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Endosulfan Damaging Effect on the Developing Kidney Types (Mesonephroi and Metanephroi) of the Chick Embryos

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ABSTRACT

This study aims to explore the effects of the organ chlorine pesticide (OCP) endosulfan on the developing kidney types of the chick embryos. Embryos aged 24 hours were treated with a single dose of 7 or 14 or 21 mg/egg (¼ or ½ or ¾ of the endosulfan LD_{50}, respectively) through the egg air space. The eggs were opened on embryonic days (EDs) 6 and 12 and the embryos were anesthetized, dissected out and the lumbar areas processed for paraffin embedding, stained with haematoxylin and eosin, and the kidney types were examined by light microscopy. In control embryos of ED 6, the only observed excretory tissue was of the mesonephric type. On ED 6, the endosulfan treatment had resulted in dose-dependent histopathological lesions included: a general delay of excretory units differentiation, reduction in the numbers of mesonephric ducts and tubules, irregular distribution of renal corpuscles, high rate of degenerative changes with atrophy and/or vacuolization of epithelial cells and excessive hemorrhage. In controls of ED 12, both mesonephric and metanephric kidneys were observed. The mesonephroi were fully developed, enlarged, and contained much more closely associated components than those of the 6-day-old stage. The effects of endosulfan on ED 12 were also dose-dependent and included increased regressive changes of mesonephroi. These changes were represented cyst-like appearance, dilatation of collecting tubules, necrosis and apoptosis, damaged renal corpuscle, and persistence of primitive excretory units. Also, the metanephros was either poorly developed or did not show any appearance. These findings suggest that endosulfan exhibits severe damaging effects on the developing kidney of the chick embryos.

Key words: Excretory units, necrosis and apoptosis, cystic kidney, regressed mesonephroi.

INTRODUCTION

Because of their persistence in the environment, pesticides are common contaminants in soil, water, and wildlife and are present in tissues of mothers and children, eggs and chicken meat samples especially in regions devoted to intensive agriculture (Ahmad *et al*., 2010). Endosulfan is a broad-spectrum OCP (insecticide and acaricide) first registered for use in the United States in 1954 to control agricultural insect and mite pests on a variety of fruits, vegetables, rice, grains, tea, coffee, cotton and also in animal farms and houses (US EPA., 2002). Most of the persistent OCPs have been removed from the market in Europe and North America, but many are still in use in Africa, Asia, and South America. Endosulfan, methoxychlor, and dicofol remain in use in the United States because their persistence and potential for bioaccumulation is lower. However, since 10 June 2010 endosulfan use in the USA is banned by the US EPA (2010)**.** It is abundant in the environment and it reaches aquatic systems through direct application, as well as spray drift and runoff from agricultural areas (Barakat *et al*., 2002; Jergentz *et al.*, 2004; Rand *et al*., 2010).

Endosulfan is a xenoestrogen and it can act as an endocrine disruptor, causing reproductive toxicity and congenital malformations in both animals and human's offspring exposed to it during pregnancy and/or lactation (Edwards *et al*., 2006; Varayoud *et al*., 2008). Maternal exposure to pesticides is associated with urogenital malformations, semen quality impairment, and testicular, prostate, ovarian, and breast cancer (Koifman *et al.*, 2002). Twenty five insecticides were tested for their toxicity to avian embryos at various concentrations using an egg injection technique. Of the two major groups studied, the organophosphorus compounds are much toxic than the organochlorine (Dunachie and Fletcher, 2008).

The embryotoxcity, neurotoxicity and teratogenicity of endosulfan on mammals (Silva and Gammon, 2009; Silva and Beauvais, 2010; Singh *et al.*, 2012), amphibians (Kang *et al*., 2008; Brunelli *et al.*, 2009) and fish (Stanley *et al*., 2009) as well as its genotoxic effects *in vitro* (Sandal and Yilmaz, 2010; Nandi *et al*., 2011) are studied. However, the data concerning the effects of endosulfan on the developing chick or avian embryos are rare and represented by some physiological, histo-chemical and teratological studies (Pourmirza, 2000; Bargar *et al*., 2001; Pushpanjali *et al*., 2005; Prakash *et al*., 2009; Mobarak and Al-Asmary, 2011). Therefore, the present study amis to find out the effects of endosulfan on the kidney types (mesonephroi and metanephroi) of the developing chick embryos on EDs 6 and 12.

MATERIALS AND METHODS

Toxicant

The pesticide used in the present study was a technical-grade endosulfan [Endocel (Batch 3, EPA Reg. No.11678-25)], in the form of a light yellow coloured liquid (35% EC) is composed of two stereo chemical

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isomers [alpha]-Endosulfan and [beta]-Endosulfan, in concentrations of approximately 70% and 30%, respectively. It was provided by Al-Wadi Agricultural Development Company, Al-Taif, K.S.A. The endosulfan was administered into the egg's air space through a minute hole without any dilutions at three dosage levels (7mg/egg, 14mg/egg and 21mg/egg) to 24 hrs-incubated eggs in volumes of 0.02, 0.04, 0.06 ml/egg, respectively. After endosulfan administration, the hole was sealed with melted paraffin wax and the eggs were re-incubated.

Egg Incubation

Fertile dark brown leghorn chicken eggs (each weighing about 55 g) were generously provided by El–Fakeeh Poultry Company, Taif, KSA. Eggs were maintained at 37.5°C in a full automatic egg incubator with full automatic control of humidity (relative 55%), egg turning, fan speed, ventilation, and alarm until the desired stages of development (24 hrs embryos, EDs 6 and 12) were reached. Eggs were candled before treatment and the unfertilized eggs were excluded from the experiments.

Experimental Protocol

Group I. LD_{50} determination

To evaluate the viability and LD_{50} of endosulfan a total of 256 (24 hrs-incubated) eggs were divided into 16 groups (16 eggs each). Dilutions of endosulfan were made in corn oil then eggs were administered different doses (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 27, 28, 30 mg/egg) of Endosulfan. The eggs were re-incubated. 24 hours following endosulfan administration. They were then opened and the survival rate of the embryos was recorded. The estimated LD_{50} of endosulfan was 28 mg/egg. The doses selected for the present study were ¼, ½, ¾ of LD_{50} (7, 14, 21 mg /egg).

Group II. Endosulfan Administration

To evaluate the developmental toxicity and teratogenicity of endosulfan, 24 hrs-incubated 480 eggs were divided into three replicates each formed of eight subgroups as follows:

Subgroup I: 20 eggs served as controls and opened on ED 6.
Subgroup II: 20 eggs administered 7 mg endosulfan/ egg at once and opened on ED 6.
Subgroup III: 20 eggs administered 14 mg endosulfan/ egg at once and opened on ED 6
Subgroup IV: 20 eggs administered 21 mg endosulfan/ egg at once and opened on ED 6.
Subgroup V: 20 eggs served as controls and opened on ED12.
Subgroup VI: 20 eggs administered 7 mg endosulfan/ egg at once and opened on ED 12.
Subgroup VII: 20 eggs administered 14 mg endosulfan/ egg at once and opened on ED 12.
Subgroup VIII: 20 eggs administered 21 mg endosulfan/ egg at once and opened on ED 12.

Tissue Processing

For histological studies, on ED 6, the whole chick embryos (control and endosulfan-treated) were sacrificed, washed in saline and fixed in aqueous Bouin's solution for 48 hrs. After fixation, the embryos were dehydrated in ascending grades of ethanol, cleared in pure terpineol for 24 hrs, and infiltrated with the embedding medium (a mixture of equal volumes of parablast and paraffin wax), and serially sectioned at 5 micrometers. On the other hand, to follow up the changes in the histological structure of the kidneys of 12- day-old embryos, the embryos were sacrificed by cervical dislocation and their abdomens were opened, washed in saline and embedded in Bouin's solution. Then, the lumbar region was dissected out, and fixed in fresh Bouin's solution for 48 hrs. Afterwards this region was processed as mentioned above. Finally, the sections (of both EDs) were stained with haematoxylin and eosin, and mounted in neutral Canada balsam. Any changes in the kidney types (mesonephroi on ED 6, mesonephroi and metanephroi on ED 12) were carefully examined with the light microscope.

RESULTS

Six-Day-Old Chick Embryos (Control and Endosulfan-Treated)

Control Embryos

The examination of the trunk region serial sections from several control 6-day-old chick embryos showed no signs of pronephroi. The only observed excretory tissue was of the mesonephric kidney type (Fig. 1). Each mesonephros was divided into an outer cortex and an inner medulla. Externally, each mesonephros was protected by a capsule made of squamous to cuboidal epithelial cells. The mesonephroi were composed of the mesonephric duct (also called the Wolffian duct), blood vessels, and excretory units (mesonephric tubules and their associated capillary tufts). In all examined embryos, the left mesonephroi were larger in size and contained much more mesonephric excretory units and interstitial cells than the right ones (Fig. 1a). The parenchyma of both mesonephric regions was occupied by some of L and/or U-shaped loops (the precursors of the uretric and collecting tubules), uretric buds, large numbers of mesonephric connecting ducts, mesonephric tubules, metanephric mesenchyme, endothelial cells, and renal corpuscles (Fig. 1b, 2a). The L and or U-shaped loops had three distinct regions; a middle part, short and long ends. The middle end known as uretric tip will differentiate into the uretric tubule, while the long and the short ends will differentiate into the proximal and the distal collecting tubules, respectively, (Fig. 1b, 2b). In the mesonephric cortex there were many cortical connecting ducts each one was a small tubule with a narrow lumen surrounded by cuboidal cells with spherical nuclei and 1-2 basophilic nucleoli. The proximal tubules lined by columnar epithelial cells with brush borders and contained one and sometimes two spherical nuclei.

Among these cells, large-sized oval to rectangular shaped cells (goblet cells) with granular cytoplasm were found. The lumen of these tubules contained vacuoles of variable size separated from each other by well distinct membranes (Fig. 2b). The distal tubules were smaller in size than the proximal ones and were lined by cuboidal cells contained large rounded nuclei surrounded by well defined nuclear membranes. A few cilia were found projecting from the inner ends of these cells into a clear lumen. Among the mesonephric components some of very small tubules (uretric tubules) lined with small cuboidal cells and narrow lumens were observed (Fig. 2b).

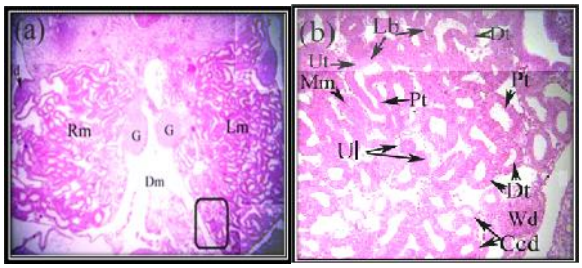

Figure 1 (a, b): Representative photomicrographs to show the mesonephroi structure of a control 6-day-old chick embryo.

(a) A transverse section displays the left (Lm) and the right (Rm) mesonephroi. The gonads (G) and dorsal mesentry (Dm) are also seen. H&E, X100.

(b) The upper part of the left mesonephros of Figure 1a magnified to display components of the cortex and medulla such as L-shaped loops (Lb) each consisted of three distinct regions; uretric tip (Ut), the proximal tubule (Pt) and the distal tubules (Dt). The metanephric mesenchyme (Mm), cortical connecting ducts (Ccd), Wolffian duct (Wd), other proximal (Pt) and distal (Dt) collecting tubules and uretric tubules (Ul) are also seen. H&E, X400.

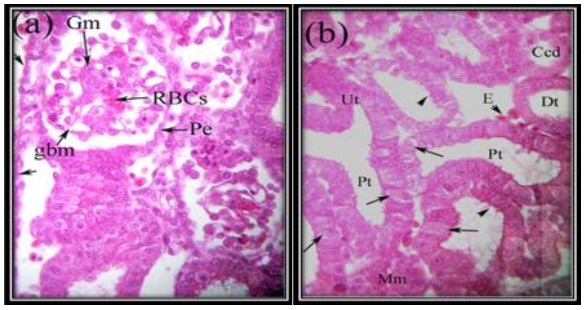

Figure 2 (a, b):

(a) The part (in small rectangle) of Figure 1a magnified to show the cellular components of the mesonephros outer covering (arrows) and the renal corpuscle constituents. Each renal corpuscle consisted of a glomerular basement membrane (gbm), the glomerular mesangium (Gm), a parietal epithelium (Pe), and red blood corpuscle (RBCs). H&E, X600.

(b) A magnified part of Figure 1a to show the cortical connecting ducts (Ccd) surrounded by some of cuboidal cells with spherical nuclei and 1-2 basophilic nucleoli, the proximal (Pt) tubules consisted of columnar epithelial cells with brush borders (arrow heads) and large-sized goblet cells (arrows) are found among them, and distal (Dt) collecting tubules lined by cuboidal-shaped cells with large rounded nuclei. Uretric tubules (Ut) and endothelial cells (E) are also seen. H&E, X600.

Low Endosulfan Dose (7 mg egg^{-1}) Treated Embryos

In few embryos, this dose resulted in a marked decrease of the mesonephric excretory units and interstitial cells. Also, there was developmental delay of the cellular components of the connecting ducts and collecting tubules with a general delay of the L/ and or U-shaped bodies differentiation (Fig. 3a). In addition, a dilatation of the Wolffian ducts, blood vessels, and the mesonephric collecting tubules and excessive hemorrhage, extravasation of blood among components of both mesonephroi was observed (Fig. 3b).

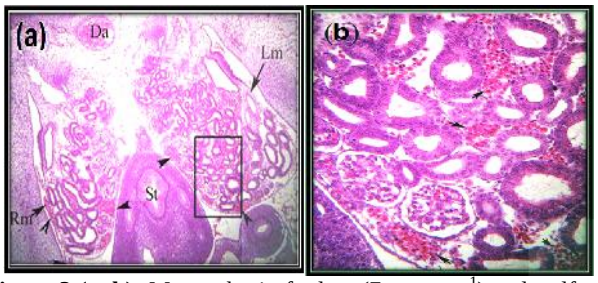

Figure 3 (a, b): Msonephroi of a low (7 mg egg^{-1}) endosulfan dose-treated 6-day-old chick embryo.

(a) Shows the left mesonephros (Lm) smaller in size and contained much more mesonephric excretory units than the right one (Rm). Both mesonephroi are smaller in size than the control. Excessive hemorrhage (arrows heads) is noted within the coelom and among mesonephric excretory units. The dorsal aorta (Da) and the stomach are also seen. H&E, X100.

(b) The part (in rectangle) of Figure 3a (left mesonephros) magnified to show the hemorrhagic changes (arrows heads) among normally appeared mesonephric excretory units. H&E, X400.

Mid-Endosulfan Dose (14 mg egg^{-1}) Treated Embryos

In all treated embryos this dose caused a general reduction in the number of mesonephric ducts and tubules. This decrease was contaminant with an increase in number of the undifferentiated L and / or U-shaped bodies (Fig. 4a). There was excessive hemorrhage in the peritoneal cavity and among all components of the mesonephroi. Also, some of the connecting ducts and collecting tubules displayed damage of their lining epithelium associated with hemorrhage. In some embryos, urinary tissue was only represented by earlier and very primitive mesonephric excretory units and interstitial cells as compared to controls. The epithelial cells in most of the mesonephric connecting ducts and collecting tubules showed degenerative changes (programmed cell death or apoptosis) with no mitotic figures (Fig. 4b).

High-Endosulfan Dose (21 mg egg^{-1}) Treated Embryos

In such treated group, the mesonephroi exhibited a high rate of degenerative changes, dilated tubules with atrophy and/or vacuolation of their epithelial cells and pronounced enlargement of the tubules (Fig. 5a). There were degeneration of the renal corpuscular glomerular basement membrane, the glomerular mesangium, parietal

epithelium and pyknotic nuclei of the red blood corpuscle. The corpuscles exhibited few capillaries in which the red blood corpuscles coagulated forming large blood clot and small Bowman's space were also detected (Fig. 5b).

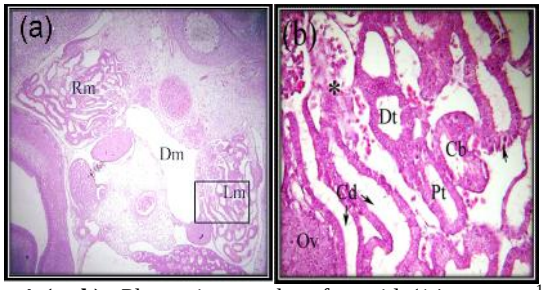

Figure 4 (a, b): Photomicrographs of a mid (14 mg egg^{-1}) endosulfan dose-treated 6-day-old chick embryo.

(a) The mesonephroi (left, Lm and right, Rm) are widely spaced and the dorsal mesentry (Dm) is highly dilated. Each mesonephros contained some poorly developed excretory elements. H&E, X100

(b) The part (in rectangle) of the left mesonephros of Figure 4a magnified to show undifferentiated comma-shaped body (Cb), degenerative changes (arrow) with few or no mitotic figures of the epithelium of proximal (Pt) and distal (Dt) collecting tubules, dilated and poorly developed connecting ducts (Cd) as well as a few poorly developed glumeruli (*). Part of the left ovary (Ov) is also seen. H&E, X400.

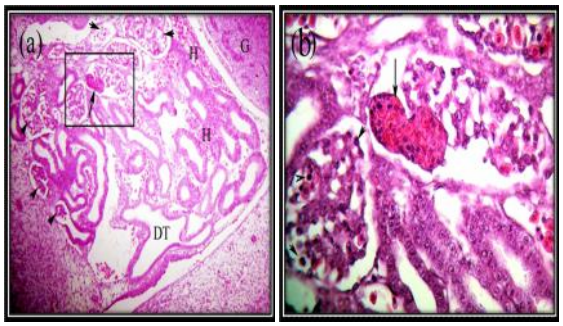

Figure 5 (a, b): Photomicrographs of mesonephric tissues from a high- (21 mg egg^{-1}) endosulfan dose-treated 6-day-old chick embryo.

(a) Shows the right mesonephros contained some L-and U-shaped bodies and few highly dilated (Dt) connecting ducts with atrophy of their epithelial cells, renal corpuscles are randomly distributed (arrow heads) and poorly developed with blood coagulation in one of them (arrow). Hemorrhage (H) is seen in between the tubules. H&E, X200.

(b) The part (in rectangle) of Figure 5a magnified to display the damage of the renal corpuscles with and pyknotic nuclei (arrow heads) of red blood corpuscles and a clear blood clot (arrow) within the renal corpuscle. H&E, X600.

Also, the mesonephros contained some of the undifferentiated comma-shaped bodies, renal vesicles and highly dilated poorly developed mesonephric connecting ducts and collecting tubules as well as abnormally located poorly developed glumeruli (Fig. 6a). Furthermore, tubular-interstitial damage that resulted in the loss of renal tubules and the accumulation of extracellular remains among kidney excretory units with malformed dilated tubules was detected (Fig. 6b).

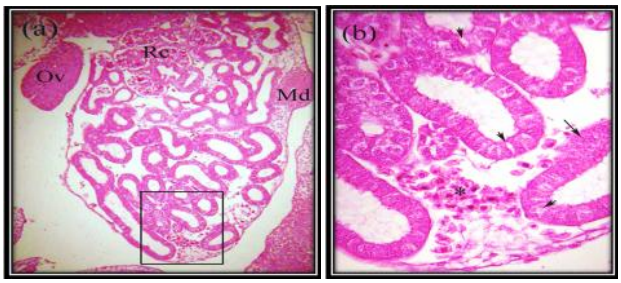

Figure 6 (a, b): Photomicrographs of mesonephric tissues of another high- (21 mg egg^{-1}) endosulfan dose-treated 6-day-old chick embryo.

(a) Illustrated the right mesonephros contains some of the undifferentiated comma-shaped bodies, renal vesicles and highly dilated poorly developed mesonephric connecting ducts and collecting tubules as well as abnormally located poorly developed glumeruli (Rc) in the dorsal cortex. H&E, X200

(b) The part (in rectangle) of Figure 6a magnified to show the poorly developed mesonephric connecting ducts and collecting tubules with wide spaces present among their lining epithelial cells (arrow heads). There is an accumulation of cells in the outer surface (arrow) of one the collecting tubules and abnormal red blood corpuscles (*) among them. H&E, X600

Twelve- Day-Old Chick Embryos (Control and Endosulfan-Treated)

Control Embryos

Such embryos contained both mesonephric and metanephric types of the developing kidney. The mesonephroi were fully developed, enlarged, more compacted and had much more closely associated components than those of the previously mentioned 6-day-old stage (Fig. 7a, 8a). In such control embryos, no signs of regression were detected in any of the examined mesonephroi (Fig. 7b). Clusters of adrenal cells organized as irregular cords were found in the upper outermost part of the mesonephros, while the gonads were well individualized as organs and protruded into the coelomic cavity (Fig. 8a).

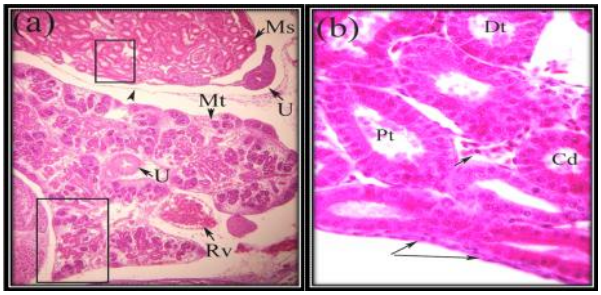

Figure 7 (a, b): Kidney types of a control 12-day-old chick embryo

(a) A transverse section displays the lower part of the left mesonephros (Ms) and the metanephros (Mt) below it, with their associated ureters (U) and renal vein (Rv). Both kidney types are separated by a thin connective tissue membrane (arrow head). H&E, X100.

(b) An outer cortical part (in rectangle) of the mesonephros

of Figure 7a magnified to show the outer protective capsule (arrows), closely associated collecting proximal (Pt) and distal (Dt) tubules, connecting ducts (Cd) with intercellular tissue among them (short arrow). H&E, X400.

The renal corpuscles were more developed than those of 6-day-old mesonephroi, each consisted of a glomerulus and an intact Bowman's capsule (Fig. 8b). The mesonephric medulla was crowded by proximal tubule consisted of cylindrical to high cuboidal cells, frequently denominated prismatic cells, with high brush border. The distal tubule lined by cuboidal cells with a generally apical nucleus (Fig. 9b). The collecting system of the metanephros exhibited multiple divisions (darkly and lightly- stained) that made of metanephric vesicles, metanephric cups, S-shaped nephrons, comma-shaped bodies, mesenchymal cells and more mature nephrons; consisted of Bowman's capsule and renal glomerulus; collecting tubules and developing ureters (Fig. 9c).

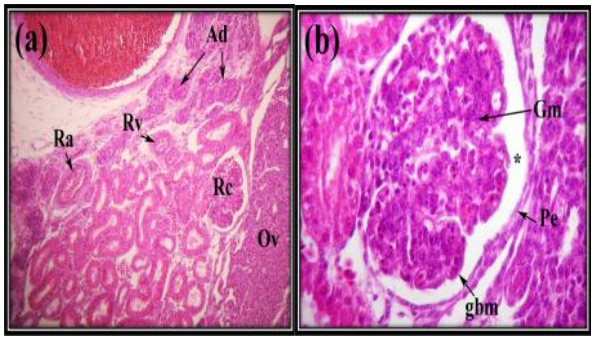

Figure 8 (a, b):

(a) A transverse section displays the upper part of the previous mesonephros with clusters of adrenal cells (Ad) organized as irregular cords, while the ovary (Ov) is well individualized as an organ and is protruded into the coelomic cavity. A renal corpuscle (Rc), renal venules (Rv), renal arteriole (Ra) are seen in the outer cortex. H&E, X100.

(b) The renal corpuscle of Figure 8a magnified to show compacted normal components made of a parietal epithelium (Pe) at a distance (*) from the glomerular basement membrane (gbm), the glomerular mesangium (Gm). H&E, X600.

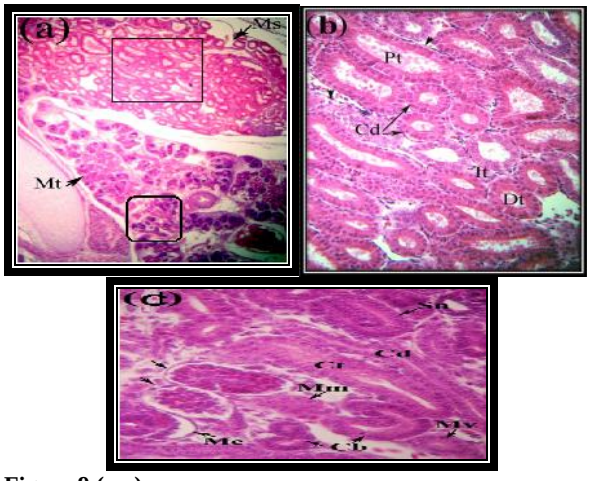

Figure 9 (a-c):

(a) The same 12-day-old control embryo displays cortical and medullary parts of the mesonephros (Ms) and terminal part of the metanephros (Mt). H&E, X100.

(b) The medullary part (in square) of Figure 9a magnified to show more compacted mesonephric proximal (Pt) and distal (Dt) tubules, connecting ducts (Cd) externally protected by a thin sheath of squamous epithelium (arrow heads). H&E, X400.

(c) The part (in rectangle) of Figure 9a magnified to show other metanephros components as S-shaped nephron (Sn), comma-shaped bodies (Cb), metanephric vesicle (Mv), metanephric cup (Mc), metanephric mesenchymal cells (Mm), Bowman's capsule and renal glomerulus (arrows), collecting tubules (Ct) and connecting duct (Cd). H&E, X400.

Low Endosulfan Dose (7 mg egg^{-1}) Treated Embryos

Both meso- and metanephric kidney types were present. The mesonephros exhibited slightly to moderately dilated collecting tubules, damaging of renal corpuscles, appearance of hemorrhage, extracellular spaces and dense interstitial tissue among mesonephric components, and atrophy of epithelium of the collecting tubules (Fig. 10a and 11a). The inner components of the renal corpuscles were depleted or separated by large spaces and sometimes exhibited irregular arrangements (Fig. 10b, 11). However, the metanephroi were quite normal except the formation of a few empty spaces among its components (Fig. 10a).

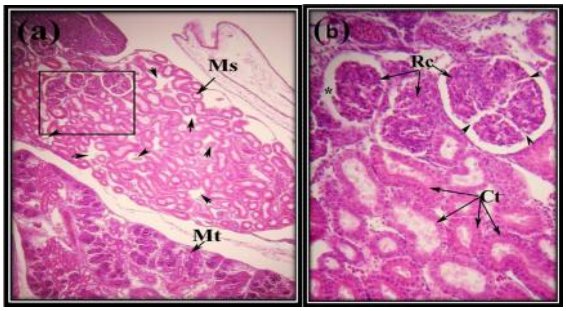

Figure 10 (a,b): Representative photomicrographs of kidney types of a low (7 mg egg^{-1}) endosulfan dose-treated 12-day-old chick embryo.

(a) A transverse section displays the left mesonephros (Ms) and the metanephros (Mt) below it, with their components appeared quite normal except the appearance of some extracellular spaces among the mesonephric components (arrow heads). H&E, X100.

(b) The part (in square) of Figure 10a magnified to show three adjacent renal corpuscles (Rc) the inner components of the largest one are separated by large spaces (arrow heads), the middle one is irregular elongated, and the third with wide space (*). However, the collecting tubules (Ct) appeared normal. H&E, X400.

Mid-Endosulfan Dose (14 mg egg^{-1}) Treated Embryos

The mesonephroi became much smaller in size and contained many dilated and much less collecting tubules as well as widely spaced excretory units than the control (Fig, 12a). Among these tubules there were remains of degenerated ones, appearance of spaces within their epithelium, injury of intercellular tissue as well as acidophilic inflammatory cells among the tubules (Fig

12b). Also, the collecting tubules lining epithelium showed shrinkage and atrophic changes and cellular apoptosis. Degenerated metanephric mesenchyme, damaged renal corpuscle, and primitive excretory units as L-shaped body and uretric bud were also observed (Fig. 12c). The metanephros showed a size reduction and decrease in its excretory units.

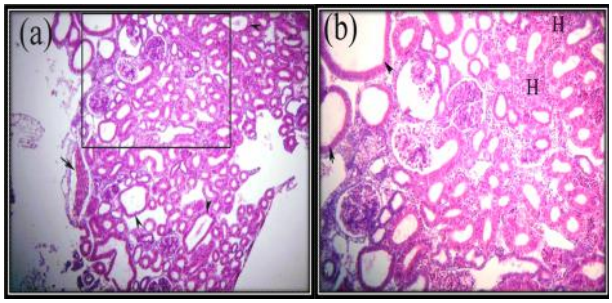

Figure 11 (a, b): Photomicrographs of mesonephros of another low (7 mg egg^{-1}) endosulfan dose-treated 12-day-old chick embryo.

(a) A transverse section displays the left mesonephros with moderately dilated collecting tubules (arrow heads). One of these tubules showed fibroses (arrow). H&E, X100.

(b) The part (in rectangle) of Figure 11a magnified to show damaged renal corpuscles, hemorrhage (H) and dense interstitial tissue among mesonephric components, and atrophy of the epithelial lining of collecting tubules (arrow heads). H&E, X200.

High-Endosulfan Dose (21 mg egg^{-1}) Treated Embryos

In embryos received high endosulfan dose the prevalent kidney type was the mesonephros since the metanephros was either poorly developed or did not show any appearance (Fig. 13a). Acceleration of regressive changes and cyst formation were evident in the mesonephros of all examined embryos. These changes were severe and sometimes were similar to those observed in the previously mentioned changes. The epithelium of the renal tubules exhibited program-med cell death or apoptosis and strongly acidophilic cytoplasm with signs of local inflammation and vacuolization in their interstitial cells (Fig. 13b).

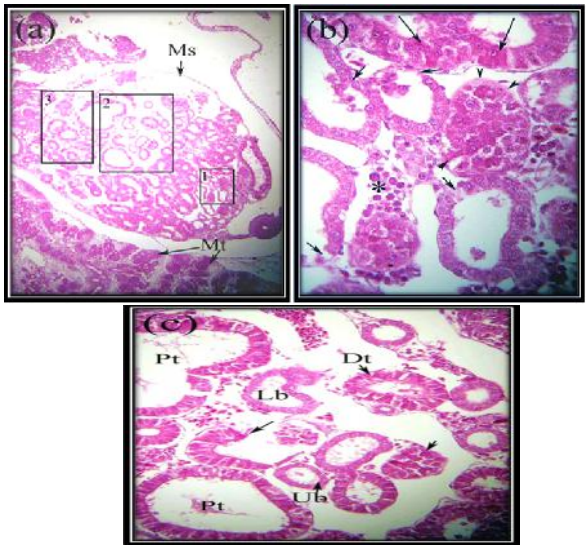

Figure 12 (a-c): Kidney types of a mid-(14 mg egg^{-1}) endosulfan dose-treated 12-day-old chick embryo.

(a) The left mesonephros (Ms) contained many dilated and much less collecting tubules as well as widely spaced excretory units. The metanephros (Mt) also shows a decrease in its excretory units. H&E, X100.

(b) The part (in rectangle 1) of Figure 12a showing widely spaced collecting tubules with remains of degenerated ones (arrow heads), appearance of spaces within the epithelium of the tubules (short arrows), injury of intercellular tissue (*). Acidophilic inflammatory changes (long arrows) are also present in the epithelium of some tubules. H&E, X400.

(c) Another part (in rectangle 2) of Figure 12a magnified to show the mesonephric medulla has widely spaced excretory units with no or a few damaged interstitial cells. The proximal tubules (Pt) are dilated and lined by atrophic epithelium. The distal tubule (Dt) showed shrinkage of its cells. Degenerated metanephric mesenchyme (short arrow), damaged renal corpuscle (long arrow), L-shaped body (Lb) and uretric bud (Ub) are also seen. H&E, X400.

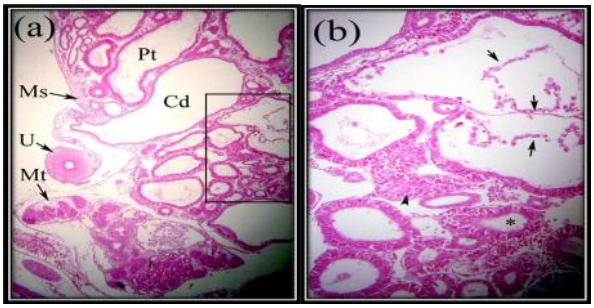

Figure 13 (a, b): Kidney types of a high (21 mg egg^{-1}) endosulfan dose-treated 12-day-old chick embryo

(a) A transverse section displays and medullary parts of the mesonephros (Ms) with cystic highly dilated components including connecting ducts (Cd), proximal tubules (Pt) and other excretory units. The metanephros (Mt) is highly reduced in size. H&E, X200.

(b) The medullary part (in rectangle) of Figure 13a magnified to show damaged wall of two adjacent mesonephric proximal (arrows) and distal (*) tubules with vacuolation of its lining epithelium, abnormal degenerated comma-shaped body (arrow head). H&E, X400.

DISCUSSION

The observed histological structure of control 6-day-old chick embryos's mesonephros is in agreement with Diaz-Ruiz *et al.*, (1993). In embryos received (low or mid)-endosulfan treatment a dose-dependent reduction of the mesonephroi size, decrease of the mesonephric excretory units and interstitial cells, as compared to controls were investigated. Also, a general delay of the L and/or U-shaped bodies differentiation; dilatation of the Wolffian ducts, blood vessels, and the collecting tubules; excessive hemorrhage and extravasations of blood was observed among components of both meson-ephroi. Similarly, histopathological alterations in kidn-eys of the rat fetuses following maternal treatment with low to moderate doses of endosulfan were reported by Singh *et al.*, (2008). On the other hand, the high endosulfan dose

had resulted in maintaining primitive excretory units; poorly developed mesonephric connect-ing ducts, collecting tubules and glumeruli; atrophy and vacuolation of epithelial cells and pronounced enlarge-ment of the tubules. Dubale and Shah (1981) declared that the renal tubules are the first to be affected by pesticidal stress and the process of tissue destruction is a function of dosages. In controls of 12-day-old embryos both mesonephric and metanephric types of kidney were observed. The mesonephroi were fully developed, enlar-ged, more compacted and contained much more closely associated components than those of the previously mentioned 6-day-old stage. These results are similar to those presented by Ditrich (2005). The effects of endo-sulfan on ED 12 were also dose-dependent and included acceleration of regressive changes in the mesonephroi and the metanephros was either poorly developed or did not show any appearance as well as there was an acceleration of cyst growth and tubular dilatation.

These changes could be possibly resulted from the oxidative damage of kidney tissue macromolecules by reactive oxygen species generated during the metabolism of endosulfan or by the induction of a substance which lowers the intracellular calcium concentration as proposed by Osten *et al*., (2009)

The acceleration of regressive changes and cyst formation in the mesonephroi which were observed on ED 12 could be due to the inability of the mesonephroi of endosulfan- treated embryos to reach their optimum rate of development as did control ones. Furthermore, the poor metanephroi development and/or the delay of their appearance might be the result of endosulfan-damaging effect on mesonephroi and their precursors mesenchymal cells.

In addition, the programmed cell death or apoptosis was observed as a result of endosulfan toxicity. It can be inferred that the present damage to renal tissue with endosulfan treatment might have occurred through the induction of apoptosis. This result is in agreement with Jia and Misra (2007) who proposed that endosulfan and zineb induced apoptotic and necrotic cell death *in vitro*. Also, Singh *et al.*, (2012) reported that citrinin and endosulfan administration to pregnant rats caused apoptosis in an additive manner because there was significant increase in the apoptotic cells percentage in kidneys, whereas fetal liver was comparable to endosulfan group, indicating that endosulfan was more nephrotoxic than hepatotoxic in fetuses and it was both hepatotoxic and nephrotoxic in pregnant rats. The swelling of renal tubules, local inflammation and vacuolization following endosulfan exposure were evident in the present study. Similarly, Choudhary *et al.* (2003); Altinok and Capkin (2007) reported changes like vacuolation of epithelial cells of renal tubules and pronounced enlargement of the kidney tubules, odenoma and glomerulus deposits at higher sublethal concentration and prolonged exposure to endosulfan, that represent a support for the present observations.

Endosulfan toxicity to specific target organ such as kidney in the form of size reduction and decrease in its excretory units, obtained in the present study, provide a support to the previously obtained body swellings and edemas formation and other deleterious effects of endosulfan on the developing chick embryo (Mobarak & Al-Asmary, 2011). The results of the present study clearly indicate that endosulfan had damaging effects on kidney types of the developing chick in both EDs. These findings open a new area for further research regarding the potential toxicity of this environmental contaminant through dietary exposure which might cause deleterious effects in animal and human populations. The study also recommends banning the use of this pesticide in Egypt and other Arab countries.

REFERENCES

AHMAD, R., N. M. SALEM, AND H. ESTAITIEH. 2010. Occurrence of organochlorine pesticide residues in eggs, chicken and meat in Jordan. Chemosphere **78**: 667-671.

ALTINOK, I., AND E. CAPKIN. 2007. Histopathology of rainbow trout exposed to sublethal concentrations of methiocarb or endosulfan. Toxicology and Pathology. **35**(3):405-410.

BARAKAT, A. O., M. KIM, Y. QIAN, AND T. L. WADE. 2002. Organochlorine pesticides and PCB residues in sediments of Alexandria Harbour, Egypt. Marine Pollution Bulletin **44**(12):1421-1434.

BARGAR, T. A., G. I. SCOTT, AND G. P. COBB. 2001. Uptake and distribution of three PCB congeners and endosulfan by developing white leghorn chicken embryos (*Gallus domesticus*). Archives of Environmental Contamination and Toxicology **4**: 508-514.

BRUNELLI, E., I. BERNABO, C. BERG, K. LUNDSTEDT-ENKEL, A. BONACCI, AND S. TRIPEPI. 2009. Environmentally relevant concentrations of endosulfan impair development, metamorphosis and behaviour in *Bufo bufo* tadpoles. Aquatic Toxicology **91**: 135-142.

CHOUDHARY, N., M. SHARMA, P. VERMA, AND S.C. JOSHI. 2003. Hepato and nephrotoxicity in rat exposed to endosulfan. Journal of Environmental Biology **24**(3):305-308.

DIAZ-RUIZ, R., P. TOM, X. CULLER, AND J. DOMINGO. 1993. Imunnunohistochelnical localization of transforming growth factor and epidermal growth factor-receptor in the mesonephros and metanephros of the chicken. Cell Tissue Research **271**:3-8.

DITRICH, H. 2005. Renal structure and function in vertebrates. In: Biological Systems in Vertebrates, edited by Dutta H.M. and Kline D.W., vol. **2**, p. 1-163. Enfield, NH: Science.

DUBALE, M. S., AND P. SHAH. 1981. Histopathology of the kidney of the fish *Channa punctatus* exposed to cadmium. Journal of Animal Morphology and Physiology **28** (1-2): 166-171.

DUNACHIE, J. F., AND W. W. FLETCHER. 2008. An investigation of the toxicity of insecticides to birds egg using the egg injection technique. Anneals of Applied

Biology **64**: 409-423.

EDWARDS, T. M., B. C. MOORE, AND L. J. GILLETTE. 2006. Reproductive dysgenesis in wildlife: A comparative view. International Journal of Andrology **29**: 109-121.

JERGENTZ, S., H. MUGNI, C. BONETTO, AND R. SCHULZ. 2004. Runoff-related endosulfan contamination and aquatic macroinvertebrate response in rural basins near Buenos Aires, Argentina. Archives of Environmental Contamination and Toxicology **46**: 345-352.

JIA, Z. H., AND P MISRA. 2007. Developmental exposure to pesticides zineb and/or endosulfan renders the nigrostriatal dopamine system more susceptible to these environmental chemicals later in life. Neurotoxicology **28**(4):727-735.

KANG, H. S., M. C. GYE, AND M. K. KIM. 2008. Effects of endosulfan on survival and development of *Bombina orientalis* (Boulenger) embryos. Bulletin of Environmental Contamination and Toxicology **81**: 262-265.

KOIFMAN, S., R. J. KOIFMAN, AND A. MEYER. 2002. Human reproductive system disturbances and pesticide exposure in Brazil. Cad Saude Publica **18**: 435-445.

MOBARAK, Y. M., AND S. A. AL-ASMARY. 2011. Endosulfan impacts on the developing chick embryos: Morphological, morphometric and skeletal changes. International Journal of Zoological Research **7**(2): 107-127.

NANDI, S., P. S. GUPTA, S. C. ROY, S. SELVARAJU, AND J. P. RAVINDRA. 2011. Chlorpyrifos and endosulfan affect buffalo oocyte maturation, fertilization and embryo development *in vitro* directly and through cumulus cells. Environmental Toxicology **25**(1): 57-67.

OSTEN, L., M. KUBITZA, A. R. GALLAGHER, J. KASTNER, H. OLBRICH, U. DE VRIES, F. KEES, B. LELONGT, S. SOMLO, AND H. OMRAN. 2009. Doxycycline accelerates renal cyst growth and fibrosis in the *pcy*/*pcy* mouse model of type 3 nephronophthisis, a form of recessive polycystic kidney disease. Histochemistry and Cell Biology **132** (2): 199-210,

POURMIRZA, A. A., 2000. Toxic effects of malathion and endosulfan on chick embryo. Journal of Agricultural Science and Technology **2**: 161-166.

PRAKASH, P. J., G. RAJASHEKHAR, H. KRISHNAPPA, S. M. SULAIMAN, AND K. RAO. 2009. Effect of endosulfan 35% Ec on the egg laying and egg shell thickness in Japanese quails. Research Journal of Environmental Toxicology **3** (3): 140-146.

PUSHPANJALI, A. K., R. L. PAL, A. PRASAD, S. K. PRASAD, S. A. KUMAR, AND S. B. JADHAO. 2005. *In ovo* embryotoxicity of α-endosulfan adversely influences liver and brain metabolism and the immune system in chickens. Pesticide Biochemistry and Physiology **82**: 103-114.

RAND, G. M., J. F. CARRIGER, P. R. GARDINALI, AND J. CASTRO. 2010. Endosulfan and its metabolite, endosulfan sulfate, in freshwater ecosystems of South Florida: A probabilistic aquatic ecological risk assessment. Ecotoxicology **19**: 879-900.

SANDAL, S., AND B. YILMAZ. 2010. Genotoxic effects of chlorpyrifos, cypermethrin, endosulfan and 2,4-D on human peripheral lymphocytes cultured from smokers and nonsmokers. Environmental Toxicology **26**: 433-442.

SILVA, M. H., AND D. GAMMON. 2009. An assessment of the developmental, reproductive and neurotoxicity of endosulfan. Birth Defects Research (B). Developmental and Reproductive Toxicology **86**: 1-28.

SILVA, M. H., AND S. L. BEAUVAIS. 2010. Human health risk assessment of endosulfan. I: Toxicology and hazard identification. Regulatory Toxicology and Pharmacology **56**: 4-17.

SINGH, N. D., A. K. SHARMA, P. DWIVEDI, A. G. TELANG, M. KUMAR, AND R. D. PATIL. 2012. Studies on apoptotic changes in combined toxicity of citrinin and endosulfan in pregnant wistar rats and their fetuses. Toxicology International **19**(2): 138-143.

SINGH, P. B, V. SINGH, AND P. K. NAYAK. 2008**.** Pesticide residues and reproductive dysfunction in different vertebrates from north India. Food Chemistry and Toxicology **46**(7): 2533-2539.

STANLEY, K. A., L. R. CURTIS, S. L. SIMONICH, AND R. L. TANGUAY. 2009. Endosulfan I and endosulfan sulfate disrupts zebrafish embryonic development. Aquatic Toxicology **95:** 355-361.

US EPA. 2002. Reregistration eligibility decision for endosulfan. EPA 738-R-02-013. Pollution, Pesticides and Toxic Substances (7508C), United States Environmental Protection Agency.

US EPA. 2010. Action to terminate endosulfan. United States Environmental Protection Agency.

VARAYOUD, J., L. MONJE, T. BERNHARDT, M. MUÑOZ-DE-TORO, E. H. LUQUE, AND J. G. RAMOS. 2008. Endosulfan modulates estrogen-dependent genes like a non-uterotrophic dose of 17beta-estradiol. Reproductive Toxicology **26**: 138-145.

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التأثير الضار للإندوسلفان على نوعى الكُلي النامية (المتوسطة والبعدية) لأجنة الكتكوت

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الملخـــص العربـــي

تهدف هذه الدراسة إلى التعرف على الآثار الضارة لمبيد الآفات (الكلورية العضوية) إندوسلفان على نوعى الكُلي النامية لأجنة الكتكوت. تم علاج الأجنة عمر ٢٤ساعة بجرعة واحدة من الإندوسلفان قدرها ٧ مجم أو ١٤ مجم أو ٢١ مجم / بيضة فى الفراغ الهوائى للبيضة ولمرة واحدة، ثم فتح البيض في اليومين السادس و الثانى عشر من عمر الأجنة، وخدرت الأجنة وشرحت ثم أخذت المناطق القطنية للطمر فى شمع البارافين، وصبغت بالهيماتوكسيلين والأيوسين، وفحصت أنواع الكُلي عن طريق المجهر الضوئي.

فى المجموعة الضابطة عمر ستة أيام كانت الأنسجة الإخراجية المتكونة ممثلة فقط بالكُلي المتوسطة، وقد أدت المعاملة بالاندوسلفان إلى حدوث أضرار مرضية نسيجية فى الأجنة عمر ٦ أيام اشتملت على تأخر عام لتمايز الوحدات الإخراجية ونقص فى أعداد قنوات الكُلي المتوسطة وأنبيبياتها الجامعة، والتوزيع الغير منتظم لكريات الكُلي وحدوث بعض التغيرات التحللية ونزيف دموي بين مكونات الكُلي وضمور الطلائية المبطنة للانبيبيات الجامعة. أما في أجنة المجموعة الضابطة فى اليوم الثانى عشر من العمر فقد ظهرت مرحلتين من مراحل تطور ونمو الكُلي (الكُلي المتوسطة والكُلي البعدية)، حيث إكتمل نمو الكُلي المتوسطة وأصبحت كبيرة فى الحجم وأكثر تماسكا وإحتوت على مكونات إخراجية أكثر، بالمقارنة بالمجموعة الضابطة عمر ستة أيام. كان تأثير الإندوسلفان فى عمر١٢ يوم أيضاً يعتمد على تركيز الجرعة المستخدمة، واشتملت على تغييرات تنكسية للكُلي المتوسطة تمثلت فى ظهور تحوصل لمحتوياتها وإتساع للأنيبيبات الجامعة وتنكرز وموت مبرمج لطلائيتها وتلف للكريات الكلوية و إستمرارية الوحدات الإخراجية البدائية، كما وجد أن تطور الكُلي البعدية كان متدنى وأحياناً لم يحدث لها أى ظهور. من تلك النتائج يتضح أن الإندوسلفان قد أحدث آثاراً ضارة شديدة على الكُلي النامية لأجنة الدجاج.