Prenatal and Postnatal Development of the Adrenal Gland of Albino Rats: Light and Electron Microscopic Study

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Abstract: Background: The dexamethasone (Dx) is a synthetic glucocorticoid that cross the placenta especially in the late pregnancy and affects the development and maturation of most of the fetal organs. And thus, the Dx is widely used drug in premature labor to enhance development of the fetal lung surfactant, decreasing the postnatal mortality from respiratory death syndrome. **This study was carried out to** This work was amid to investigate the effects of dexamethasone injected subcutaneously to pregnant mother in late pregnancy, on the development of suprarenal gland. Both in the fetuses aged 20 days of gestation and n rats pups aged 14 days postnatally. **Material & Methods:** Thirty-Six Adult (24 female and 12 male) albino rats, ranged in weight between 200-250 gm, used in this study. The pregnant rats were divided into two main groups: **1- Control group:** 12 pregnant rats were injected subcutaneously with 0.3 ml of (0.9%) saline on the day 16, and 1.0ml of (0.9%) saline on day17 of pregnancy. **2- Experimental group:** 12 pregnant rats were injected subcutaneously on day 16 with Dx, 1.5 mg/kg body weight and 0.5 mg/kg body weight on day 17 of pregnancy. Scarifications occur on the prenatal day 20 of gestation to the pregnant rats (6 rats from each group) and 14th days after birth to rat pups. The adrenal glands specimens were prepared for light and electron microscopic examinations. **Results:** In the present study, by 20 days, there were relative increase thickness of Capsule, cortex and medulla, more demarcation between the cortex and medulla than the previous age. DZ cells arranged in groups underneath the capsule with appearance of dark and light cells. The FZ cells arranged in irregular cords with blood sinusoids in between. Vacuolated cytoplasm is common within the cells of this zona. The adrenocortical cells represented more lipid and mitochondrial contents than the previous ages but still the FZ cells show increase the lipid and mitochondrial contents than that of DZ. The treated groups showed decrease the collagenous contents of the capsule and the stroma connective tissue with increase the connective tissue cells poor demarcation between the cortex and medulla with random distribution of the adrenocortical cells within both Dz and FZ with ill differentiation between both zones. Appearance of microcyst like spaces and eosinophilic masses within the FZ. Dark and light cell appearance with predominance of the dark cells. The medullary cells arranged in groups in relation to the blood sinusoids. Profile of degenerated cells affecting both DZ and FZ cells, the degeneration profile includes cyst-like vesicles of degenerated endoplasmic reticulum, degenerated mitochondria, few lipid droplets and myelin like figures. In the present study by 14 days after birth, the control groups showed a well-developed an outer connective tissue capsule. The connective tissue fibers are well formed within the capsule and around the parenchymatous blood vessels. The cortex is formed of three concentric zones: Zg, Zf and Zr. There is increase in the thickness of all zones in comparison with the previous ages. The outer Zg is formed polygonal cells with darkly stained nuclei and vacuolated cytoplasm. The cells arranged either in globules and arches. Zf is the largest of the three zones its cells are arranged in parallel cords with blood sinusoids in between. The Zr is the smallest zone; its cells are arranged in reticular pattern. The medulla is distinct and apparently more developed than the previous ages. The medullary cells are small eosinophilic cells with vesicular nuclei with dark and light cell affinity. It is rich in blood vessels and sinusoids. Zg cells lipid droplets are many, and multiple mitochondria and endoplasmic reticulum. Zf cells are larger than that of the Zg with multiple lipid droplets and mitochondria. In the treated group: the cells of Zf showed few lipid droplets containing myelin figurers and multiple number of mitochondria. **Conclusion:** these results are very helpful for clinician deal with the adrenal gland diseases and those who deal with premature deliveries.

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Keywords: development, adrenal gland, Dexamethasone, premature deliveries.

1. Introduction:

The dexamethasone (Dx) is a synthetic glucocorticoid that cross the placenta especially in the late pregnancy and affect fetal organ maturation **Roberts and Dalziel (2006); Ritic et al (2014)**. Therefore, the Dx is widely used drug in premature

labor to enhance lung surfactant, reduce the postnatally respiratory distress syndromes and hence decrease the postnatal premature deaths **Manojlovic-Stojanoski et al (2006) Ricci (2017)**. Dx affects many organs in way to enhance lung maturations and this may help in programing of postnatal disease, such as hypertension, diabetes…. etc.

2. Materials and Methods:

Thirty-Six Adult (24 female and 12 male) albino rats, ranged in weight between 200-250 gm, used in this study. The female rats placed in quarantine for one week prior to breeding housed in plastic cages in a controlled environment at 23-25 °C, light- dark cycle (14:10 H), and free access to water and balanced diet. All aspects of the research were complied with the protocols approved by Local Ethical Committee of Faculty of Medicine, Benha University. After acclimatization to laboratory conditions two females kept overnight with one male to allow matting; the presence of spermatozoa in the vaginal smear in the next morning is an indicator of the day zero of pregnancy (GD 0). The pregnant rats were divided into two main groups: **1- Control group:** 12 pregnant rats were injected subcutaneously with 0.3 ml of (0.9%) saline on the day 16, and 1.0ml of (0.9%) saline on day17 of pregnancy. **2- Experimental group:** 12 pregnant rats were injected subcutaneously on day 16 with Dx, 1.5 mg/kg body weight and 0.5 mg/kg body weight on day 17 of pregnancy. Scarifications occur on the prenatal day 20 of gestation to the pregnant rats (6 rats from each group) and 14th days after birth to rat pups. The adrenal glands specimens were prepared for light (H & E and Masson's trichrome stains) and transmission electron microscopic examinations.

3. Results

Prenatal control group (Aged 20 days):

Light microscopic examinations of suprarenal gland of rat fetus aged 20 days of gestation of control group reveal the outer connective tissue capsule is formed of many connective cells and fibers (Fig. 1). The capsular cells exhibit spindle shape and eosinophilic cytoplasm. Their nuclei are elongated and darkly stained (Figs. 2 & 3). The collagen fibers are uniformly distributed throughout the capsule and forming the thin parenchymatous background of the gland and around the blood vessels (Fig. 4). The cortex is thicker and more developed than the previous age and surrounded the medulla. The cortex is composed of smaller outer DZ and larger inner FZ. The Medulla is apparently demarcated from the surrounding cortex, with some cortical cells are seen in between the outer medulla (Fig. 1). The DZ is formed of small closely packed cells that arranged in groups of cells around blood sinusoids (Fig. 2). DZ cells has eosinophilic cytoplasm with few tiny vacuoles. The nuclei of DZ cells are small and some are dark stained while others show vesicular large nuclei fill most of the cytoplasm (Fig. 3). The FZ cells are arranged in anastomotic cords (of one to three cell thickness) radially running (towards the medulla). The cords of FZ cells are separated from each other by blood sinusoids which appear wide spaces. The FZ cells appear with large central nuclei and eosinophilic, vacuolated cytoplasm (Figs. 2, 3). **By electron microscope** the cytoplasm of the adrenocortical cells contains larger and more numerous mitochondria than the previous age. The mitochondria are elongated and numerous in the DZ (Fig. 5) but oval or round and more numerous in the FZ. The lipid droplets are few, and small-sized, in DZ but they exhibit an apparent increase in number and diameter and appear in groups of cells in the FZ. The nuclei are oval to rounded in shape and contain dark dispersed chromatin. The endoplasmic reticulum is abundant and fill the cytoplasm (Fig. 6).

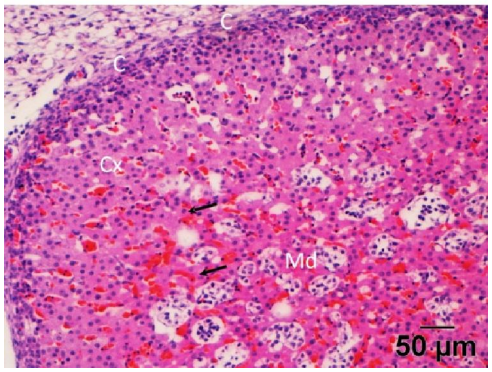

Fig. 1: A photomicrograph of a section of adrenal gland of control rat fetus aged 20 days of gestation shows: connective tissue capsule (C). Cortex (Cx) surround a central medulla (Md) which formed of masses of cells. Some cortical cells deepen in between the outer medulla (arrows) **H & E, X100**

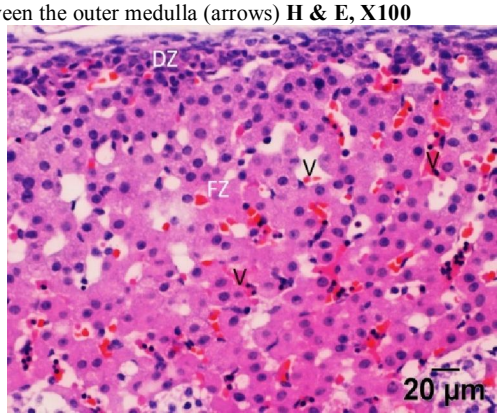

Fig. 2: A higher magnification of the previous one, shows: definitive zone (DZ), and fetal zone (FZ). The DZ forms a small subcapsular band of closely packed eosinophilic cells with large dark nuclei. The FZ are much wider than the DZ forming the main bulk of the gland. The FZ cells are arranged in branching cords separated by blood vessels (V). the FZ cells are large, eosinophilic cells with oval to rounded nuclei. **H & E, X200**

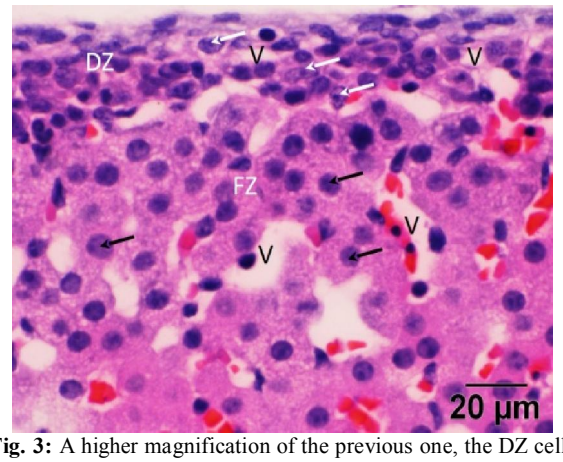

Fig. 3: A higher magnification of the previous one, the DZ cells are packed together and tend to form glomeruli or arches of cells separated by blood vessels (V). They are small cells with vesicular nuclei and surrounded with small rim eosinophilic cytoplasm (white arrows). The FZ cells are eosinophilic, foamy cytoplasm and vesicular nuclei (black arrows). They arranged in cords separated with blood vessels (V). **H & E, X400**

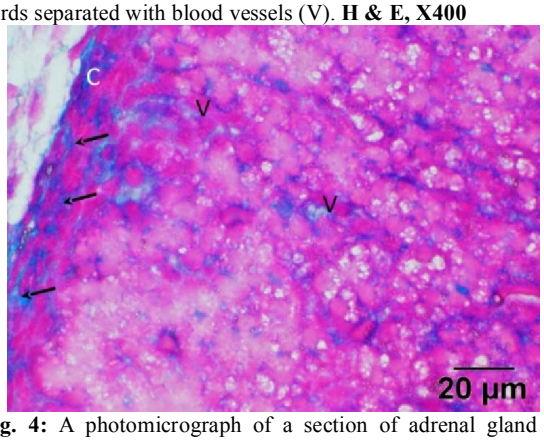

Fig. 4: A photomicrograph of a section of adrenal gland of control rat fetus aged 20 days of gestation, shows: thick capsule (C) with collagenous fibrous (arrows) that fill the whole thickness of the capsule. Minimal parenchymatous collagenous fibers around the blood vessels (V). **Masson's trichrome, X400**

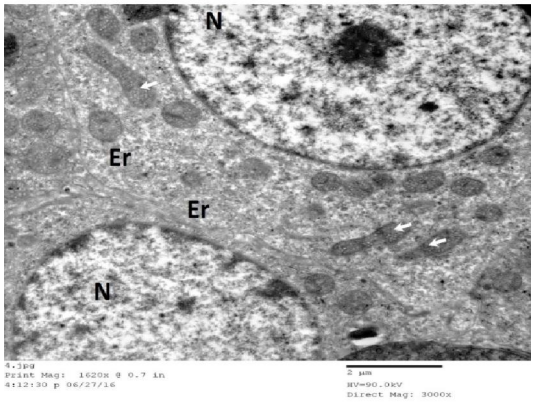

Fig. 5: Transmission electron micrograph of adrenal gland of fetus aged 20 days of gestation of a control mother, shows: cortical cells of DZ show nucleus (N) with electron-dense chromatin, numerous elongated mitochondria (white arrows) with vesicular cristae. Abundant endoplasmic reticulum (Er) are distributed throughout the cytoplasm. **EM, X1200**

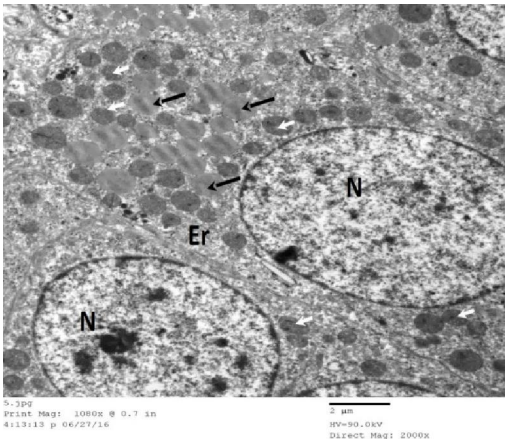

Fig. 6: Transmission electron micrograph of adrenal gland of a control fetus aged 20 days of gestation, shows: cortical cells of FZ show nucleus (N) with electron-dense chromatin, numerous oval to rounded mitochondria (white arrows) with dense matrix. Variable sized lipid droplet (black arrows) with homogenous electron dens matrix. Abundant endoplasmic reticulum (Er) are distributed throughout the cytoplasm. **EM, X2000**

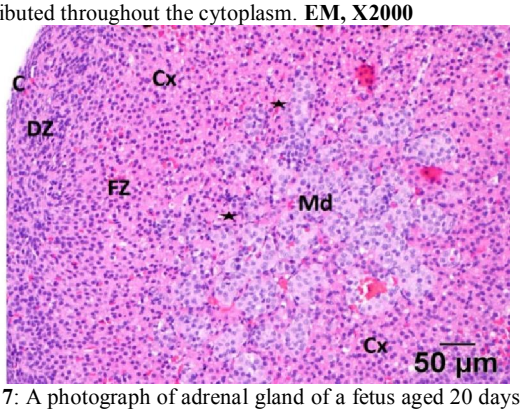

Fig. 7: A photograph of adrenal gland of a fetus aged 20 days of gestations of Treated group, shows: outer capsule (C). The cortex (Cx) surrounds a central medulla (Md). Increase thickness of DZ with disturbed arrangement of its cells. Loss of normal arrangement of the FZ cells. Columns formed by the cortical cells (stars) are seen in between the medullary cells **H & E, X100**

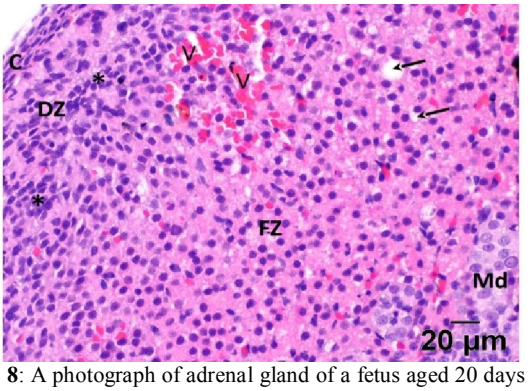

Fig. 8: A photograph of adrenal gland of a fetus aged 20 days of gestations of Treated group, shows: outer capsule (C). Areas of degenerations in the form of variable sized microcyst-like spaces (arrows) within the FZ. Areas of overcrowding of cells of the Dz (*). The medulla (Md) formed of group of basophilic cells with large oval nuclei. There is area of congested blood vessels with RBCs (V). **H & E, X200**

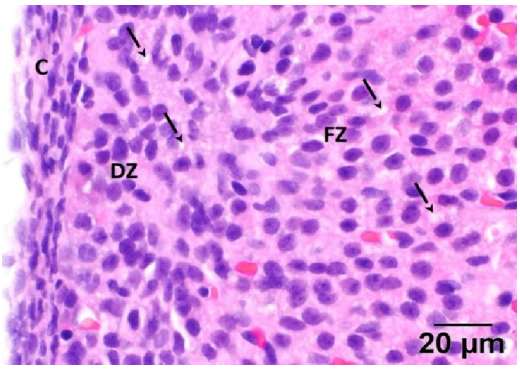

Fig. 9: A photograph of adrenal gland of fetus aged 20 days of gestation of Treated group, shows: Disturbed architecture of the adrenal gland. DZ cells shows Large number of cells with dark nuclei and eosinophilic cytoplasm. Few cells of DZ contains few vacuoles of variable sizes (arrows). FZ cells are large eosinophilic cytoplasm with large nuclei, some are dark and the other are light stained. Most of cells of FZ shows variable size vacuoles (arrows). **H & E, X400**

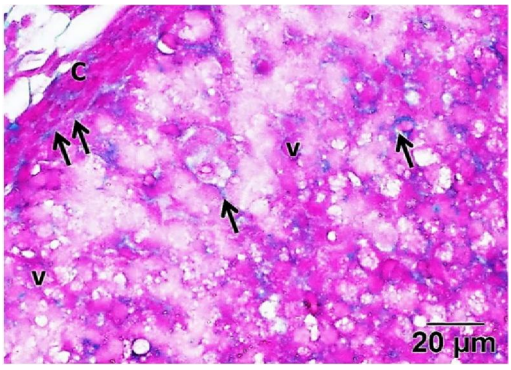

Fig. 10: A photomicrograph of adrenal gland of a fetus aged 20 days of gestation of a Treated group, shows: little amount of collagen fibers (arrows) within the capsule (C) and around the parenchymatous of blood vessels (V) **Masson's trichrome, X400**

Prenatal treated group (Aged 20 days):

Light microscopic examinations of gland of rat fetus aged 20 days of gestation of Treated group reveal an outer relatively thick capsule surrounded the gland (Fig. 7 & 8). The capsule is composed of cells and fibers with an apparent increase of cellular elements (Fig. 8 & 9). The cells are eosinophilic with elongated dark nuclei (Fig. 9). The collagenous fibers are distribution through the capsular and stromal layers are minimal (figs 10). The cortex surrounds the medulla and shows the DZ and FZ with poorly demarcation in between (Fig. 7). There is loss of the arrangement of the cortical cells in both the DZ and the FZ in comparison with the controls. Some areas of the DZ shows overcrowding of cells (which extended to the FZ) more than the others (Figs7 & 8). The FZ are seen with scattered microcyte-like spaces (Fig. 8). The FZ shows small areas without nuclei and appear as an eosinophilic mass that show tiny vacuoles. Cells with dark stained nuclei and cells with light stained nuclei are observed in the FZ with apparent predominance of the dark cells (Fig 9). The blood sinusoids are scattered in between the adrenocortical cells throughout the gland. The medullary cells are small light basophilic cells with dark basophilic nuclei. They are arranged in small groups or nests around blood sinusoid (fig. 8). **By electron microscope** the cortex shows multiple necrotic cells. Cells of DZ show multiple cysts-vesicles of the dilated endoplasmic reticulum, degenerated mitochondria and few myelin like figures, and lipid droplets. Their nuclei are large, oval with dispersed chromatin (Fig 11). Cells of FZ show oval central nuclei with peripheral dispersed chromatin. Their cytoplasm shows multiple mitochondria with electron-dens matrix, few variable sized lipid droplets, vesicles of degenerated endoplasmic reticulum (fig. 12).

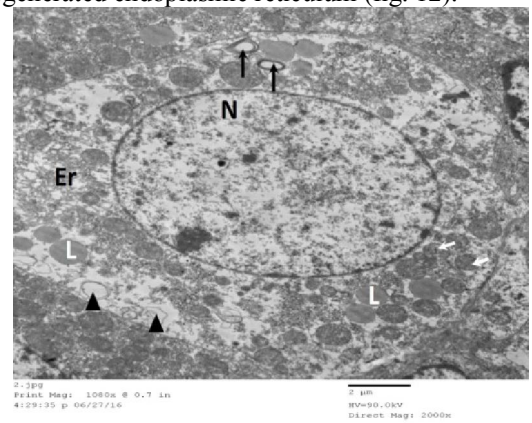

Fig. 11: Transmission electron micrograph of adrenal gland of a fetus aged 20 days of gestation of a Treated group shows: cortical cells of DZ with degenerated cytoplasmic organelles, myelin figures (black arrows), Few lipid droplets (L), mitochondria (white arrows), vesicles of degenerated endoplasmic reticulum (arrowheads). Large oval nucleus (N) with dispersed chromatin. **EM, X2000**

Postnatal control group (Aged 14 days):

Llight microscopic examinations of suprarenal gland of control group aged 14 days old, reveal well-developed an outer connective tissue capsule is formed of connective tissue cells and fibers with predominance of the cells (Figs. 13, 15 and 16). The collagenous fibers layers are seen well-formed and scattered throughout the capsular (Figs 17 & 76). The stromal collagenous fibers are seen well-formed and surrounding the parenchymatous blood vessels (Fig 76).

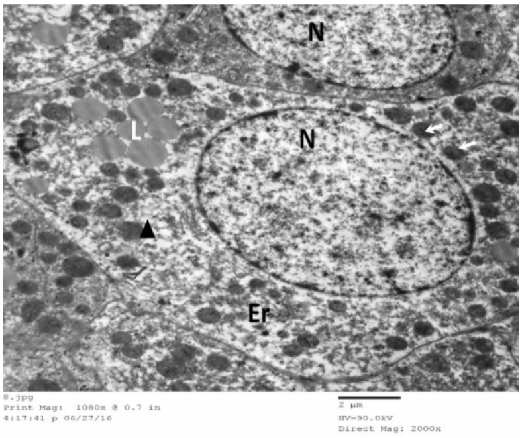

Fig. 12: Transmission electron micrograph of adrenal gland of a fetus aged 20 days of gestation of a Treated group shows: cortical cells of FZ with degenerated cytoplasmic organelles, few lipid droplets, cyst-like structure (arrowheads). Mitochondria with electron- dense matrix (white arrows). The nuclei (N) with peripheral dispersed chromatin. **EM, X2000**

The cortex is formed of three concentric layers: Zg, Zf and smaller Zr. The Zg is formed the outer layer of the cortex with relative increase in its width in comparison with the previous ages. The Zg cells are arranged in two layers outer subcapsular and inner layer adjacent to the Zf. They are Polygonal cells with darkly stained nuclei and vacuolated cytoplasm. The Zf is the largest one of the three zones. Its cells are arranged in parallel cords with blood sinusoids in between. The Zf cells are polygonal with central vesicular nuclei and eosinophilic vacuolated cytoplasm. The Zr is the smaller inner layer of the cortex that surrounds the medulla. The Zr is missed in some areas. Its cells are arranged in reticular cords. The dark and light cells are differentiated with predominance of the dark cells (Figs. 14 & 16). The medulla is distinct from the cortex and apparently more developed than the previous ages. The medullary cells are small eosinophilic cells with small vesicular nuclei that show dark and light staining affinity with predominance of the dark cells. They are arranged in groups and Blood vessels form a network of blood sinusoids and veins in between the medullary cells (Fig 15). **By electron microscope:** The cortical cells of Zg of the control groups show large nucleus with peripheral dispersed chromatin and two nucleoli. The lipid droplets are many and variable sizes with moderate electron density. The mitochondria are multiple and oval to rounded in shape. Endoplasmic reticulum is multiple (Fig. 18). The Zf cell is larger than that of the Zg and shows oval nuclei with peripheral dispersed chromatin and two nucleoli. The lipid droplets are multiple with moderate electron densities. Mitochondria are multiple and of variable sizes & shape (Fig 19).

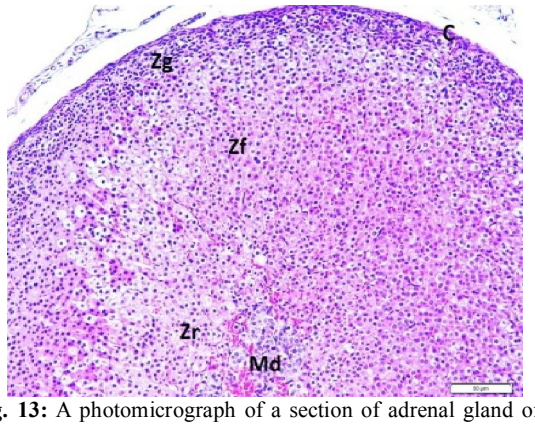

Fig. 13: A photomicrograph of a section of adrenal gland of a rat pup aged 14 days of a control mother, shows: outer connective tissue capsule (C). medulla (Md) is centralized and surrounded with cortex (Cx). The cortex is composed of ill-distinct three zones: zona glomerulosa (Zg), zona fasciculata (Zf) and zona reticularis (Zr). **H & E, X100**

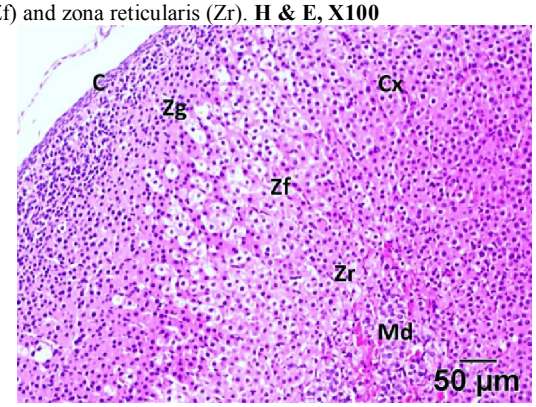

Fig. 14: A photomicrograph of a section of adrenal gland of a rat pup aged 14 days of a control mother, shows: outer connective tissue capsule (C). medulla (Md) is centralized and is surrounded by the cortex (Cx). The cortex is composed of ill-distinct three zones: zona glomerulosa (Zg), zona fasciculata (Zf) and zona reticularis (Zr). **H & E, X200**

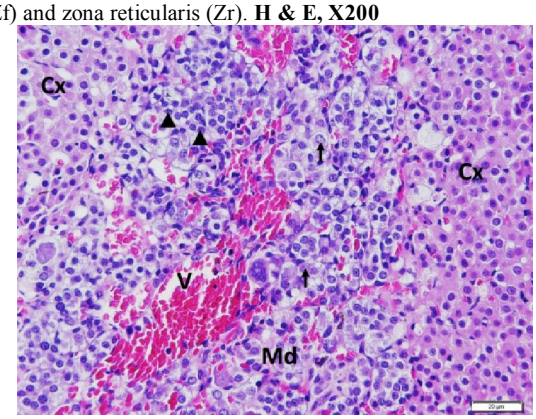

Fig. 15: A photomicrograph of a section of adrenal gland of a rat pup aged 14 days of a control mother, shows: medulla (Md) is centralized and surrounded with cortex (Cx). The medullary cells arranged in globules and their nuclei are vesicular and show both dark (arrowheads) and light (arrows) staining affinity with predominance of dark cells. Blood vessels (V) are wide and filled with RBCs. **H & E, X200**

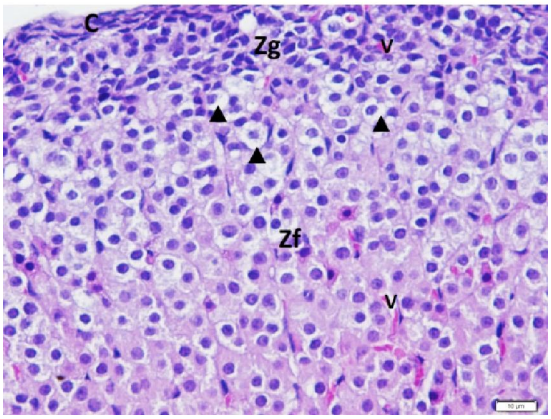

Fig. 16: A photomicrograph of a section of adrenal gland of a rat pup aged 14 days of a control mother; shows: increase width of Zg, the Zg cells are arranged into two layers outer subcapsular and inner layer. The cell cords of Zf run away from the capsule (C). The junctional zone between Zg and Zf shows many cells with large cytoplasmic vacuole (arrowheads). The blood vessels (V) run in between the Zg cells and cords of Zf. **H & E, X400**

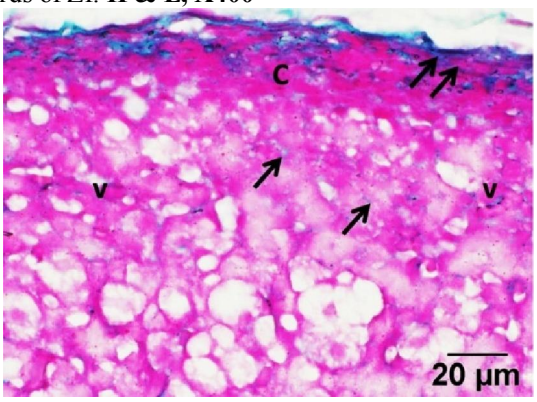

Fig. 17: A photomicrograph of a section of adrenal gland of a rat pup aged 14 days of a control mother; shows: Increase amount of collagen (double arrows) within the structure of the capsule (C). Scattered amount of collagenous fibers (arrows) within the parenchymal blood vessels (v). **Masson's trichrome, X400**

Postnatal treated group (Aged 14 days):

light microscopic examinations of suprarenal gland treated group aged 14 days old, reveal an outer connective tissue capsule is formed of connective tissue cells and fibers with predominance of the cells (Figs. 20, 21 and 22). The collagenous fibers are distributed within the capsule (especially its outer part). It is minimal within the gland and around the blood vessels (Figs 23 & 83). The cortex is well distinct from the medulla and is formed of three zones: Zg, Zf and smaller Zr. The Zg is formed of the outer layer of the cortex. The Zg cells are arranged in two layers outer subcapsular and inner layer adjacent to the Zf. They are polygonal cells with darkly stained nuclei and vacuolated cytoplasm. The width of Zf is the largest one of the three zones. Its cells are arranged in parallel cords with one or two cell-thick which are separated by blood sinusoids. The Zf cells are polygonal to oval with central vesicular nuclei and eosinophilic vacuolated cytoplasm. The Zr is the smaller inner layer of the cortex that surrounds the medulla. The Zr is missed in some areas. Its cells are arranged in reticular cords. The dark and light cells are differentiated with predominance of the dark cells (Figs. 21 & 22).

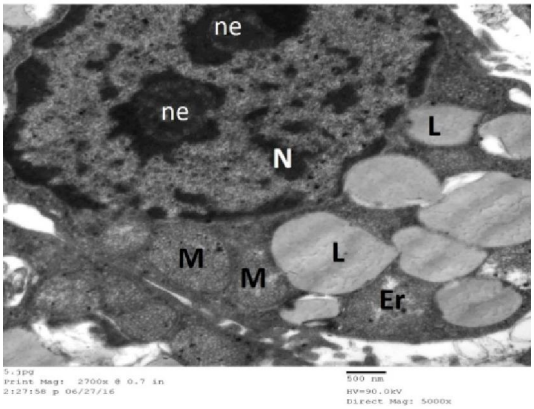

Fig. 18: A Transmission electron micrograph of adrenal cortex of a rat pup aged 14 days of a control mother, shows: cortical cell of zona glomeruloza shows: large central nucleus (N) with peripheral dispersed chromatin and two nucleoli (ne). Lipid droplets (L) of variable size, Mitochondria (M) and endoplasmic reticulum (Er). **EM, X5000**

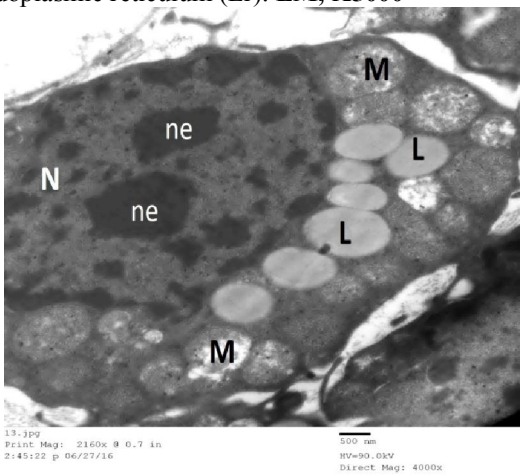

Fig. 19: A Transmission electron micrograph of adrenal cortex of a rat pup aged 14 days of a control mother, shows: cortical cell of zona fasciculata shows: large nucleus (N) with dispersed chromatin and two nucleoli (ne). Lipid droplets (L) of variable size, Mitochondria (M). **EM, X4000**

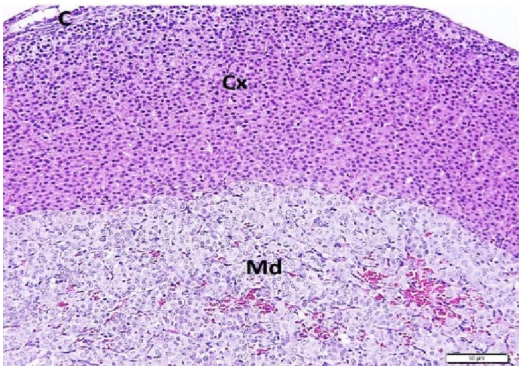

Fig. 20: A photomicrograph of a section of adrenal gland of a rat pup aged 14 days of a Treated group; shows: outer connective tissue capsule (c), cortex (Cx) and medulla (Md). **H & E, X100**

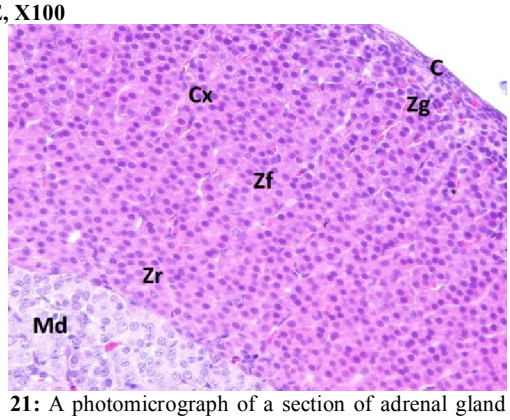

Fig. 21: A photomicrograph of a section of adrenal gland of a rat pup aged 14 days of a Treated group; shows: outer connective tissue capsule (c), zona glomerulosa (Zg) forms a small band of cells underneath the capsule, zona fasciculata (Zf) forms the large band of the cortex, zona reticularis (Zr) forms a small band around the medulla (Md). **H & E, X200**

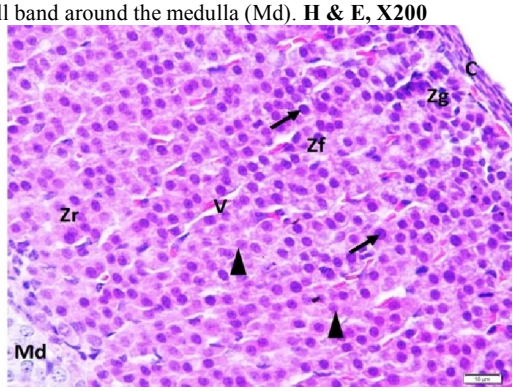

Fig. 22: A photomicrograph of a section of adrenal gland of a rat pup aged 14 days of Treated group; shows: capsule (C) with connective tissue cells and fibers. zona glomerulosa (Zg) formed of eosinophilic cells with central dark nuclei. Zona fasciculata (Zf) shows eosinophilic cells with central rounded or oval vesicular nuclei and vacuolated cytoplasm. These cells arranged in cords separated with blood sinusoids (v). The zona reticularis (Zr) is the smaller and deepest layer of the cortex. The medulla is formed of group of small cells with vesicular nuclei and granular cytoplasm. These cells are grouped together on reticular background and separated with blood sinusoids (v). Note, dark cells (arrow) and light cells (arrowheads). **H & E, X400**

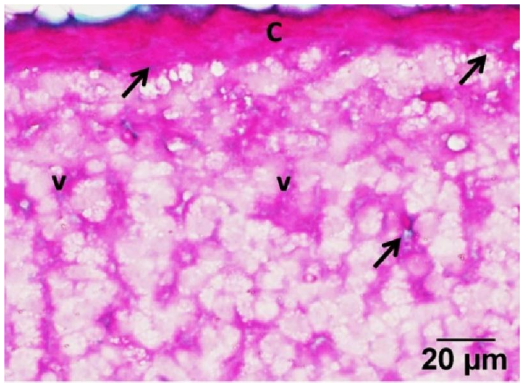

Fig. 23: A photomicrograph of a section of adrenal gland of a rat pup 14 days of Treated group; shows: collagen fibers (arrows) distribution within the capsule (C) and around the parenchymatous blood vessels (v). **Masson's trichrome, X400**

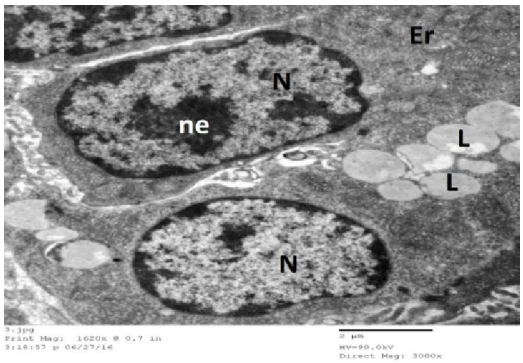

Fig. 24: A Transmission electron micrograph of adrenal cortex of a rat pup aged 14 days, of a Treated group, shows: cortical cells of zona glomerulosa with a nucleus (N) containing peripheral dispersed chromatin and nucleoli (ne). Lipid droplets (L) are few in numbers and variable sizes. Endoplasmic reticulum (Er) is multiple. **EM, X1500**

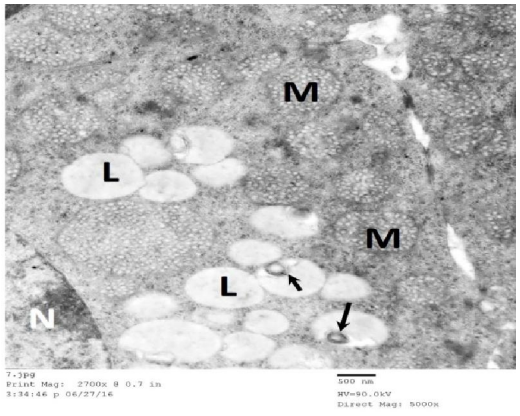

Fig. 25: A Transmission electron micrograph of adrenal cortex of a rat pup aged 14 days, of a Treated group, shows: cortical cell of zona fasciculata with a nucleus (N), lipid droplet (L) are large and few. Some lipid droplets show myelin figures (arrows). Mitochondria (M) are numerous and vesicular type. Part of the nucleus (N) with peripheral dispersed chromatin. **EM, X5000**

By electron microscope: The cortical cells of the treated group aged 14 days at the junctional zone between the Zg and Zf, reveal variable size of lipid droplets that seen large homogenous moderate electron densities in the apical part of the cells in the Zf and of moderate sizes and numbers in the Zg. The nuclei are small and basal with peripheral dispersed chromatin (Fig 24). The cells of Zf show myelin figures within lipid droplets of variable sizes and homogenous mild electron dense. Multiple mitochondria are of variable in shape and size (Fig 25).

4. Discussion:

In this study, by age 20 days of gestation, the control groups showed growing adrenal gland in the form of a relative increase in the thickness of capsule, cortex and medulla. Also, a more demarcation between the cortex and medulla than the previous age. DZ cells arranged in groups underneath the capsule with appearance of dark and light cells. The FZ cells arranged in irregular cords with blood sinusoids in between. Vacuolated cytoplasm (which indicates increase lipid contents) was common within the cells of this zona. the adrenocortical cells were represented more lipid and mitochondrial contents than the previous ages but still the FZ cells showed increase the lipid and mitochondrial contents than that of DZ. The endoplasmic profiles were abundant giving the cells the estrogenic producing-cell profile. These results were in agreement with those of **Matini et al (1999) and Xing et al (2015). Matini et al (1999)** who suggested that in rat, the differentiation between the cortex and medulla occurs around birth. **Xing et al (2015)** encapsulation of the adrenal primordium the mesenchymal cells form the FZ and this was followed by development of the DZ cells under the capsule in the prenatal period, this occurs both in human and rodents (before 16 days of gestation). **Walker and McCormick, (2009)** demonstrated that the onset of differentiation of the adrenal gland into cortex and medulla and the production of the steroidogenic enzymes, responsible for hormonal production, occurs during the fetal life in many Mammals. The secretion of ACTH of the fetal pituitary was essential for the morphological and functional maturation of the adrenal gland both in fetal and postnatal life. In rat, fetal encephalectomy leads to atrophy of the adrenal gland.

In this study, the treated groups showed a decrease of the collagenous fibers with increase the connective tissue cells of the capsule while the stroma connective tissue fibers were minimal. Also, there was a poor demarcation between the cortex and medulla and loss of control distribution of the adrenocortical cells within both Dz and FZ and ill-differentiation between both zones. Appearance of microcyst like spaces and eosinophilic masses within the FZ. Dark and light cell appearance with predominance of the dark cells. The medullary cells arranged in groups in relation to the blood sinusoids. the profile of degenerated cells was affecting both DZ and FZ cells, the degeneration profile includes cyst-like vesicles of degenerated endoplasmic reticulum, degenerated mitochondria, few lipid droplets and myelin like figures. These results were in agreement with that of **Hristic et al (1995); Manojlovic-Stojanoski et al (2006)** who stated that treatment of pregnant rats with Dx, on the day 16 of gestation, affect the proliferation of fetal adrenocortical cells as well as the ACTH cell of the pituitary gland resulting in increased the glucocorticoid level and developmental malformation of the HPA axis. The mechanisms under which these atrophic changes occur, are questionable? They may includea direct effect of the DX and/or interference of the negative feedback mechanism on the ACTH secretion by the pituitary gland. So, the inhibition of growth and loss of architecture of the adrenocortical cells seen in this study were due to cross of the DX to the placenta and incorporation to the fetal blood affecting the hypothalamus-pituitary-adrenal (HPA) axis, stimulation of the catabolic process, suppression of the adrenocortical cell proliferation, stimulation of secretion of the somatostatin hormone according to **Hristic et al (1995) and Kalafatic et al (2000).** DX crosses the placenta and suppresses the secretion of the ACTH from the pituitary gland because of negative feedback mechanism; also, this suppression was followed by stimulation of the proliferation of the ACTH-cells as well as cortisol synthesis and release during the neonatal periods according to **Kalafatic et al (2000)**, **Sangma et al (2008); Kim et al (2009); El-nahla et al (2011); Tischler et al (2014); Xing et al (2015); Vinson (2016) and Pignatti et al (2017).**

In the present study, the control groups at age 14 days old showed a well-developed an outer connective tissue capsule. The connective tissue fibers were well formed within the capsule and around the parenchymatous blood vessels. The cortex was formed of three concentric zones: Zg, Zf and Zr with no anatomical borders in between. There was increase in the thickness of all zones in comparison with the previous ages. The outer Zg was formed polygonal cells with darkly stained nuclei and vacuolated cytoplasm. The cells arranged either in globules and arches. Zf was the largest of the three zones its cells were arranged in parallel cords with blood sinusoids in between. The Zr was the smallest zone; its cells were arranged in reticular pattern. The medulla was distinct and apparently more developed than the previous ages. The medullary cells were small eosinophilic cells with vesicular nuclei with dark and light cell affinity. It was

rich in blood vessels and sinusoids. Zg cells lipid droplets were many, and multiple mitochondria and endoplasmic reticulum. Zf cells were larger than that of the Zg with multiple lipid droplets and mitochondria. **(Yamamoto et al, 1986; Hristic et al, 1995)**.

In this study the treated groups by 14 days old showed well-developed capsule and cortical layers as in control groups. The cells of Zf show myelin figures within the lipid droplets and multiple number of mitochondria. These results were in agreement with that of **Hristic et al (1995)** and **Xu et al (2011). Hristic et al (1995)** studied the effect of dexamethasone on the development of adrenal gland in rat. Marked changes in the histology and physiology of the fetal adrenal gland occurs due to exposure of the pregnant rats to dexamethasone during late pregnancy, where, this period is considered as a critical period for the development of the hypothalamo-pituitary-adrenal system in the fetuses. These changes continue up to 14^{th} day of postnatal life.

The mechanisms by which the Dx exert the previous changes are discussed by **Xu et al (2011)** who evaluated the possible mechanisms of developmental toxicity induced by dexamethasone in mic. They suggested that the administration of dexamethasone to the pregnant mother in late pregnancy induces fetal developmental toxicity. The mechanisms of this fetal toxicity include depression of the adrenal function and placental glucocorticoid barrier.

Moreover, the decrease in the birth weight and programs adult hypertension is induced by injection of dexamethasone (Dx) at the prenatal period in rats. Where, increase of serum Dx (glucocorticoids) concentration inhibits the secretion of corticotropin-releasing hormone (CRH) and ACTH from the hypothalamus and pituitary gland through negative feedback mechanisms. Then, the reduction in the secretion of glucocorticoids is important to maintain the internal environment of the body. Thus, Dx has no direct effect on the steroidogenesis on the adrenal gland **(Powers et al, 2010, Xu et al, 2011).**

Conclusion:

The results of the present study demonstrate that the development of a functioning adrenal gland occurs before 20 days prenatally where the adrenal gland appeared consists of capsule, cortex and medulla. Appearance of the definitive and fetal zones. the cortical cells containing lipid droplets, mitochondria and profiles of steroid secreting cells. This is followed by gradual and steady increase in the volume and numbers of the adrenocortical cells and appearance of well-defined cortical zones, Zg, Zf and Zr on the day 14 after birth. Dx, administered to pregnant mother during the day 16 and 17 (as short course treatment) of pregnancy, crosses the placenta and affects the development of the adrenal gland especially during the perinatal period. Its maximum effects were seen in the 20 days of gestation. These effects include disturbance of the arrangement of cells with focal necrosis and degeneration of the cortical cells. These effects were also, present in the day 14 postnatally, but at the ultrastructure level.

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