecal layer and follicular cells and improvement of different ovarian structures such as the appearance of well developed vitelline membrane and zona radiata. Moreover, both vitamins accelerate the maturation of follicles and improve the general ovarian structures.

This study proved that the administration of a combined dose of vitamins C and E with the daily diets and drinking water in the poultry farms especially those near to the sites of pollution of the sewage and industrial wastes, is highly recommended.

Key words: Lohaman chickens – lead – cadmium – vitamins C & E – ovarian structure – LM - TEM

INTRODUCTION

Pollution is still the main problem of the world today. As a result of modernization and construction of different manufactories of oil refining and painting industries, the pollutants have become widely distributed in the air, soil and water.

Water is the primary route of human and animal exposure to the pollutants such as chemical leaching from stores of hazardous waste (Yang *et al.*, 1989). The

Industrial waste, leaching of minerals from natural deposits, accidental spills and leaks….etc are the major contributors of ground water pollution (Yang and Rauckman, 1987).

Lead and cadmium are among the most common heavy metal pollutants, which contribute to environmental pollution because of their worldwide natural presence in coal, mineral oils, batteries and in several chemical industries (Gerhardsson and Skerfving, 1996). Pesticides are considered the primary sources of lead and cadmium pollution (WHO, 1984 & 1992). Moreover, the hazards of these two metals are not only due to their high toxicity, but also to the low rate of elimination from the consumer's body, and often unchanged, for long periods (Allen, 1995).

The toxicity of these metals becomes more complex owing to successive exposure of different physical, chemical, biological and psychological factors in the environment. Moreover, in the majority of the investigations, a single pollutant was used, but this is not the case in nature, where more than one contaminant is usually present. These contaminants do not act individually on the organism, but they interact with each other in a way that may result in modification of their toxicological effects (El-Dawi and Elbadry, 2001; Abdel-Salam, 2003; El-Dawi, 2004).

Many studies have suggested that antioxidants may play an important role in abating some hazards of heavy metals, especially lead and cadmium (Gurer and Ercal, 2000 ; Abdel-Salam, 2003). It is important to underline the importance of an adequate vitamin intake in the prevention and treatment of cadmium and lead intoxication (Pace and Iannucci, 1994). Ascorbic acid (vitamin C) and α-tocopherol (vitamin E) are known as antioxidants and free radical scavenging nutrients and provide potential protection against cell damage by oxidative stress (Anderson and Theron, 1990).

Birds are considered valuable indicators of heavy metals contamination, because they occupy a wide range of trophic levels in different food chains and are thereby exposed to different concentrations of heavy metals in their food (Gochfeld and Burger, 1982). The concentrations of safe levels of substances in poultry drinking water have been estimated (Vohr,1980). However, the accuracy of these values needs further evaluation.

It is difficult to evaluate the impact of water pollution on the poultry industry (Vodela *et al*., 1997). A review of literature, revealed limited information on the effects of water impurities on the reproductive performance, egg quality and very scarcely on the gonadal structure of chickens.

Fry (1995) stated that the range of chemical pollutants effects on adult birds leads to reduced fertility and suppression of egg formation. He also determined the types of pollutants that cause reproductive effects; such as organochlorine and organophosphate pesticides, petroleum hydrocarbons and heavy metals. Also, Vodela *et al.* (1997) studied the effect of drinking water containing a low concentration of chemical mixture (arsenic, benzene, cadmium, lead and trichloroethylene) and a high 10 times greater than the low one, on the Broiler breeder hens. They found that at low concentration of this mixture, egg production and egg weight were significantly decreased, whereas the percentage of embryonic morality was increased. In mammals, on the other hand, Priya *et al.* (2004) studied the effect of lead and cadmium both alone or in combination on the follicular (granulosa) cells of the ovary of rat and suggested that these metals cause a reduction in LH and FSH binding, which significantly alters steroid production *in vitro* and exerts a direct influence on follicular cells structure and function. Also, Nampoothiri and Gupta. (2006) reported that lead and cadmium are reproductive toxicants, which accumulate in granulosa cells of the ovary of rat.

Therefore, the objective of this study was to determine the effects of mixture of two (lead and cadmium) on the ovarian structure of the adult Lohaman chickens and to throw light on the possible ameliorative impacts of vitamins C and E, separately and in combination as antioxidants in treating the heavy metal toxicity. Chicken were selected for these studies because of their economic importance as a farm commodity, their potential use as a model to estimate sensitivity of wild avian species to water contaminated with a mixture of chemicals.

MATERIAL AND METHODS

Water collection and analysis

Three water samples were collected from the ground water of three different sites of Premman area at Jeddah, Kingdom of Saudi Arabia. Premman area is the collection site of the sewage wastes, industrial wastes, accidental spills and leaks and water drainage. The ground water of these sites is the main source of the animal drinking water and usually supplies different animal farms in these areas.

The water analysis was carried out according to APHA (1985). One ml of concentrated nitric acid/liter water was added to preserve the water sample, until the time of analysis. The samples were prepared for analysis in the Central Laboratory, Faculty of Science Ain Shams University. Perkin Elmer Series 3100 Graphic Furnace Atomic Absorption was used for analysis of lead, whereas Perkin Elmer, Series 6000 Flame Atomic Absorption Spectrophotometer was used for analysis of cadmium, in the test ground water. The average concentration for each heavy metal from each site was calculated and the results of analysis are shown in table (1).

Pollutants

Two heavy metals lead and cadmium acetate were used. The concentrations of the heavy metals, the international permissible levels (USEPA, 1991) and the selected levels were 0.17 mg /l of lead and 0.06 mg /l of cadmium are shown in table (1). These concentrations were added to the drinking water and the diet (5 ml of the previous concentration of both heavy metals to 10 Kg/diet,.

Antioxidants

Vitamins C (L-ascorbic acid) and E (alpha-tocopherol) were used separately and in combination with lead and cadmium salts. Also, both vitamins were used together without combination with any of the heavy metals.

The concentrations of vitamins C and E were 250 mg/kg of diet, and 200 mg/kg of diet, respectively were added to the diet according to Sahin *et al.* (2002a).

Table 1: Average concentrations of lead and cadmium in the ground water from different sites at Jeddah, their international levels and the selected concentrations.

Sites of the test ground water	Average conc. of heavy metals (mg) ± SD		International Permissible conc. (mg) *USEPA		Selected conc. (mg) ± SD	
	Lead mg\l	Cadmium mg\l	Lead mg\l	Cadmium mg\l	Lead mg\ l	Cadmium mg\ l
Site I	0.23 ± 0.05	0.11 ± 0.03	0.37	0.085	0.17	0.06
Site II	0.17± 0.02	0. 06 ± 0.008				
Site III	1.10± 0.07	0.2 ± 0.005				

* USEPA = United State Environmental Protection Agency (1991)

Animals and housing

Thirty hundred and sixty chicks of Lohmann LSL (Leghorn Selected Layer), *Gallus domesticus* (one day old, with an average body weight 50 gm) were delivered from Arab Poultry Breeders Company "OMMAT" at Jeddah City, Kingdom of Saudi Arabia. It must be noted that this number of chicks were used for different studies including the ovarian structure.

The chicks were randomly placed in 6 floor pens and exposed to day light and acclimatized for one week prior to initiation of the experiment. Each pen contained 60 chicks and the chicks were fed daily with a basal diet (Broiler Starter FM, manufactured by ARASCO (30001) Feed Mill, Dammam, Saudi Arabia) and drinking tap water. After acclimatization 300 healthy chicks were selected for the experiment, weighed separately, at the beginning (average body weight was 85 gm) and divided randomly into 50 chicks/ pen.

Experimental design and feeding

The chicks were classified into 6 groups as follow:

- **G I** :was fed daily with basal diet (control group).
- **G II** :was fed daily with basal diet supplemented with lead and cadmium at concentrations of 0.17 mg /l and 0.06 mg/l, respectively.
- **G III** :was fed daily with basal diet supplemented with lead, cadmium at the above mentioned concentrations and vitamin C at a concentration of 250 mg/kg of diet
- **G IV** :was fed daily with basal diet supplemented with lead, cadmium at the above mentioned concentrations and vitamin E at a concentration of 200 mg/kg of diet
- **G V** :was fed daily with basal diet supplemented with lead and cadmium at the above mentioned concentrations and vitamins C&E at the above mentioned concentrations.
- **G VI** :was fed daily with basal diet supplemented with vitamins C & E at the bove mentioned concentrations.

All treated groups were provided daily with contaminated drinking water with lead and cadmium, whereas G I and GVI was provided with normal tap water.

Histological and ultrastructural examinations

Dissection and excision of ovary

At the end of treatment, seven of female chickens of each group were selected randomly and a mid-ventral longitudinal incision was made Then the ovary of each chicken were immediately excised and weighed. At the end of the treatment (20 weeks) portions of each dissected ovary were cut into small pieces and immediately fixed.

Light microscopy

Pieces of each ovary were immediately fixed in a freshly prepared aqueous Bouin's fluid and 10% formalin solution for 24 hours. The tissue pieces were then dehydrated through upgraded series of ethyl alcohol, cleared in either cedar wood oil or terpineol, and embedded in parablast (+). Sections of ovary were cut at 5-7 μm in thickness and then stained using Harris' hematoxylin, according to Humason (1979) with eosin as a counter-stain. The specimens were mounted using DPX on clear slides and examined by Olympus Binocular light microscope.

Transmission electron microscopy (TEM)

The dissected specimens were cut into small pieces and fixed in cold (4°C) 2% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) for 2 hours, according to Milloning (1964) and post-fixed (2 hours at 4°C) in OsO_4 for electron microscopy. The samples were then dehydrated, treated with propylene oxide, infiltrated and embedded in Epon 812 for 24 hr. Semi-thin sections were cut with the RMC-MT7 ultra-microtome and stained with toluidine blue. Ultra-thin sections (silvery) were cut, using a diamond knife and stained with uranyl acetate and lead citrate. Cupper grids were then examined using a Joel 1200 Ex II transmission electron microscope at the Department of Zoology, Fac. of Science, Ain Shams University and using Joel EM at The Regional Center for Myocolgical and Biotechnology of Al-Azhar university, Nasr City.

RESULTS

A- Gross and microscopic anatomy

Control group (GI):

The ovary is irregular in shape and pinkish in colour and is situated on the left side of the abdominal cavity close to the median line of the body. It lies ventral to the cranial part of the kidney and obscures the adrenal glands. The ovary is attached to the dorsal body wall by a fold of the peritoneum; the mesovarium, which is formed of fibrous connective tissue, smooth muscles, blood vessels and nerves, together forming ovarian stalk.

The ovarian microscopic structure shows a well defined cortex and medulla. The cortex contains few small follicles and numerous large ones; whereas the medulla contains bundles of smooth muscles, loose connective tissue and numerous thick walled blood vessels (Fig.1).

The ovum is generally composed of outer thecal layer and underneath is the follicular layer that surrounds the ooplasm. The thecal layer is mainly composed of fibrocytes and collagen fibers intermingled together in the small ovum, however in the large one it could be differentiated into an outer theca externa of swollen fibrocytes and an inner theca interna of flattened fibrocytes. The thecal layer contains islets of light cells, the thecal glands and some scattered blood vessels and capillaries. Underneath the thecal layer is found a single layer of the follicular cells, which are mainly cuboidal and columnar cells in the small follicles, whereas pseudostratified and stratified in the intermediate and large-sized ones, respectively The ooplasm is finely granulated and contains scattered acidophilic granules at the periphery underneath the follicular layer (Fig.2).

The interstitial tissue appears slightly fibrotic and degenerated with many blood vessels that can be observed beside the follicles.

Treated Groups

- **Cadmium and lead-treated group (GII):**

The most prominent feature of this group is the presence of great numbers of large ovarian follicles, besides others of different sizes. However, the thecal layer of the larger follicles appears compact and slightly degenerated in scattered areas. Some of the follicular cells show vacuolization. The vitelline membrane and spotted areas of zona radiate appear in some large follicles, but disappear in others, in which small peripheral vacuoles are noticed (Figs.3&4).

The ooplasm of the large follicles is characterized by the presence of numerous vacuoles. Most of these vacuoles contain acidophilic droplets (yolk droplets) of different sizes (Fig.12). The interstitial tissue shows highly congested blood capillaries as well as highly degenerated and fibrotic tissues at different areas.

- **Cadmium, lead and vitamin C-treated group (GIII):**

The thecal layer of the large ovarian follicles of this group are characterized by spotted differentiated and undifferentiated areas, scattered islets of some degenerated thecal glands are also present Signs of degeneration and vacuolization have been observed in some follicular cells, however, compact and darkly stained follicular cells, as well as dark vitelline membrane are noticed in the larger follicles. Interstitial tissue is scanty with many degenerated and fibrotic areas. Moreover, large, collapsed vacuoles with acidophilic droplets are observed beside scattered congested blood capillaries (Fig. 5).

- **Cadmium, lead and vitamin E-treated group (G IV):**

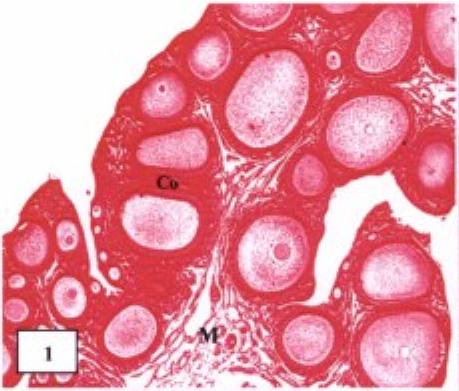
The general ovarian structure of this group is more or less similar to that of G I. However, many of the ovarian follicles of various sizes are surrounded with undifferentiated thecal layer, which contains islets of thecal glands. Many of the follicular cells are degenerated in follicles of different sizes. However, the vitelline membrane persists in the larger follicles, and the basement membrane is prominent and darkly stained in most follicles. The ooplasm of the follicles of this group lacks the vacuolization and the acidophilic droplets in the vacuoles. The interstitial tissue contains many edematous and fibrotic areas (Fig.6).

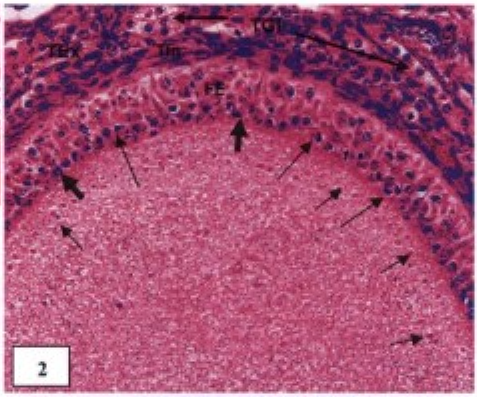
- **Cadmium, lead and vitamins C and E-treated group (GV):**

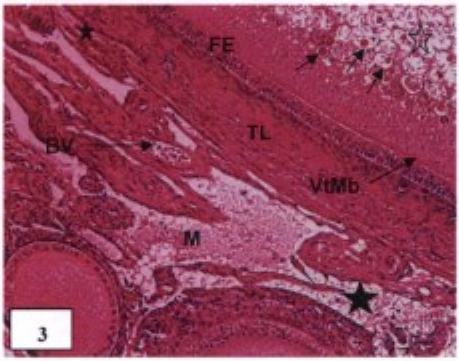
The ovarian follicles of this group are healthier than those of the previous groups; the thecal layer is slightly differentiated into theca externa and theca interna, and contains many clusters of thecal glands. The follicular cells are well differentiated and formed mainly of columnar and pseudostratified epithelium. The vitelline membrane is well defined and preceded by an interrupted layer of vacuoles, zona radiata in the large follicles. The absence of the vacuolization and yolky droplets from the finely granulated ooplasm is the prominent feature of this group. The interstitial tissue appears with many congested blood vessels and scattered areas of fibrotic tissue (Fig.7).

- **Vitamins C and E -treated group (GVI):**

The ovarian follicles appear healthier than those of GV. The thecal layer is well differentiated into theca externa and theca interna, the follicular cells are formed of a well differentiated pseudostratified layer, zona radiata and vitelline membrane. The vacuolization of the ooplasm with numerous yolky acidophilic droplets is greater to the extent that it reaches the periphery of the ooplasm. The interstitial tissue appears normal with some scattered congested blood vessels (Fig. 8).







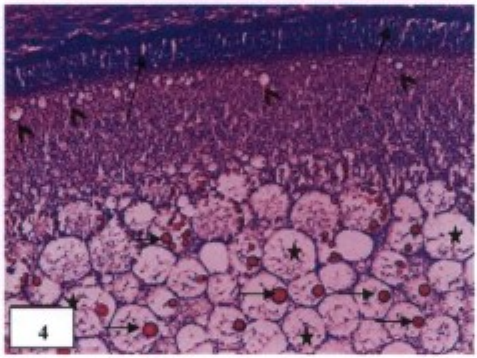
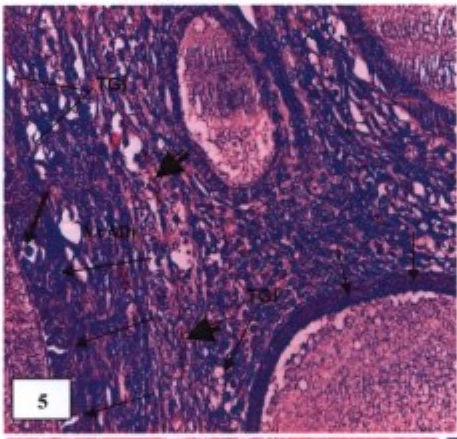


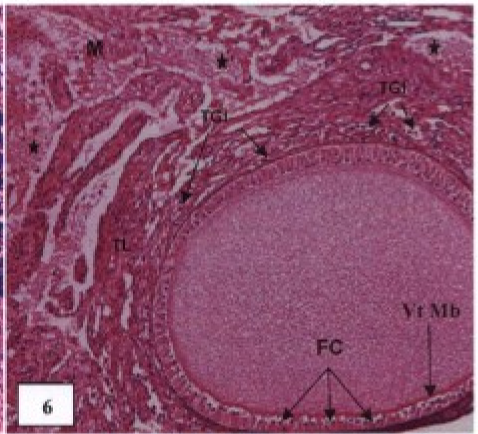
Fig. 1: Photomicrograph of a T.S of the ovary (GI), showing the structure of the cortex and the medulla. X 320

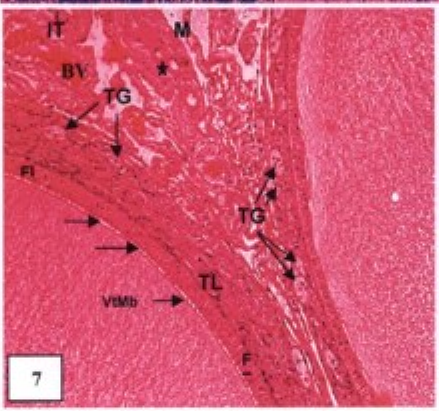
Fig. 2: Photomicrograph of a part of a T.S of the ovary (GI), showing the thecal layers (theca externa and interna), the follicular epithelium is formed of pseudostratified and stratified epithelia. Notice some pyknotic nuclei (long arrow) of the follicular cells and others showing mitotic activity (thick arrow). Notice the scattered large acidophilic granules at the periphery of the ooplasm (short arrow). X 640

Fig. 3: Photomicrograph of a T.S of the ovary (GII), showing part of the medulla (M). Notice the degenerated thecal layer (small black asterisks) and well developed vitelline membrane. The ooplasm of follicles has many large vacuoles (white asterisks) with acidophilic droplets (yolk droplets) (arrows). Notice, the degenerated areas of adipose tissue (large black asterisks) and congested blood vessels (B.V). X 320

Fig. 4: Photomicrograph of a T.S of the ovary (GII), showing the ooplasm of a follicle with many large vacuoles (*) with different sizes of yolk droplets (arrows). Notice the vacuolization of some follicular cells (long arrow), also the subfollicular vacuoles (arrowhead) of the ooplasm. X 640







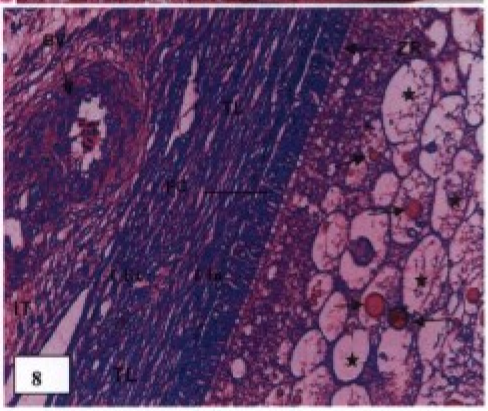


Fig. 5: Photomicrograph of a T.S of the ovary (GIII), showing some degenerated follicular cells (arrows) whereas others appear compact and darkly stained. Notice, islets of degenerated thecal glands, congested blood capillaries (thick arrows) and darkly stained vitelline membrane. X 640

Fig. 6: Photomicrograph of a T.S of the ovary (GIV), showing the medulla and the structure of an ovarian follicle. Notice the undifferentiated thecal layers with numbers of thecal glands, the edematous tissue (*), and the degenerated follicular cells with many vacuoles. Notice also the well defined vitelline membrane. X 320

Fig. 7: Photomicrograph of a T.S. of the ovary (GV), showing the healthy structure of the ovarian follicle. Notice, the thecal layers containing islets of thecal glands, well developed follicular layer and vitelline membrane. Notice also the interrupted vacuolar layer, zona radiata and homogenous ooplasm. The medulla (M) has a fibrotic area (*) and the interstitial tissue contains congested blood vessels (B V). X 320

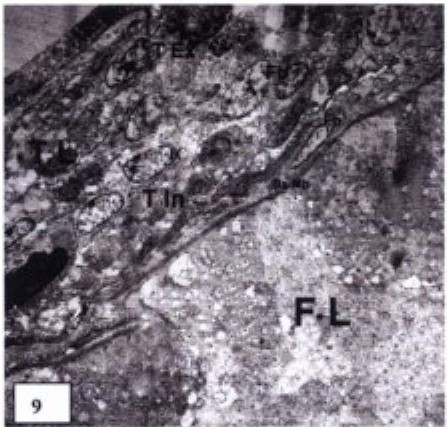
Fig. 8: Photomicrograph of a T.S. of the ovary (GVI), showing well differentiated thecal layer, well developed pseudostratified follicular epithelium and zona radiata. Notice also the vacuoles of the cytoplasm containing acidophilic yolk droplets (arrows) and the congested blood vessel (B.V.) in the interstitial tissue (IT). X 640

B. Ultrastructural study

Control group (G I):

The thecal layer (TL) of the small ovarian follicles (primary oocytes) is mainly formed of 2-3 layers of fibrocytes (Fb), whereas in the growing (secondary) oocytes and the mature follicles, the thecal layer is mainly formed of about 4-8 layers of fibrocytes and thecal interstitial cells. The thecal layer is differentiated into outer theca externa (TEx) and inner theca interna (TIn). The outer theca externa is composed of swollen fibrocytes and collagen fibers, whereas the inner theca interna is formed of flattened fibrocytes. The fibrocyte contains large more or less rounded nucleus (N) with clear nucleolus (Nu) and heterochromarin nuclear clumps, whereas the cytoplasm contains dense and great minute bodies and scarce rough endoplasmic reticulum (RER). The thecal interstitial cells (IC) are scarce, opaque and smaller in size than the fibrocytes and contain oval nuclei, as well as lipid droplets and mitochondria (Mt). Moreover, islets of large thecal glands (TGl) containing many variable-sized vacuoles were observed scattered in between the thecal cells. A number of scattered congested blood capillaries (BCp) is usually present in between the thecal cells. (Fig. 9)

The follicular layer (FL) of the small ovarian follicles appears as a single layer of cuboidal cells located between the basement membrane and the cytoplasm. In the growing (secondary) oocytes, the follicular cells begin to elongate than that of the primary oocytes and the nuclei of both stages are rounded with chromatin clumps and nucleoli. However, in the mature ovarian follicles, the follicular cells are formed of pseudostratified layer and their nuclei are more active and many of these nuclei appear mitotically active (Fig. 10).



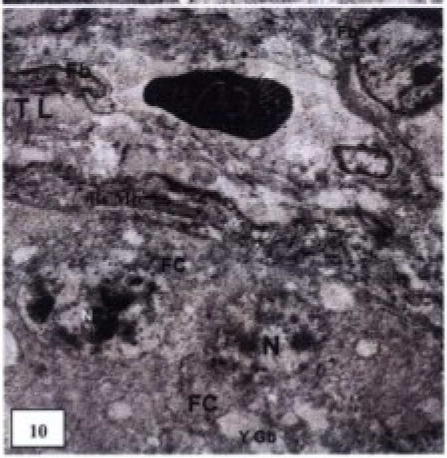


Fig. 9: Transmission electron micrograph (TEM) of an ovarian section of GI, showing the structure of the thecal layer. Notice the theca externa and theca interna, the fibrocytes and the interstitial cells. Notice also the structure of the elongated follicular cells. X 8000

Fig. 10: TEM of an ovarian section of G I, showing the structure of the follicular cells (FC) of the primary oocytes. X 6000

The follicular cells of the previous stages contain vacuoles and many cytoplasmic organelles. At the basal area of cytoplasm, several cisterns of RER, as well as clusters of dense bodies were observed.

Numbers of mitochondria (Mt) and Sometimes, Golgi stacks (Go S) were noticed. Traces of vitelline membrane can be observed between the follicular cells and the ooplasm (Fig. 27). The ooplasm (Op) of the large and mature ovarian follicles contains many vacuoles of varying sizes; the larger ones contain the precursor of yolk globules. These vacuoles and the yolk globules are usually located at the periphery of the cytoplasm. Also, numbers of small mitochondria and scarce small RER have been observed scattered in this area (Fig. 11).

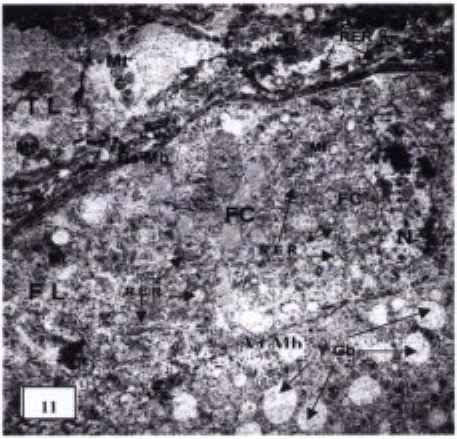
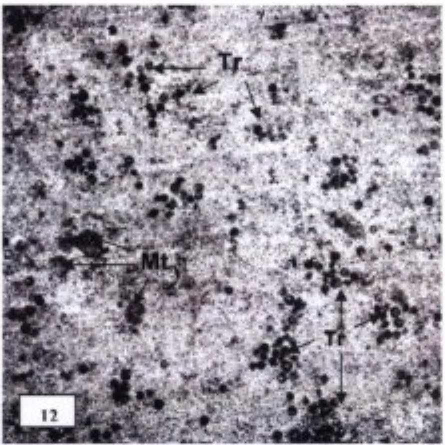


Fig. 11: TEM of an ovarian section of G I, showing the structure of the follicular cells (FC) of the mature follicles. Notice RER in spotted area of the follicular cells and small mitochondria (Mt). Notice also traces of vitelline membrane at the tips of the follicular cells. X 16000

In the growing ovarian follicles, transosome-like bodies are detected near the apical area of the follicular cells and then spreading during development in the ooplasm. However, in the mature follicles the transosomes are markedly reduced. Each transosome consists of a dense curved plate (appears as semicircular dense layer) with rows of ribosome-like particles loosely attached to the inner surface (Figs. 12 & 13).



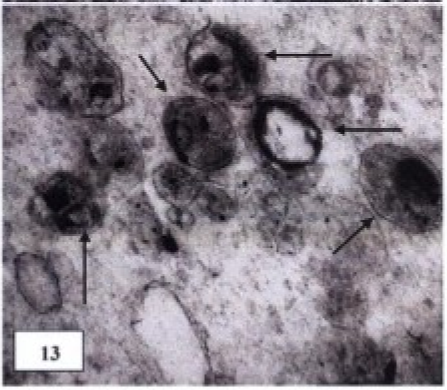


Fig. 12: TEM of an ovarian section of G I showing the structure of the ooplasm (Op) of the growing follicles. Notice great numbers of scattered small dense bodies, transosome-like bodies and scattered mitochondria (Mt). X 6000

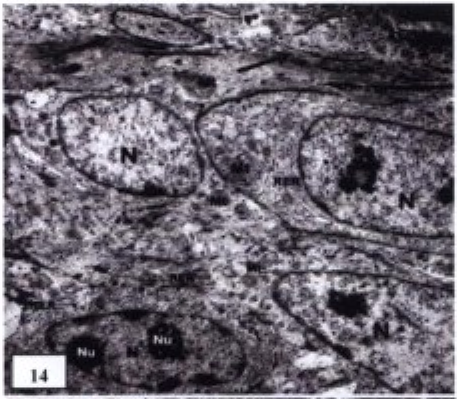
Fig.13: TEM of an ovarian section of G I showing the structure of the ooplasm (Op) of the growing follicles. Notice, structure of the transosome-like bodies at different stages (arrows). X 24000

Treated Groups

- **Cadmium and lead- treated group (GII):**

Most of the ovarian components of this group appear more healthy than those of G I.

In the thecal layer, the fibrocytes of this group is characterized by their prominent cell and nuclear membranes. Some nuclei of the interstitial cells have two nucleoli. Extensive RER and mitochondria are well developed in most of the fibrocytes and some interstitial cells. The thecal glands become enlarged and their components are more prominent On the other hand, signs of degeneration have been observed in the cytoplasm of some fibrocytes and thecal glands, especially at the periphery of the thecal layer (Figs.14&15).



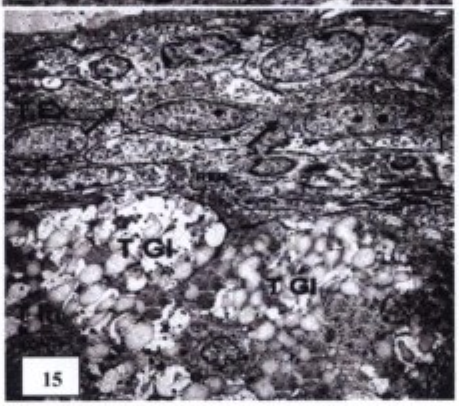
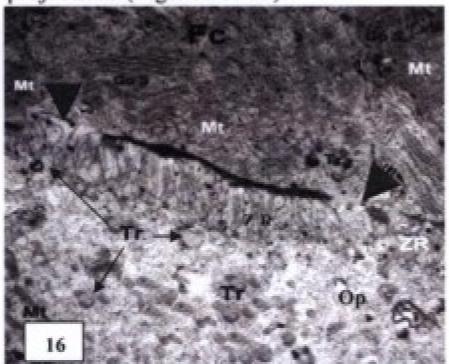


Fig. 14: TEM of an ovarian section of G II, showing the structure of the thecal layer. Notice the vivid structure of the fibrocytes and the interstitial cells. Notice two nucleoli of an interstitial cell. X 14000

Fig. 15: TEM of an ovarian section of G II, showing the structure of the thecal layer. Notice the thecal glands and their numerous vacuoles. Notice the fibers (arrow).X 6000

In the follicular layer, most of the follicular cells contain extensive and well developed cisterns of RER, clusters of dense bodies, large numbers of mitochondria (Mt), Golgi stacks (Go S), microtubules and a well developed network of microfilaments have been observed. A well developed vitelline membrane is prominent in this group; however, it is disconnected at intervals. Also, numerous microvilli (zona radiata) and apical projections of the follicular cells protrude into the cytoplasm. The transosomes appear beneath the apical plasma membrane of the follicular cells and usually concentrated near the apical projections (Figs. 16 & 17).



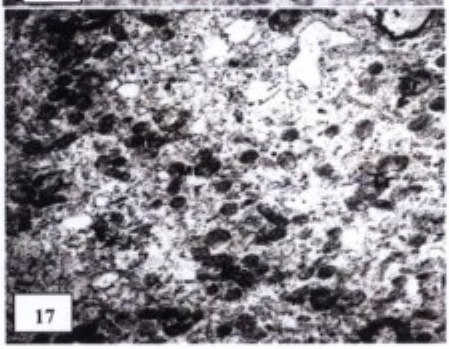


Fig.16: TEM of an ovarian section of G II, showing the structure of the follicular cells (FC). Notice the interrupted vitelline membrane (arrowhead), micrprojections (zona radiata) ZR and the transosome-like bodies at the apical cells and in the ooplasm. X 16000

Fig.17: TEM of an ovarian section of G II, showing the structure of the ooplasm (Op). Notice the great numbers of the mitochondria (Mt) and aggregations of transosome-like bodies. X 16000

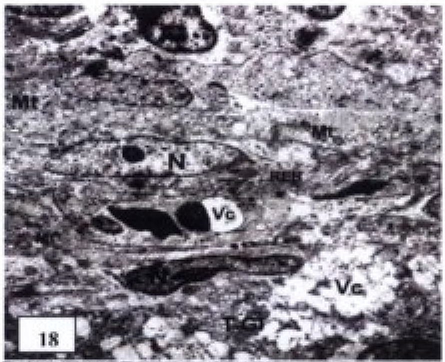
The ooplasm of the growing follicles is characterized by the presence of great number of mitochondria, which are disappeared in the mature follicles, as well as vacuoles of different sizes The transosome-like bodies are also detected near the apical area of the follicular cells (Fig.17).

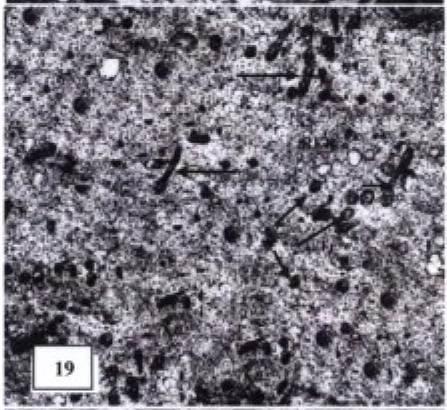
▪ Cadmium, lead and vitamin C-treated group (GIII):

In the thecal layer, both the theca externa (TEx) and theca interna (TIn) are ill-defined, and the borders of most cells are generally disappeared. Degeneration and vacuolization have been also observed in many fibrocytes and thecal glands. In the follicular layer, their cytoplasmic organelles (Mt, RER and Go S) are few than those of GI or GII. Moreover, the zona radiata are not clear. The transosome–like bodies can not be detected at the periphery of these cells. The ooplasm of this group is nearly similar to that of GII. However, great number of healthy and degenerated Mt, scattered transosomes, and RER, as well as absence of large vacuoles and yolk droplets are characteristic features of this group (Figs.18 &19)

▪ Cadmium, lead and vitamin E-treated group (GIV):

The thecal layer appears healthier than that of GIII. The borders of many cells are well developed and the cytoplasmic organelles, such as RER and mitochondria, are observed in many cells of the thecal layer. Prominent fibers are noticed scattered in the thecal layer in between their cells. In the follicular layer, the follicular cells are similar to those of GIII, however prominent membranous structures were observed at the tips of the cells and closely attached to the vitelline membrane. Such membranous structures may represent microprojections that will form the zona radiata. The ooplasm appears homogenous and contains few scattered mitochondria and minute vesicles of RER. The absence of large vacuoles and yolk droplets is a characteristic feature of this group (Figs. 20 & 21).





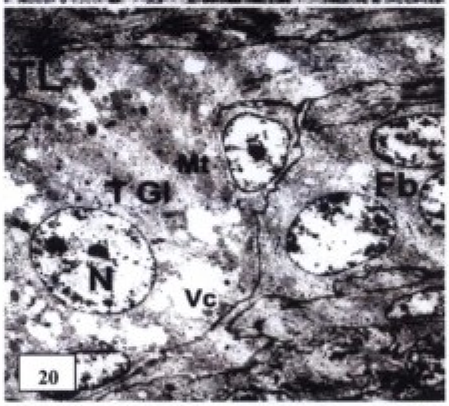


Fig. 18: TEM of an ovarian section of G III, showing the structure of the thecal layer. Notice the undifferentiated thecal layer, degeneration and vacuolization of the fibrocytes and rupture of the borders of different cell types. X 10000

Fig. 19: TEM of an ovarian section of G III, showing the structure of the ooplasm (Op). Notice the numbers of healthy mitochondria (Mt), scattered transosome-like bodies and RER. X10000

Fig. 20: TEM of an ovarian section of G IV, showing the structure of the thecal layer. Notice the RER and mitochondria (Mt) of the fibrocyte. Notice also two nucleoli (Nu) in the nuclei of the fibrocytes. X 18000

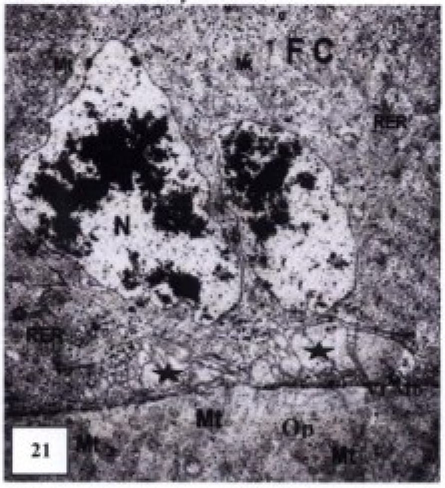


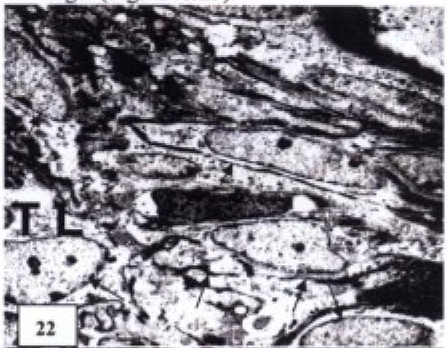
Fig.21: TEM of an ovarian section of G IV, showing the structure of the follicular cells (FC) and ooplasm (Op). Notice extensive membranous structure (*) at the tips of the follicular cells near (Vt Mb) and few scattered mitochondria (Mt) in the follicular cells and the ooplasm (Op). X 16000

▪ Cadmium, lead and vitamins C and E-treated (GV):

The thecal layer of this group is characterized by cohesion of the thecal cells, well developed borders of many cell types, extensive appearance of fibers in different directions, vivid thecal gland with prominent vacuoles and presence of numbers of congested blood capillaries. The structure of the follicular layer and its basement membrane are well developed and greatly similar to those of G II and G III. Also, both the vitelline membrane and zona radiata are greatly developed. However, the transosome–like bodies can not be detected in this group. The structure of the ooplasm is typically similar to that of GI and GIV (Figs. 22 &23).

▪ Vitamins C and E- treated group (GVI):

The thecal layer is more healthy than that of the previous groups. This is represented by clear borders of their cells and their methodically active nuclei, in addition bundles of fibers are more prominent and thicker than those of G V The follicular layer structure is typically similar to that of GV, however their cellular components are more prominent The ooplasmic structure is nearly similar to that of G II and G IV, in the presence of great number of well developed mitochondria, dispersed in the ooplasm and numbers of vacuoles. However, the vacuoles and yolk globules, in this group, are extensive and the yolk droplets are larger (Figs. 24&25).



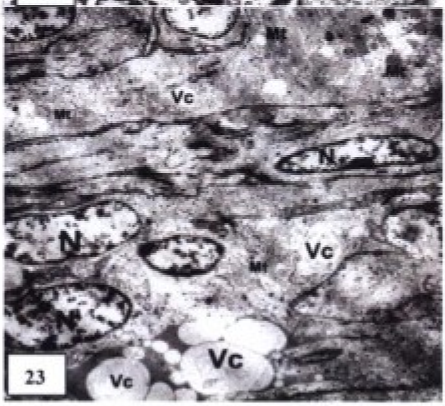
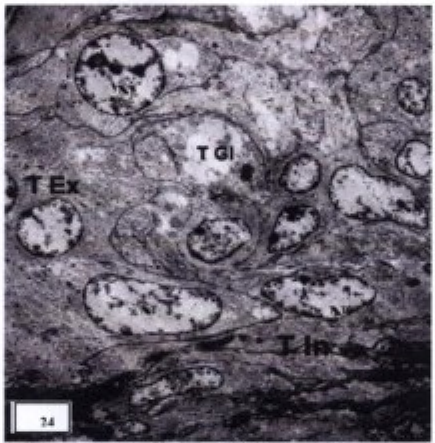


Fig.22 TEM of an ovarian section of G V showing the general structure of the thecal layer. Notice the cohesion of the thecal layer and prominent outlines of different cell types (arrows). X 10000

Fig.23: TEM of an ovarian section of G V showing the structure of the thecal layer. Notice the vacuoles (Vc) of the thecal gland (TGl) and scattered mitochondria (Mt). X 8000



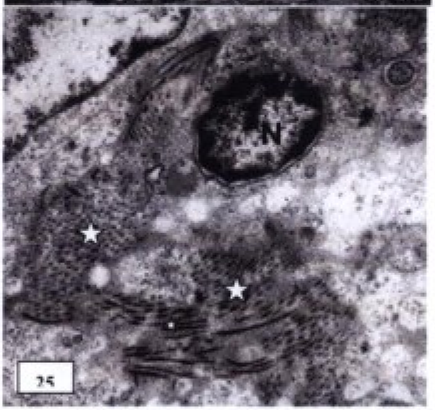


Fig.24: TEM of an ovarian section of G VI, showing the general structure of the thecal layer. Notice the clear borders of the different cell types and the mitotically active nuclei (arrows). X 6000

Fig.25: TEM of an ovarian section of G VI, showing the general structure of the thecal layer. Notice prominent fibers (.) in different direction in the thecal layer. Notice also the nucleus of a fibrocyte containing extensive chromatin clumps. X 8000

DISCUSSION

In the present investigation, the ovary of the Lohman chicken, is formed of well defined cortex, containing few small growing follicles beside many large ones; and medulla, which contains thick walled blood vessels, smooth muscle fibers and loose connective tissue. Such structure is generally similar to those of other species of birds **(Deol, 1955; Forgo *et al.*, 1988; Gupta *et al.*, 1988; Guraya, 1989).** On the other hand, the main detailed structures of the normal large ovarian follicles, of the present investigation, are the outer thecal layer, follicular layer and ooplasm. The thecal layer is hardly differentiated into theca externa and theca interna and contains islets of light cells (thecal glands). The follicular layer, is formed mainly of cuboidal and columnar cells in the small follicles, whereas pseudostratified and stratified in the larger ones. The ooplasm, on the other hand, is finely granulated with scattered large acidophilic granules at the periphery. These structures were extensively investigated by light and transmission electron microscopes in the domestic fowl **(Wyburn *et al.*, 1965; Dahl, 1971; Rothwell and Solomon, 1977; Perry *et al.*, 1978),** the Japanese quail **(Kudryavtsev *et al.*, 1982),** the domestic duck **(Gorbick and Gabaeva, 1975; Deray, 1978),** the domestic goose **(Forgo *et al.*, 1988; Kovacs *et al.*, 1992).**

The studies on exposure of the birds, especially the hens, to the heavy metals alone or combined with other pollutants and their impacts on the ovarian structures are scarce, in contrast to other animals, especially the mammals. The light and ultrastructural microscopic studies of the ovary of *Lohman* chicken, showed marked histopathological changes after the treatment with lead and cadmium (GII, GIII, and GIV) compared with GI, GV and GVI. More great changes were observed in GII than the other treated groups. These changes were mainly represented by increasing the number of the growing ova. Moreover, edematous and fibrotic areas in the interstitial tissue, undifferentiation of the thecal layer, degeneration of the thecal glands and vacuolization of the follicular cells were the main symptoms of toxicity in most groups treated with lead and cadmium (GII, GIII, and GIV). These findings are greatly similar to those reported by many investigators who used different heavy metals or mixed pollutants in many vertebrate species such as fishes **(Kime,1995; Aditya *et al.*, 2002)**, rat **(Priya *et al.*,2004; Nampoothiri and Gupta, 2006)**. Most probably, the degeneration of the thecal glands, in the present investigation, leads to the increase of the progesterone hormone secretion which helps in acceleration of ovarian growth. This is confirmed through the physiological studies in fishes **(Thomas, 1990; Kime, 1995)** in rats **(Priya *et al.*, 2004; Nampoothiri and Gupta, 2006)**. In contrast, **Heindel *et al.*(1995)** and **Vodela *et al.*(1997)** stated that no histopathological changes could be detected in the ovary and testis in the experimental animals (mice, rats and rabbits) treated with drinking water containing low concentration of a chemical mixture of heavy metals and volatile organic chemicals.

In the present study, the vitelline membrane was nearly absent in GI, but it was observed in some large follicles of GII and GIII, whereas it was well defined in GIV and GV. However, the vitelline membrane and zona radiata were well developed in GVI treated with vitamins C and E only. It is noteworthy that, in these groups (GII, GIII, GIV, GV & GVI), the annulated lamellae which are found in some of their large follicles are greatly similar to those described in the follicular cells of the goose **(Kovacs *et al.*, 1992)** and also reported in the duck **(Deray, 1978)** and have not been reported, so far as we are aware, in the hen ovarian follicles. **Kessel (1989)** stated that the function of these annulated lamellae is unknown. Most probably, these annulated lamellae act as accessory channels between the follicular cells and the ooplasm.

In the present investigation, light microscopy showed the presence of many vacuoles with acidophilic droplets in the ooplasm of GII, GIII GVI only, indicating a progress in the development and maturation phase. However, in GVI these vacuoles were extensive and more extended to the periphery at the tips of the follicular cells. Such vacuoles have been confirmed by electron microscopy. The phenomena of early development of the reproductive organs and maturation phase have been reported by **Brody *et al.* (1980), Soller *et al.* (1984)** and **Yannakopoulos *et al.* (1995)**.

Fu *et al.* (2000) reported that the early onset of egg production in quail was the primary cause of the retinoic acid-supplemented diet, and suggested that this may reflect changes related to early sexual maturity. Also, **Sahin *et al.*(2002b)** found that diet supplemented with chromium and vitamin C increased the development of gonads and their weights. In the present study, the presence of low levels of lead and cadmium (GII), lead, cadmium with vitamin C (GIII) or vitamins C & E (GVI), most probably act as essential complementary chemicals initiative for the cytoplasmic organelles to be more anabolically active.

Recent studies indicated that transition metals act as catalysts in the oxidative reactions of biological macromolecules therefore the toxicities associated with these metals might be due to oxidative tissue damage. **Gurer and Ercal (2000), Ercal *et al.* (2001)** and **Abdel-Salam (2003)** suggested that metal-induced oxidative stress in cells can be partially responsible for the toxic effects of heavy metals; therefore antioxidants may play an important role in abating some hazards of heavy metals. **Gurer and Ercal (2000)** and **Hsu and Guo (2002)** explained that antioxidant nutrients including, vitamin E, vitamin C, vitamin B6, beta-carotene, zinc, and selenium, has a great beneficial role in lead-induced oxidative stress.

Although many researches have investigated the benefit of antioxidants in preventing most of heavy metal toxicity, the mechanism of antioxidant nutrients being effective *via* rebalancing the impaired peroxidant / antioxidant ratio are not clear **(Hsu and Guo, 2002)**.

Vitamins C and E are known as free radical scavenging nutrients. Thus, they provide potential protection against cell damage by oxidative stress **(Anderson and Theron, 1990).** Moreover, vitamin E is a fat soluble and localized in the membranous structures of the cells, whereas vitamin C is a water soluble reducing agent and important in the activities of the cells such as, regeneration of vitamin E and synthesis of collagen fibers **(McCance and Huether, 2002).**

In the present investigation, the administration of vitamin C alone with lead and cadmium diet (GIII), improved the basement membrane and the constituents of the ooplasmic organelles which were represented by the great number of elongated and healthy mitochondria and scattered transosomes. On the other hand, the administration of vitamin E alone with lead and cadmium diet (GIV), improved the cell membranes of many cells and their membranous organelles such as RER and mitochondria, and in addition the membranous structure forming the zona radiata. Such improvements were greatly obvious in the thecal cells, collagenous fibers and follicular cells. The role of vitamin C in preventing heavy metal toxicity was demonstrated by many authors. **Kapl *et al.* (1994)** explained that the addition of vitamin C could reduce the cadmium burden in animal tissues, and therefore it is considered one of the most effective factors in reducing cadmium absorption and retention of the heavy metals. **Williams (1995)** stated that vitamin C is necessary to build and maintain strong tissues in general, especially the connective tissue. It acts as cement between cells to hold them together strongly.

Niki *et al.* (1982) suggested that the protective effect of ascorbic acid does not result from direct radical scavenging but rather from the regeneration of vitamin E radicals.

In the present investigation, it was found that the combination of vitamins C and E in diet containing lead and cadmium (GV) were more effective than the addition of each of them alone (GIII and GIV). This was prominent through the decline of the edematous and fibrotic areas, preventing the degeneration of the thecal layer and follicular cells and improving the ovarian structures such as the well developed vitelline membrane and zona radiata. Moreover, in GVI, both vitamins accelerate the maturation of follicles and improve the general ovarian structures than that of all groups including GI (control group). The role of the vitamins in preventing the toxicity of the heavy metals was reported by many investigators **(Gurer and Ercal, 2000; Hsu and Guo, 2002).** On the other hand, **Appendroth and Winnefeld (1998)** suggested that when vitamins C and E were administered, the protective effect against nephrotoxicity in rat was the same as after the administration of each vitamin alone. Most probably, the vitamins have a great effect in reducing the impact of toxicity of both heavy metals.

REFERENCES

Abdel-Salam, M. E. (2003). Protective effect of folic acid and vitamin C against the toxicity of certain heavy metals in male albino rats. M.Sc. Thesis, Faculty of Science, Ain Shams University, Cairo, Egypt.

Aditya, A. K. ; Chattopadhyay, S. and Mitra, S. (2002). Effect of mercury and methyl parathion on the ovaries of *Labeo rohita* (Ham). J. Environ. Biol., 23(1):61-64

Allen, P. (1995). Accumulation profiles of lead and cadmium in the edible tissues of *Oreochromis aureus* during acute exposure. J. Fish. Biol., 47: 559- 567.

Anderson, R. and Theron, A. J. (1990). Physiological potential of ascorbate, β-carotene and α- tocopherol individually and in combination in the prevention of tissue damage, carcinogenesis and immune dysfunction mediated by phagocyte derived reactive oxidants. World Rev. Nut. Diet., 62 : 27 - 58.

APHA (American Public Health Association Standard Methods), Amereican Water Works Association, and Water Pollution Control Federation (1985).
Standard methods for the examination of water and waste water, 16^{th} ed., Washington, D.C.

Appenroth, D. and Winnefeld, K. (1998). Vitamin E and C in the prevention of metal nephrotoxicity in developing rats. Exp. Toxicol. Pathol., 50 (4-6):391-396

Brody, T.B. ; Siegel, P. B. and Cherry, J. A. (1980). Age, body weight and body composition requirements for the onset of sexual maturity of dwarf and normal chickens. Br. J. Poult. Sci., 25 : 245–252.

Dahl, E. (1971). The fine structure of the granulosa cells in the domestic fowl and the rat. Z. Zelforsch.Mik. Anat.,119:58-67.

Deol, G. S. (1955). Studies on the structure and function of the ovary of the domestic fowl (with reference to the correlation of cell changes with physiological activity). Ph. D. Thesis, University of Edinburgh. Quoted from Forgo *et al* (1988).

Deray, A. (1978). Les cellules de la granulose de Canes : Cane Pékin (*Anas platyrhynchus*), Barbarie (*Cairina moschata*) et hybride : Pékin mâle X Barbarie femelle. J. Ultrastruct. Res., 68 : 118 -135.

El-Dawi, E. F. A (2004). A comparative scanning electron microscopic study on the impact of lead and cadmium exposure on the corneal epithelium of surface and bottom teleosts. J. Egypt. Acad. Soc. Environ. Develop., (D-Environ. Studies), 5 (20) : 171 – 194.

El-Dawi, E. F. A. and Elbadry, M. (2001). Retinotoxicity and photoreceptors ultrastructural alterations in the Arabian killifish, *Aphanius dispar* exposed to lead and cadmium. J. Egypt. Acad. Soc. Environ. Develop., (D- Environ. Studies), 2 (4) : 127 - 150 .

Ercal, N. ; Gurer-Orhan, H. and Aykin-Burns, N. (2001). Toxic metals and oxidative stress part I: Mechanisms involved in metal-induced oxidative damage. Curr. Top. Med. Chem., 1(6) : 529 - 539.

Forgo, V. ; Sass, M. and Peczely, P. (1988). Light microscopic, enzyme biochemical and steroid analytical investigations of follicular atresia in the ovary of domestic goose. Acta Biol. Hung., 39: 377 - 401.

Fry, D. M. (1995). Reproductive effects in birds exposed to pesticides and industrial chemicals. Environ. Health Perspect., 103: 165 – 171.

Fu, Z. ; Kato, H. ; Sugahara, K. and Kubo, T. (2000). Organs and egg production in Japanese Quail (*Coturnix coturnix japonica*). Biol. Reprod., 63: 1795-1800

Gerhardsson, L. and Skerfving, S. (1996). Concepts on biological markers and biomonitoring for metal toxicity. *In*: "Toxicology of Metals". Change, L.; Mogos, L.; Suzuki, T. (eds.) ; Lewis Publishers, Boca Raton, New York, Tokyo.

Gochfeld, M. and Burger, J. (1982). Biological concentration of cadmium in estuarine birds of New York. Colonial Waterbirds, 5 : 116 -123.

Gorbik, T. A. and Gabaeva, N. S. (1975). Morphological alterations of the follicular epithelium in oogenesis of the duck (*Anas boschas domesticus*) and other birds. Arkh. Anat. Gistol. Embriol., 68 : 16 – 24

Gupta, S. K. ; Gilbert, A. B. and Walker, M. A. (1988). Histological study of follicular atresia in the ovary of the domestic hen (*Gallus domesticus*). J. Reprod. Fert., 82 : 219 – 225.

Guraya, S. S. (1989). Ovarian follicles of reptiles and birds. *In*: "Zoophysiology". Farner D.S. (ed.), Springer, Berlin, Heidelberg, New York

Gurer, H. and Ercal, N. (2000). Can antioxidants be beneficial in the treatment of lead poisoning? Free Radic. Biol. Med., 29 (10) : 927 - 945.

Heindel, J. J. ; Chapin, R. E. ; George, J. ; Gulati, D. K. ; Fail, P. A. ; Barnes, L. H. and Yang, R. H. (1995). Assessment of reproductive toxicity of a complex mixture of 25 ground water contaminants in mice and rats. Fundam. Appl. Toxicol.,25:9–19.

Hsu, P.C. and Guo, Y.L. (2002). Antioxidant nutrients and lead toxicity. Toxicology, 180 (1):33-44.

Humason, G.H. (1979). Animal Tissue Techniques. 4th ed., Freman and Company. London , New York.

Kapl, D. ; Weiser, H. A. and Rambeck, W. A. (1994). The influence of vitamin C on cadmium-retention in pigs. Rev. Med. Vet., 145 (4) : 291 – 297.

Kessel, R.G. (1989). The annulate lamellae from obscurity to spotlight. Electron Microsc. Rev., 2: 257 – 284.

Kime, D. E. (1995). The effects of pollution on reproduction. Rev. Fish Biol. Fisheries, 5 (1) : 52- 96

Kovacs, J. ; Forgo, V. and Peczely, P. (1992). The fine structure of the follicular cells in growing and atretic ovarian follicles of the domestic goose. Cell Tissue Res., 267 : 561 - 569.

Kudryavtsev, I. V. ; Sheina, N. I. and Vasin, V. I. (1982). A comparative electron microscope study of oocytes of the chicken and *Coturnix coturnix japonica*. Zh. Obsch. Biol., 43: 394 – 398.

McCance, K. and Huether, S. (2002). Pathophysiology. The Biological Basis for Disease. 4th ed., Mosby, St. Louis, London. pp. 826.

Milloning, G. (1964). Study on the Factors which Influence Preservation of fine Structure. *In* :Symposium on Electron Microsopy. P. Buffa ed., Consiglio Nazionale delle Ricerche, Roma; p. 347.

Nampoothiri, L. P. and Gupta, S. (2006). Simultaneous effect of lead and cadmium on granulosa cells. A cellular model for ovarian toxicity. Reprod. Toxicol., 21 (2) :179 - 185.

Niki, E. ; Tsuchiya, J. ; Tanimura, R. and Kamiya, Y. (1982). Regeneration of vitamin E from alph-chromanoxyl radical by glutathione and vitamin C. Chem. Lett., 32: 789 -792.

Pace, V. and Iannucci, E. (1994). The importance of vitamins in relation to the presence of heavy metals in food. Panminerva Med., 36(2): 80-82.

Perry, M. M. ; Gilbert, A. B. and Evans, A. J. (1978). Electron microscope observations on the ovarian follicle of the domestic fowl during the rapid growth phase. J. Anat., 125: 481 – 497.

Priya, P. N. ; Pillai, A. and Gupta, S. (2004). Effect of simultaneous exposure to lead and cadmium on gonadotropin binding and steroidogenesis on granulosa cells: an *in vitro* study. Indian J. Exp. Biol.,42(2):143-148.

Rothwell, B. and Solomon, S. E. (1977). The ultrastructure of the follicle wall of the domestic fowl during the phase of rapid growth. Br. J. Poult. Sci., 18: 605-610.

Sahin, K. ; Sahin, N. and Yaralioglu, S. (2002a). Effects of vitamin C and vitamin E on lipid peroxidation, blood serum metabolites, and mineral concentrations of laying hens reared at high ambient temperature. Biol. Trace Elem. Res., 85 (1) : 35 - 45.

Sahin, K. ; Onderci, M. ; Sahin, N. and Aydin, S. (2002b). Effects of dietary chromium picolinate and ascorbic acid supplementation on egg production, egg quality and some serum metabolites of laying hens reared under a low ambient temperature (6 degrees C). Arch Tierernahr., 56 (1): 41 - 49.

Soller, M. ; Eitan, Y. and Brody, T. (1984). Effect of diet and early quantitative feed restriction on the minimum weight requirement for onset of sexual maturity in White Rock broiler breeders. Poult. Sci; 63 : 1255 –1261.

Thomas, P. (1990). Teleost model for studying the effects of chemicals on female reproductive endocrine function. J. Exp. Zool. Suppl., 4 : 126 – 128.

USEPA (United State Environmental Protection Agency) (1991). National Priorities List Sites. Alabama 9200.5-702A, Washington. D.C.

Vodela, J. K. ; Lenz, S. D. ; Renden, J. A. ; Mcelhenney, W. H. and Kemppainen, B. N. (1997). Drinking water contaminants (arsenic, cadmium, lead, benzene, and trichloroethylene). 2. Effects on reproductive performance, egg quality, and embryo toxicity in broiler breeders. Poult. Sci., 76 : 1493 - 1500.

Vohr, P. N. (1980). Water quality for poultry use. Feedstuffs, 52 : 23 – 26.

WHO (World Health Organization) (1984). Guidelines for drinking water quality. Geneva,

WHO (World Health Organization) (1992). Environmental Health Criteria. No. 134, Geneva.

Williams, S. R. (1995). Vitamins. *In* : Basic Nutrition and Diet Therapy. 10th ed., Hills, E.A and Graham, T.J (eds.) Mosby-Year Book, Inc., St. Louis, pp.74-111.

Wyburn. G. M. ; Aitkeu, R. X. C. and Johnston. H. S. (1965). The ultrastructure of the zona radiata of the ovarian follicle of the domestic fowl. J. Anat.,99: 469-481.

Yang, R. S. H. and Rauckman, E. R. (1987). Toxicological studies of chemical mixture of environmental concern at the national toxicology program : Health effects of ground water contaminants. Toxicology, 47: 15 – 34.

Yang, R. S. H. ; Goehl, T. J. ; Brown, R. D. ; Chatham, A. T. ; Arneson, D. W. ; Buchnan, R. C. and Harris, R. K. (1989). Toxicology studies of a chemical mixture of 25 ground water contaminants 1. Chemistry development. Fundam. Appl. Toxicol., 13 : 366- 376.

Yannakopoulos, A. L. ; Christaki, E. and Florou-Paneri, P. (1995). Effect of age and carcass composition on the onset of sexual maturity in quail under normal feeding regimens. Br. Poult. Sci., 36 :771 – 777.

دراسات هستولوجية وتركيبية دقيقة على تأثير مضادات الأكسدة ضد التسمم بمعادن ثقيلة معينة فى مبيض الدجاج

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تهدف الدراسة الحالية الى تحديد تأثيرات خليط إثنين من اكثر المعادن الثقيلة إنتشاراً فى العالم وهما الرصاص والكادميوم على تركيب المبيض فى دجاج لوهمان الناضج. وتهدف الدراسة أيضاً إلى معرفة إمكانية تأثير المعالجة بفيتامينات ج ، هـ منفصلين ومتحدين كمضادات أكسدة لتحسين ومعالجة التسمم بالمعادن الثقيلة. وقد تم إختيار أقل نسب تركيز للرصاص والكادميوم والتى توجد بالفعل فى الأماكن التى اختيرت منها العينات العشوائية وتم تجهيز جرعات من خلات الرصاص (17, . مجم / لتر) و خلات الكادميوم (6, . مجم / لتر) وخلطها بالوجبات اليومية و مياه الشرب. أيضاً تم معالجة الدجاج بجرعات من فيتامين ج (250 مجم / كجم) و فيتامين هـ (200مجم/ كجم) منفصلين ومتحدين و تم خلطها بالوجبات المقدمة للدجاج. وقد تم تقسيم الدجاج الى ست مجموعات :

- الجموعة الأولى (م -1): وهى المجموعة الضابطة وتم إمدادها يومياً بوجبات ومياه شرب خالية من المعادن الثقيلة والفيتامينات.
- المجموعة الثانية (م -2): وتم إمدادها يومياً بوجبات ومياه شرب مختلطة بالرصاص والكادميوم فقط .
- المجموعة الثالثة (م -3): وتم إمدادها يومياً بوجبات ومياه شرب مختلطة بالرصاص والكادميوم وفيتامين ج.
- المجموعة الرابعة (م -4): وتم إمدادها يومياً بوجبات ومياه شرب مختلطة بالرصاص والكادميوم وفيتامين هـ .
- المجموعة الخامسة (م -5): وتم إمدادها يومياً بوجبات ومياه شرب مختلطة بالرصاص والكادميوم وفيتامين ج و هـ .
- المجموعة السادسة (م -6): وتم إمدادها يومياً بوجبات مختلطة بفيتامين ج و هـ فقط .

وقد أثبتت الفحوص بالمجهر الضوئى والإلكترونى للمبيض أن هناك تغيرات نسيجية مرضية فى (م-2 ، م – 3 ، م- 4) بالمقارنة مع المجموعات (م -1 ، م- 5 ، م – 6). وكانت أكثر وأخطر التغيرات وضوحاً فى المجموعة الثانية (م – 2) والممثلة فى الزيادة الكبيرة فى عدد البويضات ، انتفاخ وتليف بعض المناطق فى النسيج البينى مع وجود تحلل للغدد المحيطة وظهور فجوات فى الخلايا الحويصلية. وقد لوحظت هذه التغيرات أيضاً فى المجموعات م- 2 ، م – 3 ، م- 4).

وقد لوحظ بداية ظهورالغشاء المحى فى البويضات الكبيرة بالمجموعات (م -2 ، م – 3) ، بينما كان واضحاً فى (م -4 ، م- 5) . اما فى المجموعة السادسة (م – 6) والمعالجة بفيتامين ج ، هـ فقط . فقد كان الغشاء المحى و أيضا الطبقة الشعاعية أكثر نمواً و وضوحاً.

وقد لوحظ ظهور الفجوات وانتشارها فى الأوأوبلازم فى (م- 2 ، م -3). أما فى المجموعة السادسة (م – 6 والمعالجة بالفيتامين ج ، هـ فقط) فكانت هذه الفجوات أكثر انتشاراً وكثافة للحد التى تصل فيه الى قمم الخلايا الحويصلية.

وقد أدت المعالجة بفيتامين ج مع الرصاص والكادميوم الى تحسن فى بعض مكونات الأوأوبلازم مثل وضوح وزيادة عدد الميتوكوندريا ، بالإضافة الى انتشار الترانسوسومس. أما اضافة فيتامين هـ مع الرصاص والكادميوم فقد حسن من الغشاء الخلوى للعديد من الخلايا والتراكيب الخلوية مثل الشبكة الإندوبلازمية الخشنة والميتوكوندريا سواء فى الخلايا المحيطة أو الخلايا الحويصلية وأيضاً الأغشية المكونة للطبقة الشعاعية. بالإضافة الى ظهورالحزم الليفية بوضوح. أما إضافة كل من فيتامين ج ، هـ معا مع الرصاص والكادميوم فكان أكثر تأثيراً من السابقين . وذلك كان ممثلاً فى منع التحلل الواضح فى الطبقات المحيطة والخلايا الحويصلية. أيضا ساعد فى سرعة نمو وظهور الغشاء المحى والطبقة الشعاعية فى المجموعات الخامسة والسادسة بالمقارنة مع المجموعة الأولى .

وقد استنتجت الدراسة أنه لابد من إضافة مضادات أكسدة مثل الفيتامينات وخاصة فيتامين ج ، هـ الى الوجبات اليومية أو الى مياه الشرب بمزارع الدجاج القريبة من أماكن الملوثات أو التى يثبت بالتحليل أن بها معادن ثقيلة كالرصاص والكادميوم وذلك للإقلال من تركيز هذه المعادن فى البيض وليحسن من الحالة النسيجية للمبيض .