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Original Article

A Structural Study on the Effect of Sildenafil Citrate (Viagra) on the Placenta and Fetal Lung Perfusion of Albino Rat with Special Emphasis on its Fetal Safety during Pregnancy: Light and Scanning Electron Microscopic Studies

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ABSTRACT

Background: Sildenafil citrate (Viagra) has been widely used in treatment of erectile dysfunction and thereafter, for the treatment of many vascular diseases as pulmonary arterial hypertension, congestive heart failure and Reynolds's disease. Nowadays, it is introduced in the treatment of wide spectrum of pregnancy-associated diseases as placental blood flow and vascular development are critical to fetal growth and development.

Aim of the Work: To study the effect of Viagra on the structure of midterm and full term placentas, to investigate for possible teratogenic effects and to find out the role of Viagra in fetal lung perfusion in albino rats.

Material and Methods: A total of 24 pregnant albino rats were divided into 4 equal groups, six rats each: control midterm (They received normal saline and were sacrificed on the 13th gestational day), control full term (They received normal saline and were sacrificed on the 21st gestational day), treated midterm (Viagra was administered from the start of pregnancy till the 13th gestational day), and treated full term (Viagra was administered from the start of pregnancy till the 21st gestational day). The Viagra dose was 2.5mg/ Kg body weight dissolved in 5 ml saline given daily by gastric tube. Placentas and fetuses of all groups were obtained and processed for light and scanning electron microscopes studies.

Results: The midterm and full term Sildenafil-treated placentas showed hypervascularity of the basal and labyrinthine zones. Scanning electron microscopy showed presence of congested chorionic villi as well as presence of congested spiral arteries traversing the inter-villous spaces. Multiple sprouts from the spiral arteries were also observed. No apparent external or internal congenital malformations were detected in full term fetuses. Examination of the fetal-lungs of the treated groups revealed apparent increase in the diameter and the branching of the bronchi and bronchioles, decrease in the interstitial stroma with dilatation and congestion of the interstitial vessels.

Conclusion: Sildenafil citrate proved to increase the placental blood flow accompanied with increase in fetal lung perfusion without any apparent fetal teratogenicity.

Key Words: Placenta, Sildenafil (Viagra), pregnant rats, fetal lung perfusion, light microscopy, scanning electron microscopy.

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INTRODUCTION

Sildenafil citrate (Viagra) has been used widely in the last years in treatment of erectile dysfunction from various etiologies *(Medina et al., 2000)*. It selectively inhibits phosphodiesterase type 5 which is responsible for degradation of cyclic guanosine monophosphate (cGMP). During sexual stimulation, the cavernous nerves release nitric oxide (NO), which promotes cyclic guanosine monophosphate (cGMP) formation and smooth muscle relaxation in the corpus cavernosum arterioles. Sildenafil facilitates the erectile process during sexual stimulation via enhancement of the

effect of endogenous nitric oxide and increased levels of cyclic guanosine monophosphate (cGMP) leading to smooth muscle relaxation in the corpus cavernosum arterioles, and increased inflow of blood *(Corbin & Francis, 1999)*. Sildenafil citrate has a proven record of safety in humans as predicted by the pharmacological results in experimental animals; it had no effects on fertility, no genotoxic or carcinogenic effects *(Abbott et al., 2004)*. Viagra was recently used clinically for the treatment of pulmonary hypertension, congestive heart failure and Reynolds's disease *(Huang & De Santis, 2007; Mittleman et al., 2008)*. Viagra proved to be effective in improving pulmonary artery pressure, pulmonary vascular resistance, cardiac index and exercise tolerance in pulmonary arterial hypertension *(Guazzi et al., 2007)*. Moreover, *Momma et al. (2005)* reported that Sildenafil might be useful in treatment of fetal hypoxia, fetal persistent pulmonary hypertension and ductus arteriosus.

Wareing et al. (2005) and *Villanueva-García et al. (2007)* found that Sildenafil citrate improved the endothelial function of myometrial vessels and had a uterine relaxant effect. Therefore, it offered a potential therapeutic strategy in women whose pregnancies were complicated by intrauterine growth restriction and premature deliveries. Intravaginal Sildenafil has been used to improve uterine artery blood flow in patients undergoing in vitro fertilization *(Sher & Fisch, 2000)*. Moreover, intravaginal Sildenafil suppositories proved to be an interesting therapeutic and safe anti-abotive option as it significantly increases endometrial thickness *(Jerzak et al., 2008 & El Far et al., 2009)*.

The placenta is the organ that transports nutrients, respiratory gases, and wastes between the maternal and fetal systems. Consequently, placental blood flow and vascular development are essential components of normal placental function and are critical to fetal growth and development. Therefore, placental blood flow and vascular development are thus potential therapeutic targets in compromised pregnancies *(Reynolds et al., 2006)*. Pre-eclampsia is associated with insufficient adaptations of spiral arteries and is characterized by alterations in endothelial cell function *(Hutchinson et al., 2009)*. Sildenafil citrate relaxes vascular smooth muscle, resulting in modest reduction in blood pressure *(Jackson et al., 2006)*. Recently, Viagra has been used in treatment of pre-eclampsia. It produces a significant and beneficial effect on pregnancy induced vascular adaptation and fetal outcome *(Hutchinson et al., 2009)*. However, despite the several studies that reported the efficacy of Sildenafil citrate in treatment of certain maternal and fetal conditions in gestational periods, no documented histological studies on its effect on the placenta could be found in the literature. So, it became the aim of the present work to primarily study the histological structure of the midterm and full term control placentas to be able to identify the structural effect of Viagra on the midterm and full term placentas, to investigate for possible teratogenicity of the drug. Moreover, to find out the influence of Viagra on fetal lung structure and perfusion in albino rats, using the light and scanning electron microscopes.

MATERIAL AND METHODS

Animals: Twenty- four pregnant female albino rats, aged 4-5 months were used in the present study. Their average weight was 200-250 grams. They were obtained from the Medical Research Center, Faculty of medicine, Ain Shams University. Mating was allowed between adult male and female rats. The females that showed evidence of mating (the presence of vaginal plug, or a positive vaginal smear (showing sperm cells) were chosen. The day on which there was evidence of mating was recorded as day 0 of gestation. During gestation, the female rats were housed individually in plastic cage in the Medical Research Center and maintained on a standard pellet diet and were allowed free access to water.

Drugs and chemicals

Sildenafil citrate (Viagra; Pfizer, New York, NY) was used in a single oral daily dose of 2.5mg/ Kg body weight dissolved in 5 ml saline *(Buhimschi et al., 2004)* and administered by using a gastric tube.

Experimental Design: The pregnant female rats were divided into four groups, six animals each:

- **Group I (control midterm):** Normal saline was administered orally from the start of pregnancy till the 13th gestational day.

- **Group II (control full term):** received normal saline orally from the start of pregnancy till the 21st gestational day.

- **Group III (treated midterm):** Sildenafil Citrate (Viagra) was administered in the same dose and way already mentioned from the start of pregnancy till the 13th gestational day.

- **Group IV (treated full term):** Viagra was also administered in the same dose and way already mentioned but till the 21st gestational day.

All rats were kept under the same circumstances throughout the experiment. The animals were then sacrificed by an over dose of ether anesthesia in the expected date within 3 hours after administration of the last dose of the drug, as the half life of Viagra is 5 hours. The anterior abdominal wall was opened, the uterus was dissected and the placentas were obtained, collected, cross sectioned and processed for light and scanning electron microscopes studies. The fetuses of the different groups were taken and some of them were evaluated carefully by the use of scanning electron microscope for external malformations; some of them were longitudinally dissected to search for internal organ malformations. The fetal lungs were collected and processed for light microscopic study.

Light and scanning electron microscopic studies: Some specimens of the collected placentas& fetuses were fixed immediately in 10% formalin for seven days. They were processed and embedded in paraffin blocks. Serial sections 5 µm thick were sliced and stained with Hematoxylin and Eosin and Orcein stain to stain elastic tissues *(Drury & Wallington, 1980)*. The other specimens were washed twice in sterile phosphate buffer solution PBS. The specimens were then fixed in 1% glutraldehyde, 2% paraformaldehyde in phosphate-buffer at room temperature (pH 7.4) for 24 hours. The specimens were washed twice in buffered sucrose for 5 minutes each (0.1 M phosphate buffer, 5% sucrose solution). Post fixation was performed at 4° C for 60 min in phosphate-buffered 2% osmium. The specimens were dehydrated in a graded series of ethanol's (40, 50, 70, 80, 90 and twice in 100%) after rinsing in several changes in cold distilled water. Then the tissue were further dehydrated in ethanol - acetone (1:1) absolute solution for further 30 minutes, then in absolute acetone 100% for additional 30 minutes 3 times 10 minutes each, then critically point dried in CO2 drying apparatus CPD 030 and mounted on stubs, then coated with gold sputter coater SCD005. The specimens were examined and photographed with Philips Scanning Electron Microscopy XL 3 at 30 kv *(Wahlqvist et al., 1996)*.

RESULTS

Placental results:

Light microscopic examination of the control midterm placenta presented three histological distinct zones: The decidual zone formed of flat or oval cells and homogenous ground substance. The basal zone formed of trophoblasts, giant cells and clusters of vacuolated glycogen cells, all present in between maternal sinusoids (Fig. 1-A). Placental bed giant cells were larger than the adjacent trophoblasts and contained one or more large nuclei enclosed in a cytoplasm containing numerous vacuoles. Glycogen cell nests with small dark nuclei and extensively vacuolated cytoplasm were also observed (Fig. 1-B). The third zone was the labyrinthine zone which is the most extensive part of the placenta. Small islands of glycogen cells were occasionally observed in the labyrinthine zone. The maternal blood lacunae were lined by trophoblasts instead of endothelium i.e. the trophoblasts were bathed directly by maternal blood (Figs. 1-B, 2-A). Fetal capillaries were seen separated from the maternal sinusoids (lacunae) by the placental barrier. The barrier was formed of the endothelium and basement membrane of the fetal blood capillary, fetal mesenchymal tissue of the core of the chorionic villi, cytotrophoblast and syncytiotrophoblast with its fibrinoid covering. The cytotrophoblast consisted of low cuboidal cells separated from each other by well defined cell boundaries. The syncytiotrophoblast was composed of fused cells forming a syncytium (one mass of multinucleated cytoplasm that lacked any cell boundaries). It was covered by a thick extracellular eosinophilic (fibrinoid) coat (Figs. 2-A, B)

Treated midterm placentas showed more affection of the basal zone compared to the labyrinthine one. The basal area appeared studded by the trophoblastic cells which showed darkly stained cytoplasm and small dark nuclei. The

giant cells showed apparent increase in number compared to the control midterm group. Multiple vacuoles were observed in their cytoplasm. Maternal blood sinusoids in basal area appeared dilated and congested (Fig. 3). The labyrinthine area also showed dilated and congested maternal and fetal blood vessels (Fig. 4-A). The cytotrophoblastic layer as well as the extracellular matrix of the placental barrier was absent in some areas and reduced in thickness in other areas of the placental barrier compared to the control group resulting in apparent decrease in the barrier thickness (Fig. 4-B).

Examination of the control full term placenta showed moderate congestion of the blood sinusoids in the basal zones. Scattered vacuolated glycogen cell were also observed in between the basal trophoblasts. Some giant cells were also detected (Figs. 5-A, B). The labyrinthine zone showed congested maternal channels. The layer of the cytotrophoblast was hardly detected while that of the extracellular matrix of the placental barrier was absent. Occasionally, giant cells with large dark nuclei were observed nearby the villi in the labyrinthine zone. They showed degenerative changes characterized by pyknotic and bizarrely shaped nuclei. Their cytoplasm showed multiple vacuoles (Figs. 6-A, B).

Treated full term placenta revealed extensive congestion and dilatation of the maternal blood sinusoids in the basal zone. Glycogen vacuolated cells as well as dark trophoblastic cells with dark nuclei were also seen. Few giant cells were detected (Fig. 7). The labyrinthine zone showed hypervascularity in addition to massive dilatation and congestion of fetal vessels and maternal trophoblastic- lined channels (Figs. 8-A, B).

By Scanning electron microscope, the surface of the control midterm placenta showed chorionic villi covered by closely packed cells which were domed and polygonal in shape. The villous surface showed fine microvilli. Some vessels were seen traversing the surface (Figs. 9, 10).

Examination of the treated midterm placentas showed congested spiral arteries traversing the inter-villous spaces. Multiple sprouts from these arteries were observed (Figs. 11-A, B). Apparent increase in the bulging of the chorionic villi as a result of their marked congestion was noticed. Cut- sectioned villi appeared studded with blood cells (Fig. 12). Pericytes-like cells with their long processes were also observed on the surface (Fig. 13).

The control full term placenta showed well formed surface cotyledons (Fig. 14). Spiral arteries were seen on the surface (Fig. 15).

In the Viagra- treated full term group, the branches of spiral arteries appeared increased in number and diameter with the appearance of multiple sprouts (Figs. 16-A,B).

Fetal Results

Examination of the longitudinal sections of full term fetus of the Viagra- treated group showed no gross anatomical changes. No apparent external or internal congenital malformations were observed. Scanning electron microscope figures were chosen to represent these findings (Figs. 17- A, B).

Concerning the fetal lungs, the control midterm lungs appeared in the canalicular (ductal) stage. Bronchioles of various sizes were seen lined by either ciliated columnar epithelium (i.e. large bronchioles) or by cuboidal one (i.e. small bronchioles). Condensed tissue interstitium and small interstitial vessels were also observed (Figs. 18-A, B). In the midterm- treated group, there was an apparent increase in the branching and diameter of the bronchioles. A decrease in the stromal density in addition to congested and dilated interstitial blood vessels were noticed (Figs. 19-A, B).

In the full term control lungs, the terminal saccular stage of developing lung was detected. In spite of the presence of thick septa between the bronchioles, there was an apparent increase in the bronchiolar diameter compared to the control midterm lung (Figs. 20-A). Orcein- stained sections showed mild congestion of some of the interstitial vessels (Fig. 20-B).

In the full term treated group, an apparent increase in the number of the respiratory bronchioles with an apparent increase in bronchiolar diameters was evident (Fig. 21- A). In the Orcein- stained section, blood vessels appeared dilated and showed marked congestion (Fig. 21-B).

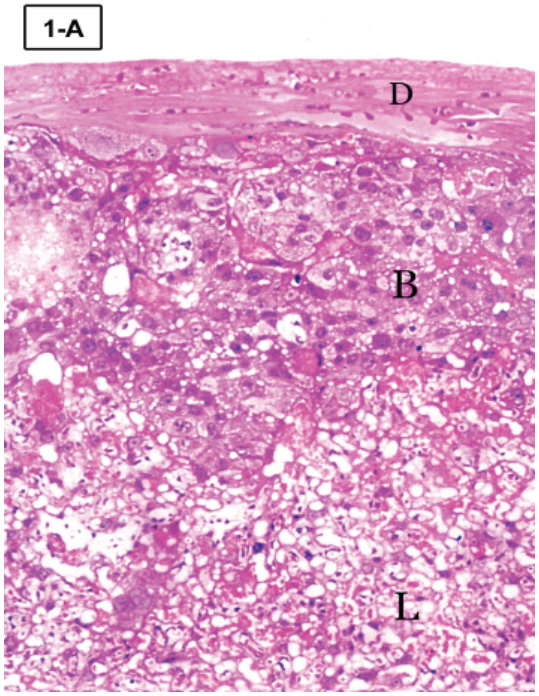

Fig. 1-A: A photomicrograph of a midterm control albino rat placenta showing the different placenta layers which are the decidual layer (D), basal layer (B) and labyrinthine layer (L). Hx.& E.; X40

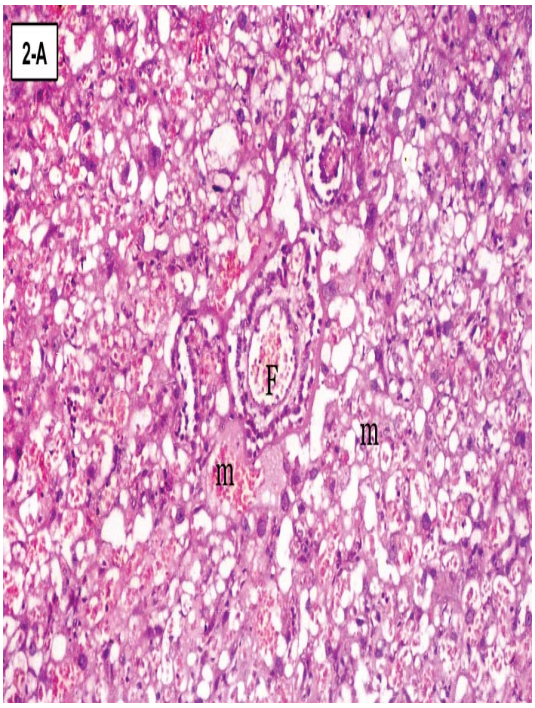

Fig. 2-A: A photomicrograph of a midterm control albino rat placenta showing the labyrinthine area with maternal trophoblastic channels (m) and fetal capillaries (F).Notice the feto-maternal barrier separating them. Hx.& E.; X100

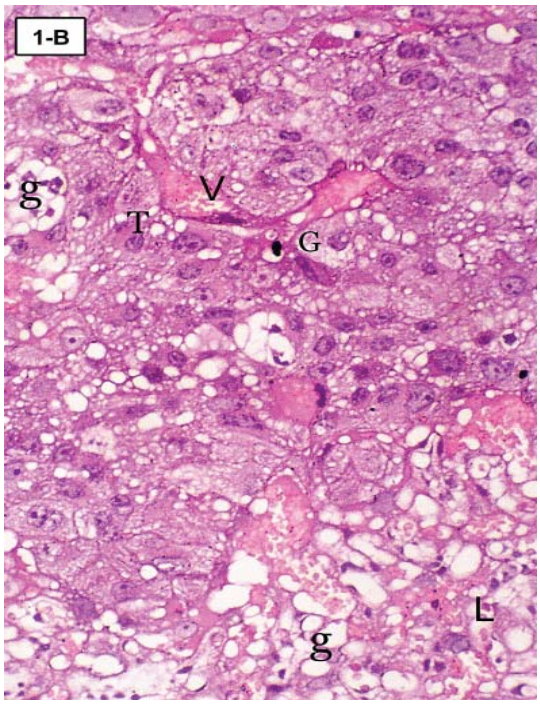

Fig. 1-B: A higher magnification of the previous section showing the different types of cells constituting the basal layer: Trophoblasts (T), vacuolated glycogen cells (g) and giant cells (G). Notice the maternal vessels (V). Note also the presence of some glycogen cells (g) in the labyrinthine layer (L). Hx.& E.; X200

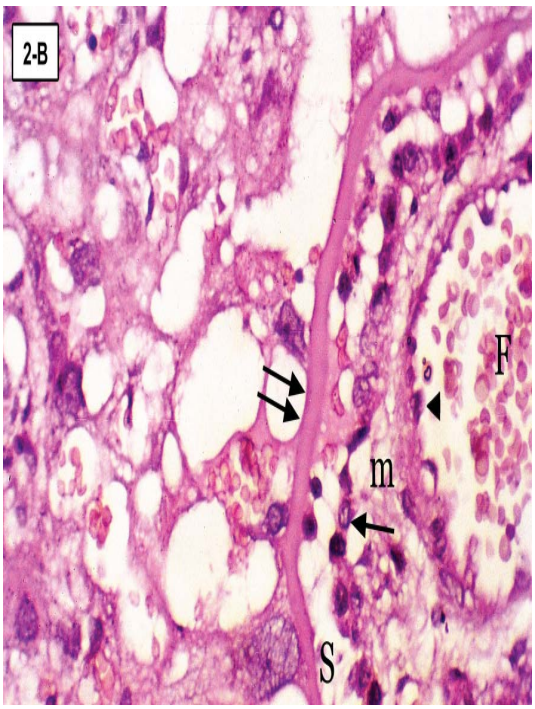

Fig. 2-B: A higher magnification of the previous section showing the layers constituting the placental barrier; endothelium and basement membrane (arrowhead) of the fetal blood capillary(F), mesenchymal core of the villous (m), cytotrophoblast (arrow) and syncytiotrophoblast (S) with its fibrinoid covering (double arrows). Hx.& E.; X400

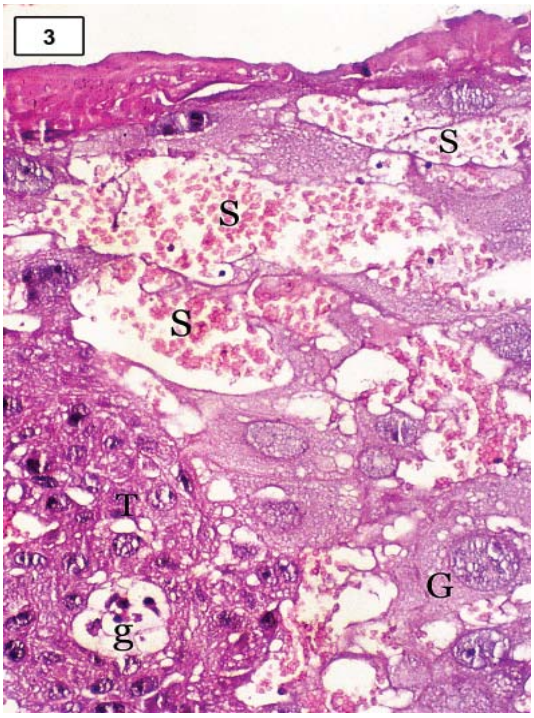

Fig. 3: A photomicrograph of a midterm treated albino rat placenta showing the excessively dilated and congested maternal blood sinusoids (s) in the basal area. Note the dark trophoblastic cells (T) and a glycogen cell nest (g). Notice also the multiple giant cells (G) with multiple cytoplasmic vacuoles. Hx.& E.; X400

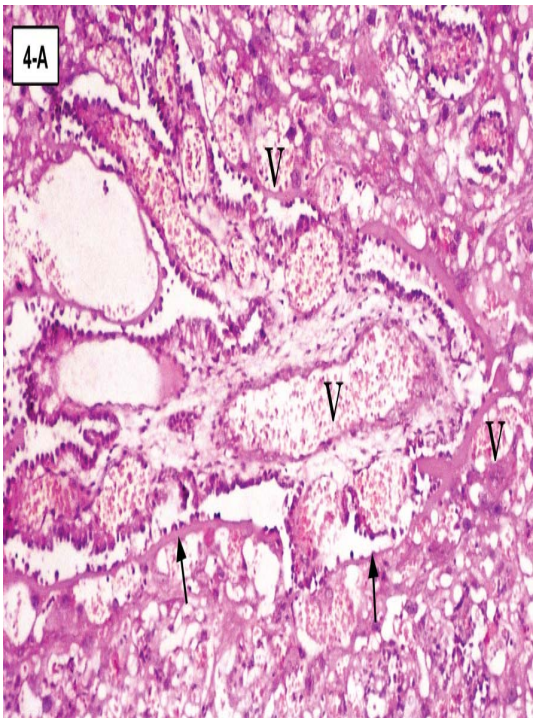

Fig. 4-A: A Photomicrograph of a midterm treated albino rat placenta showing; the dilated and congested maternal and fetal vessels blood (v) in the labyrinthine area. Note the decreased thickness or absence of the fibrinoid covering of the villous in some areas (arrows) of the placental barrier. Hx.& E.; X200

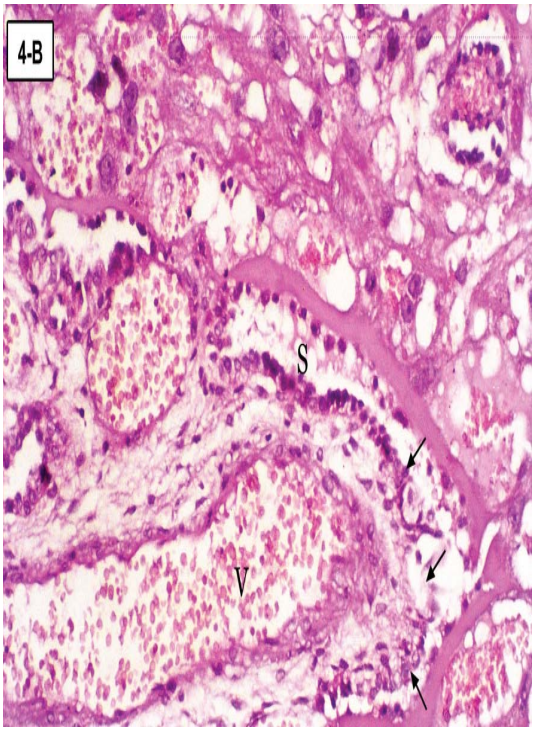

Fig. 4-B: A higher magnification of the previous section showing the layers constituting the placental barrier. Notice the decreased thickness or absence (arrows) of the layer of cytotrophoblast in some areas around the fetal blood vessel (v). Syncytiotrophoblast layer (S). Hx.& E.; X400

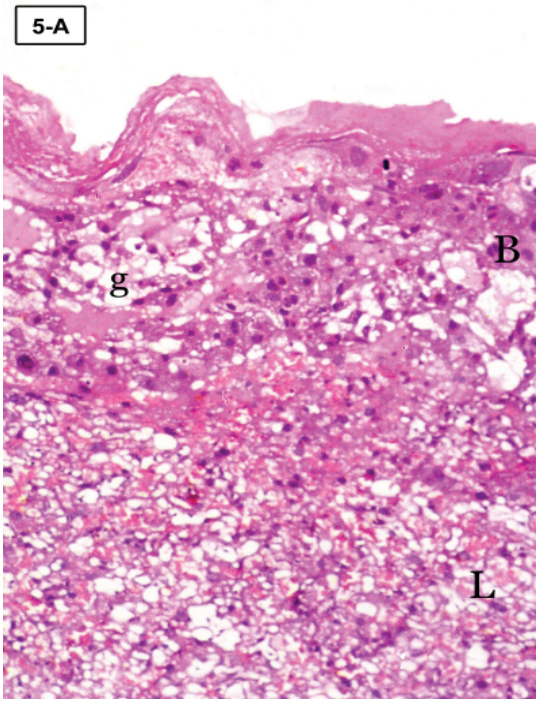

Fig. 5-A: A photomicrograph of a full term control albino rat placenta showing: The basal (B) and the labyrinthine (L) zones. Note the vacuolated glycogen cells (g). Hx.& E.; X200

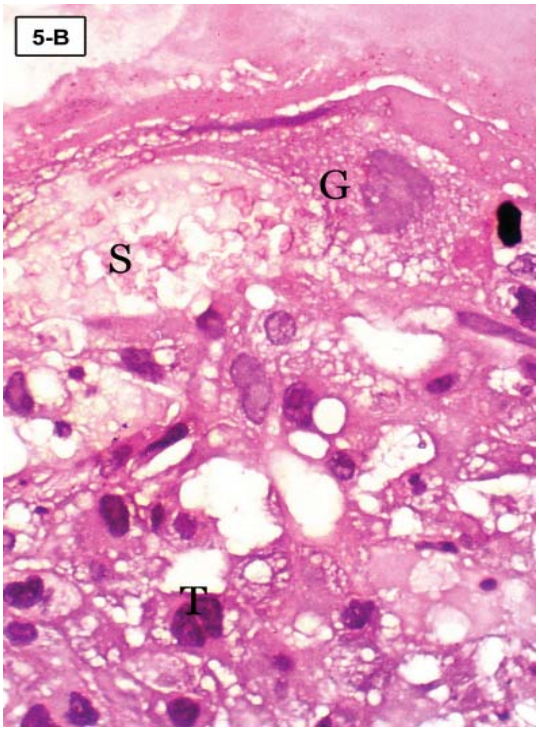

Fig. 5-B: A higher magnification of the previous section showing moderate congestion of the maternal blood sinusoid (s) in the basal area. Note the presence of some giant cells (G) with vacuolated cytoplasm. Notice also the binucleated trophoblast (T). Hx.&E.; X400

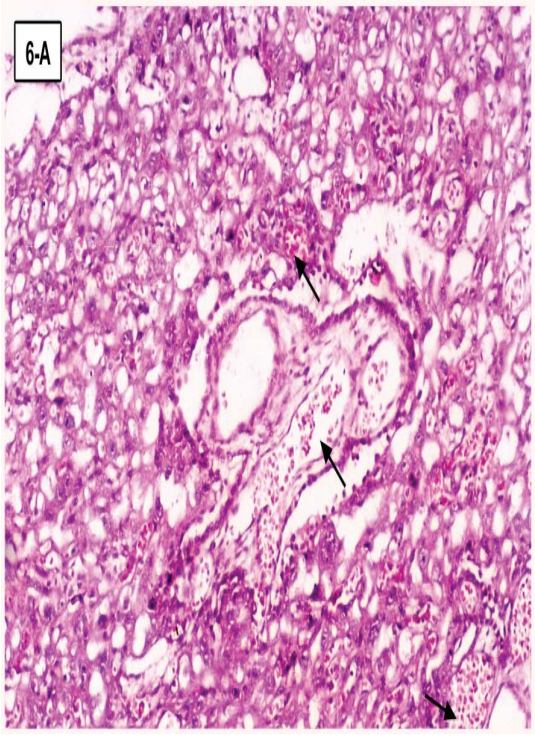

Fig. 6-A: A photomicrograph of a control full term albino rat placenta showing; well developed maternal and fetal channels (arrows) studded with blood vessel within the labyrinthine zone. Hx.&E.; X100

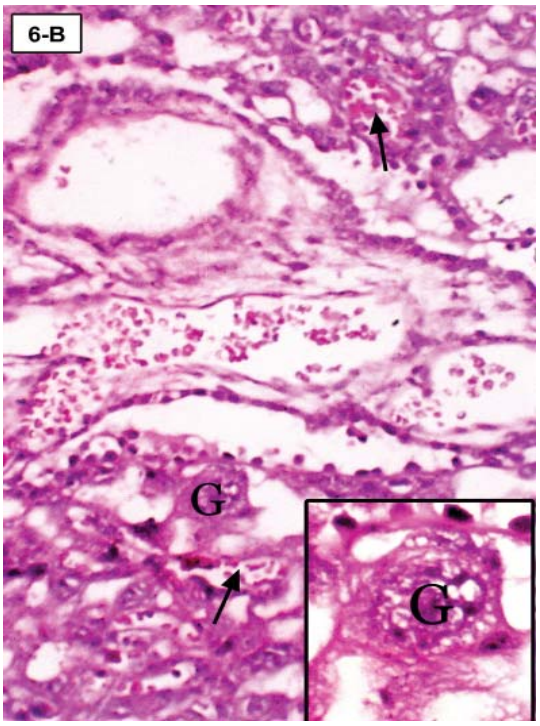

Fig. 6-B: A higher magnification of the previous section showing the absence of the fibrinoid layer of the placental barrier. Note the congested maternal channels (arrows). Notice giant cell (G) with large dark nucleus nearby the villous. The inset: High magnification showing the giant cell (G). Hx.& E.; X200 (Inset; X400)

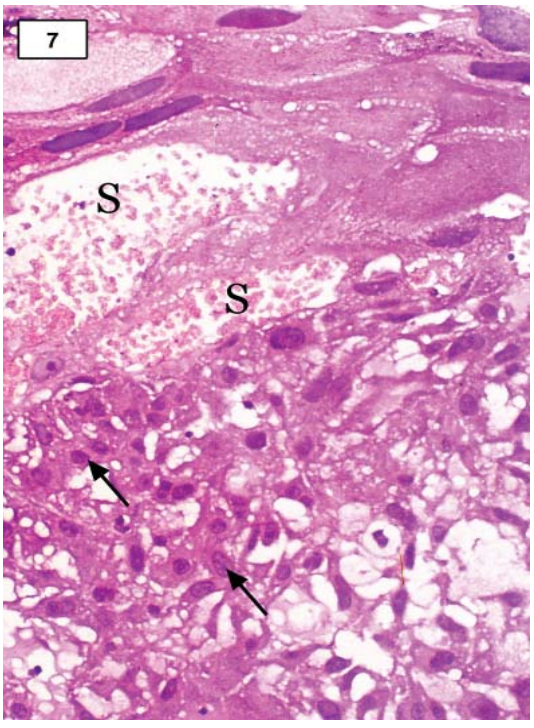

Fig. 7: A photomicrograph of a treated full term albino rat placenta showing the marked congestion and dilatation of the maternal blood sinusoids (S) in the basal zone. Note the presence of some dark trophoblasts (arrows). Hx.& E.; X400

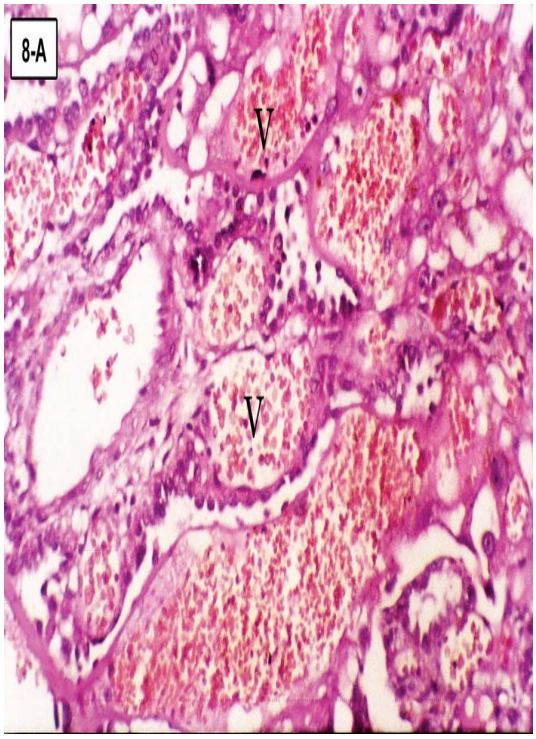

Fig. 8-A: A photomicrograph of a treated full term albino rat placenta showing congested maternal and fetal channels (v) in the labyrinthine zone. Hx.& E.; X200

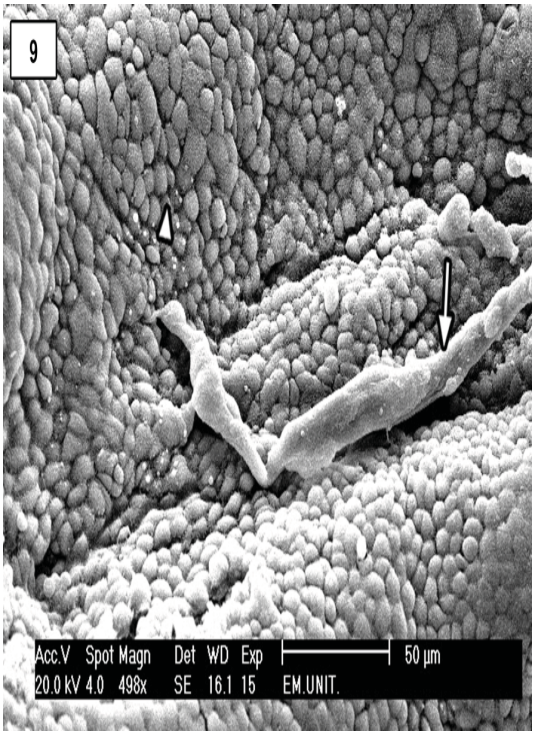

Fig. 9: Scanning electron - micrograph of a control midterm albino rat placenta showing closely packed cells covering the surface of the villi. Note the vessel on the surface (arrow). SEM; X498

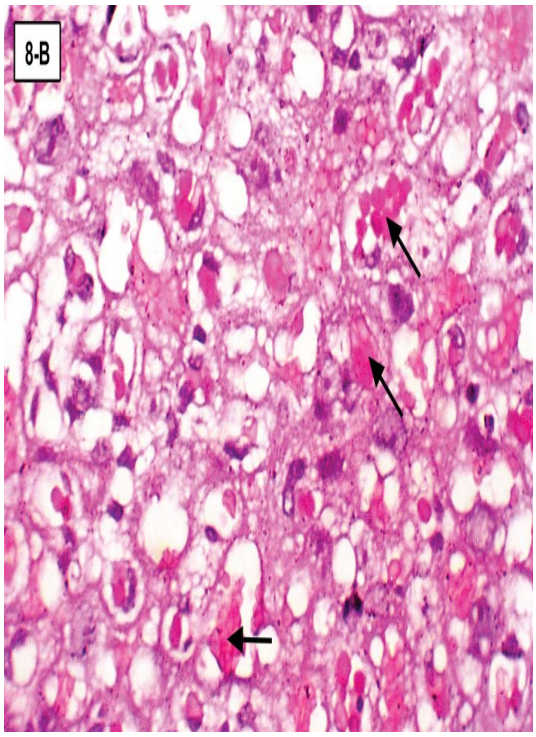

Fig. 8-B: A photomicrograph of a treated full term albino rat placenta showing congested trophoblast- lined maternal channels (arrows). Hx.& E.; X400

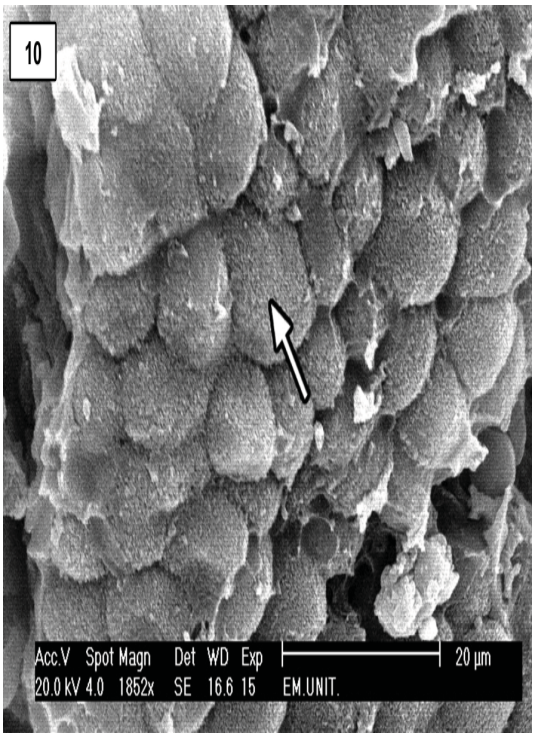

Fig. 10: Scanning electron - micrograph of a control midterm albino rat placenta showing the domed polygonal cells (arrow) with fine microvilli covering the surface. SEM; X1852

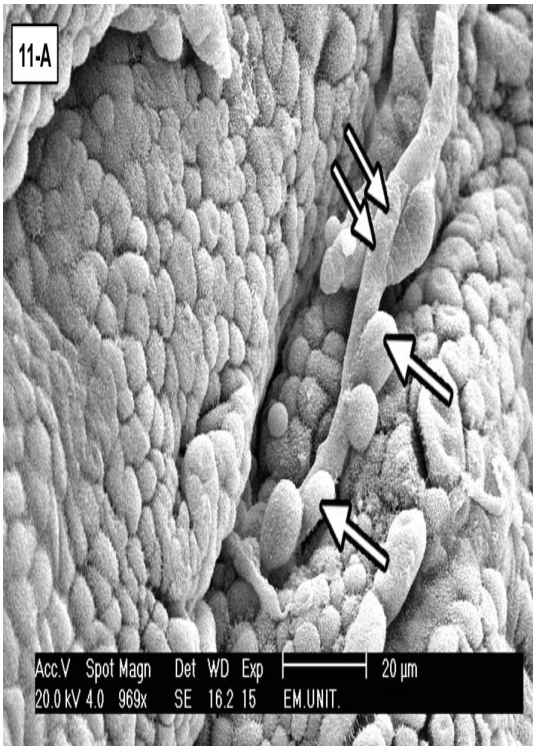

Fig. 11-A: Scanning electron - micrograph of a treated midterm albino rat placenta showing; congested spiral artery (double arrows) traversing the inter-villous space. Notice the presence of multiple sprouts (arrows) arising from the artery. SEM; X969

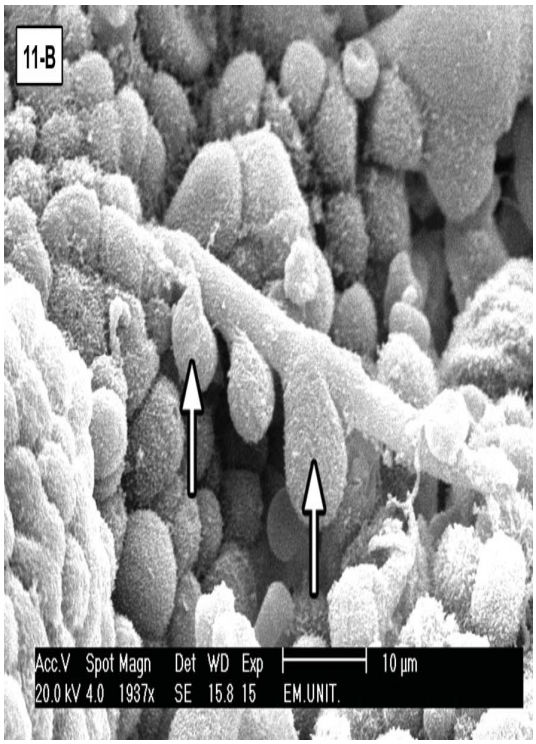

Fig. 11-B: Scanning electron - micrograph of a treated midterm albino rat placenta showing; multiple sprouts (arrow) arising from the artery. SEM; X1937

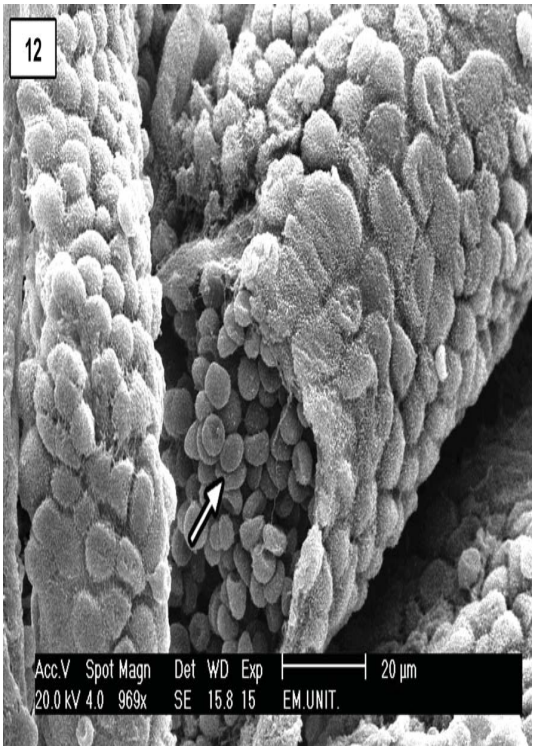

Fig. 12: Scanning electron - micrograph of a treated midterm albino rat placenta showing a villous studded with blood cells (arrow). SEM X969

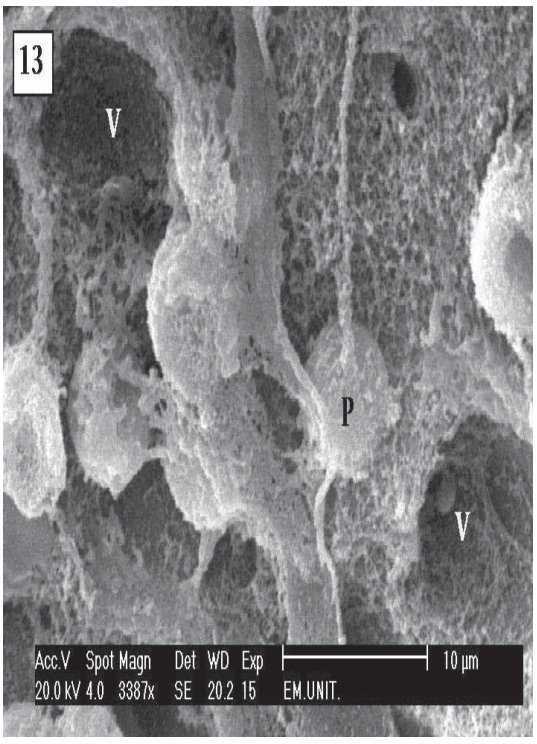

Fig. 13: Scanning electron - micrograph of a treated midterm albino rat placenta showing pericyte- like cells (P) with their long processes. Blood vessels (V). SEM; X3387

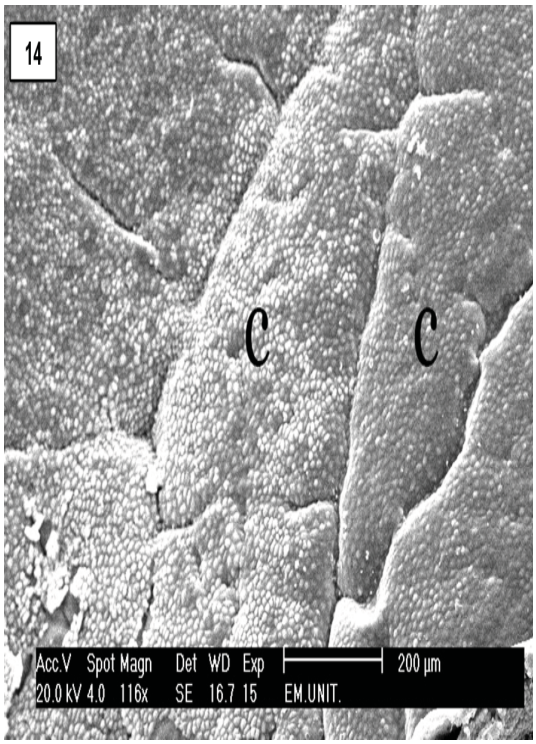

Fig. 14: Scanning electron - micrograph of a control full term albino rat placenta showing well formed cotyledons (C). SEM; X116

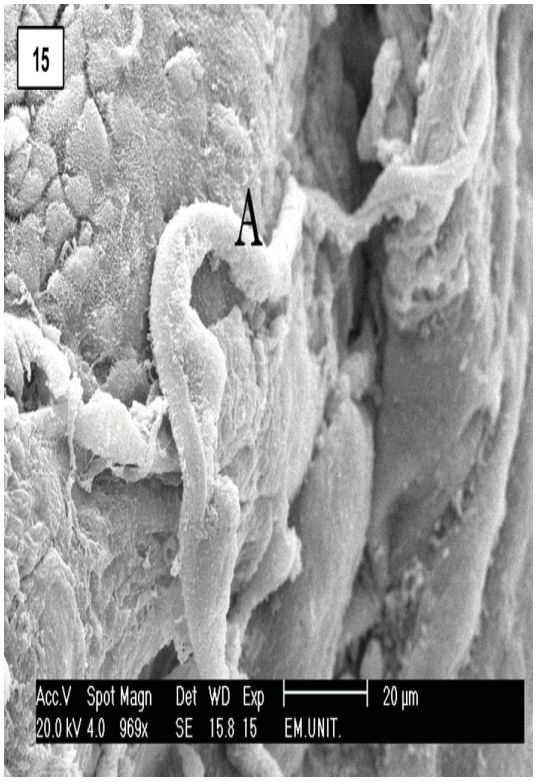

Fig. 15: Scanning electron - micrograph of a section of the placenta of a control full term rat showing tortuous spiral artery (A) traversing the surface. SEM; X969

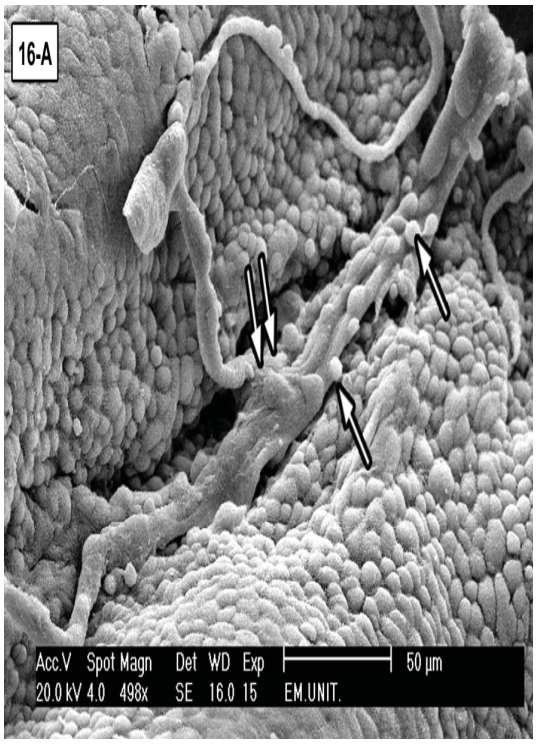

Fig. 16-A: Scanning electron- micrograph of a treated full term albino rat placenta showing: branched and congested spiral artery (double arrows) traversing the inter-villous space. Notice the presence of multiple sprouts (arrows) arising from the artery. SEM; X498

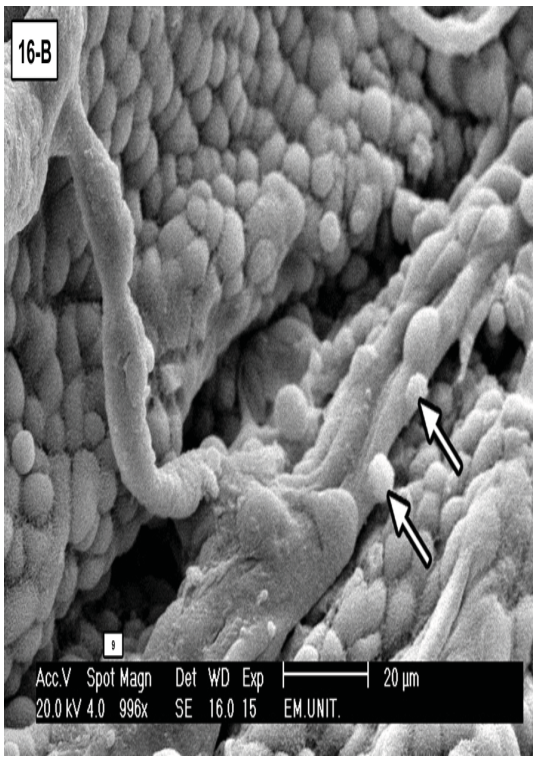

Fig. 16-B: A higher magnification of the previous section showing multiple sprouts (arrows) arising from the artery. SEM; X996

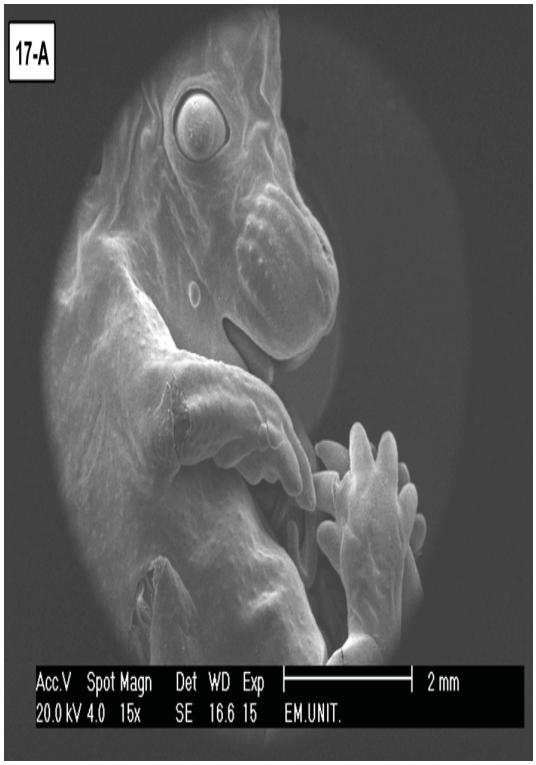

Fig. 17-A: Scanning electron- micrograph of a full term fetus of treated albino rat showing: Absence of any apparent external congenital malformations. SEM; X15

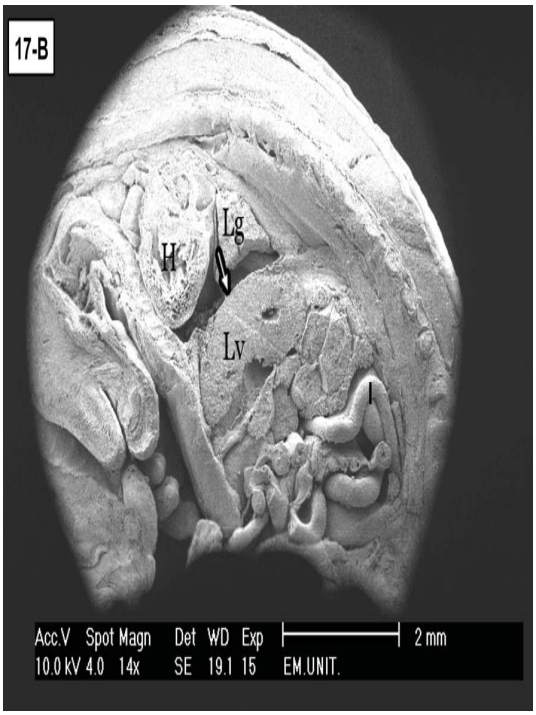

Fig. 17-B: Scanning electron- micrograph of a full term fetus of a treated albino rat showing: Absence of any apparent internal congenital malformations. Note the heart (H), Lung (Lg), diaphragm (arrow), liver (Lv) and intestine (I). SEM; X14

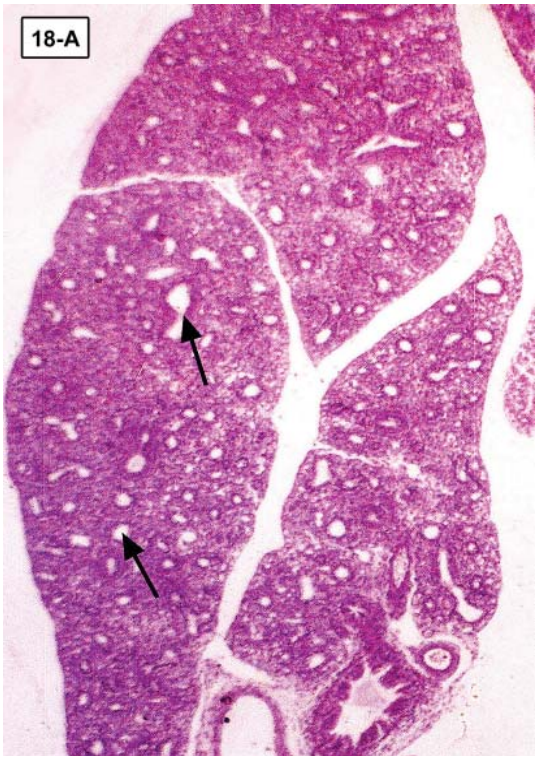

Fig. 18-A: A photomicrograph of a control midterm albino rat lung showing; many bronchioles (arrows) of variable sizes in the lung lobes. Hx.& E.; X40

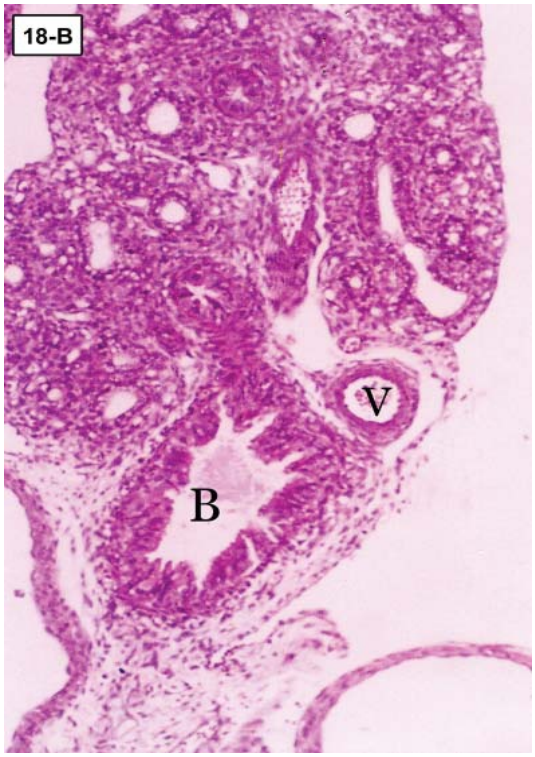

Fig. 18-B: A higher magnification of the previous section showing a large bronchus (B) and the nearby interstitial vessels (v). Hx.& E.; X200

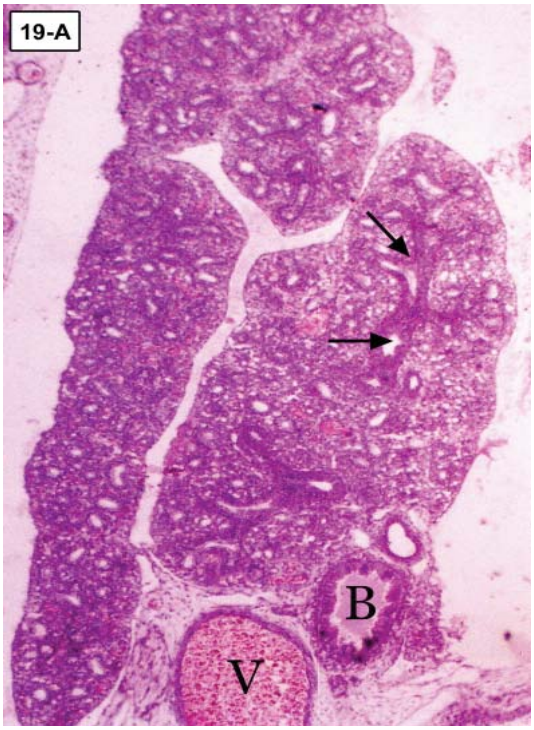

Fig. 19-A: A photomicrographs of a treated midterm albino rat lung showing; apparent increase in the branching bronchioles (arrows) compared to (Fig18-A). Note the congested vessel (V) near the bronchus (B). Hx.& E.; X40

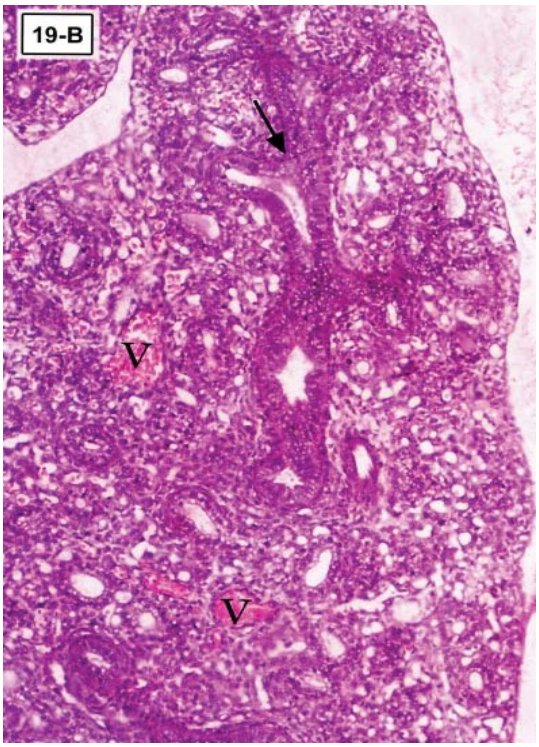

Fig. 19-B: A higher magnification of the previous section showing apparent increase in the branching bronchioles (arrows). Note the congested interstitial vessels (V) and the decreased stromal density. Hx.&E.; X200

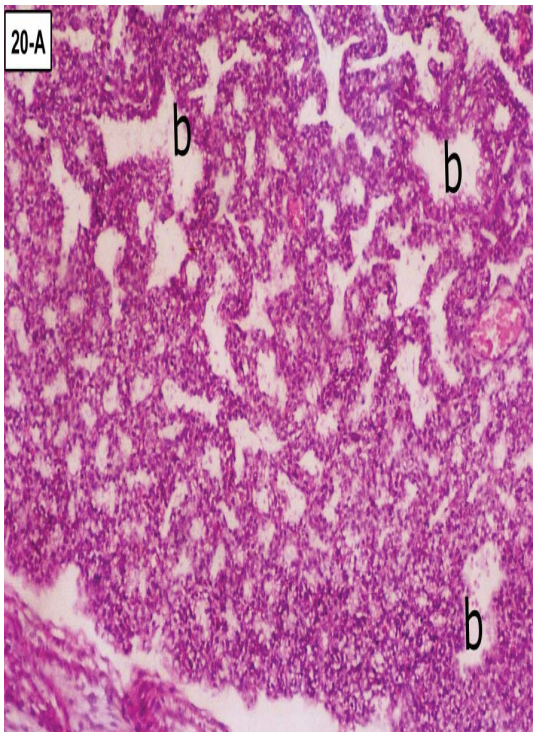

Fig. 20-A: A photomicrograph of a control full term albino rat lung showing; apparent increase in the number and diameter of the bronchioles (b) compared to (Fig.18-B). Hx.& E.; X200

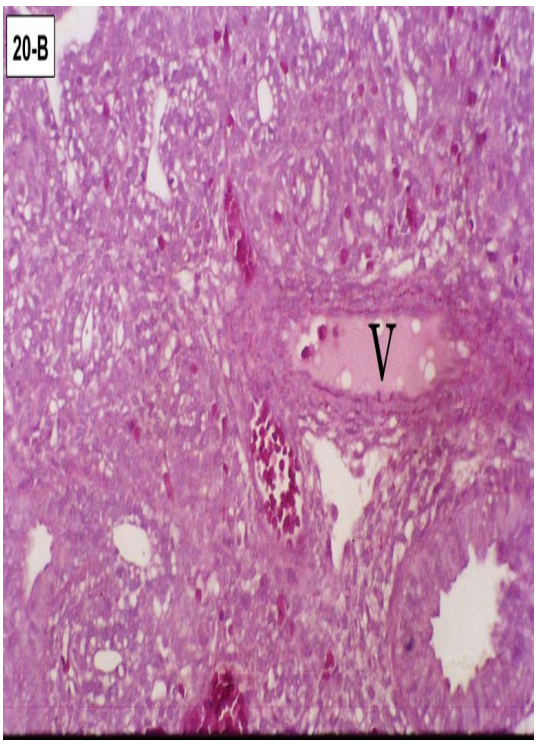

Fig. 20-B: A photomicrograph of a control full term albino rat lung showing; the interstitial blood vessel (v) occupied by plasma with minimal blood cells. Orcein; X200

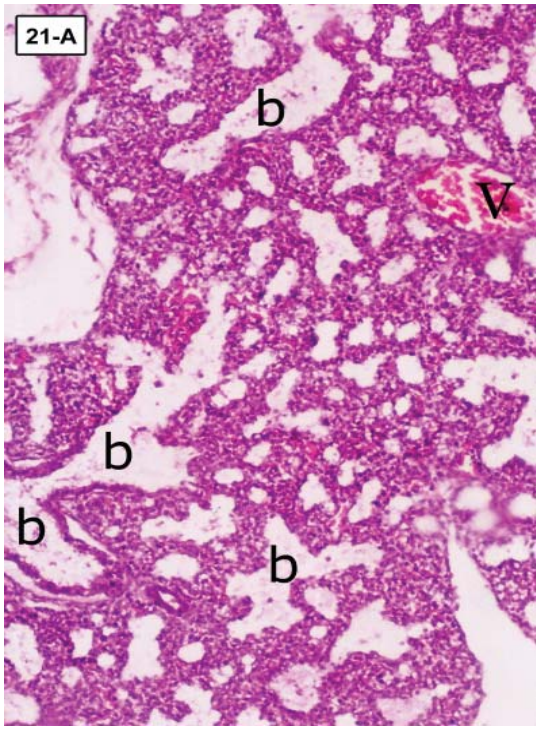

Fig. 21-A: A photomicrograph of a treated full term albino rat lung showing; apparent increase in the diameter and the branching of the bronchioles (b) compared to (Fig.20-A). Note the congested interstitial vessel (v). Hx.& E.; X200

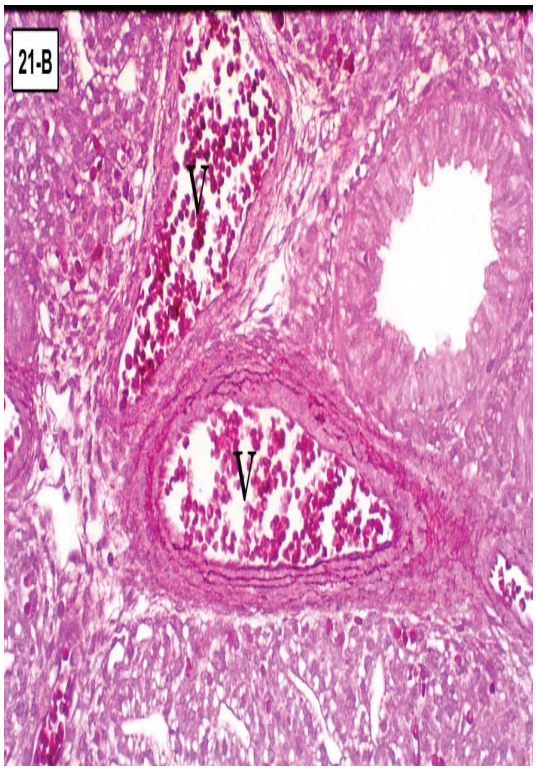

Fig. 21-B: A photomicrograph of a treated full term albino rat lung showing; marked congestion in the dilated interstitial blood vessels (v). Orcein; X200

DISCUSSION

The placenta is the organ through which respiratory gases, nutrients and waste products are exchanged between the maternal and fetal systems. The present work described primarily the normal placental structural growth during gestation. In midterm control placenta, three distinct zones were observed: the decidual, basal and labyrinthine zones. The basal zone showed mildly congested maternal sinusoids in addition to different types of cells: trophoblasts, giant and glycogen cells. The cytoplasm of the giant cells showed numerous vacuoles most probably phagosomes. Giant cells are considered as a sub-population of the extravillous trophoblasts present at the implantation site; their main function could be phagocytosis *(Pijnenborg et al., 1981)*. In early placental development, there are at least two possible fates for the interstitial extravillous trophoblasts: the first fate is trophoblastic migration into the endometrium followed by their apoptosis. The second fate is trophoblastic transformation into isolated masses of syncytium that ensure adequate local production of hormones. These two fates are critical for maintaining a normal pregnancy *(Welsh & Enders, 1987; Al Lamki et al., 1999)*. The full term control placentas showed moderate congestion of the maternal blood sinusoids in the basal zone. The labyrinthine zone also showed congested maternal channels. Apparent thinning of the placental barrier was detected compared to the placenta of midterm fetuses due to the absence of the cytotrophoblastic and the extracellular matrix layers. These observations are consistent with those of *King (1984)* who described the fine structure of the chorionic villi and found that the fetal capillaries with their surrounding trophoblasts were thinner with advancing gestation.

In the present study, scanning electron microscopy examination of the control placentas showed that the surface of the chorionic villi was covered by closely packed cells which were domed and polygonal in shape and their surface was covered by fine microvilli. In accordance with the present findings, *Burton (1986)* reported the presence of intervillous bridges in normal placenta and that microvilli covered the surfaces of these bridges and were identical to the microvilli covering the remainder of the villous surface. Spiral arteries were also seen traversing the intervillous spaces in the present work.

Hirano et al. (2002) reported that the spiral artery (SA) is an important muscular artery that maintains pregnancy through controlling the blood volume to the placenta.

Transplacental exchange is dependent upon uterine and umbilical blood flow. Moreover, blood flow rates are in turn dependent in large part upon vascularization of the placenta *(Reynolds et al., 2006)*. Fetal growth restriction (FGR) affects up to 8% of all pregnancies and has massive fetal morbidity and mortality. Impaired placental synthesis of nitric oxide (a major vasodilator and angiogenic factor) may explain the etiology of intrauterine growth retardation (IUGR) *(Wu et al., 2006)*. Sildenafil citrate is a specific phosphodiesterase- 5 inhibitor which was proved to act as a vasodilator in the fetoplacental circulation via a cGMP dependent mechanism which involves increased responsiveness to nitric oxide *(Maharaj et al., 2009)*. Sildenafil was used in the treatment of intrauterine growth retardation *(Villanueva- Garcia et al., 2007)*. The current work described the structural changes in placentas of the midterm and full term rats after maternal treatment by sildenafil citrate (Viagra). Examination of the placentas of the treated rats showed extensive congestion and dilatation of the maternal blood sinusoids in the basal zone more obvious in full term. The labyrinthine zone showed hypervascularity in addition to dilatation and congestion of fetal capillaries and maternal trophoblastic channels. Scanning electron microscopic examination of treated placentas showed apparent increase in the congestion of the villi as the sectioned villi appeared studded with blood cells.

In the current study, congested spiral arteries were seen traversing the inter-villous spaces in the treated placentas. The main ultrastructural finding was the presence of multiple sprouts arising from these arteries. Many authors described angiogenesis as being the formation of new blood vessels through sprouting from already existing blood vessels in a process involving the migration and proliferation of endothelial cells from preexisting vessels *(Patan, 2000)*. *Lee and Yeh (1986)* studied the small for gestational age placentas. The main changes observed in the fetal vasculature were the less branching of the arteries that were of variable diameters in addition to the absence of capillary bud projections. The authors suggested that the numerous capillary buds and anastomoses were characteristics of neovascularization and may be a compensatory phenomenon of the capillary network. In the present study, pericyte-like cells with their long processes were also observed on the surface of the villi. *Challier et al. (1999)* described the pericytes as large cells with extensions. Pericytes were observed lying over the microvessels that exhibited some terminal dilatations. The potential functions of the pericytes could be angiogenesis, contractility, basement membrane secretion and phagocytosis within the placental vascular bed. In the Viagra- treated placentas, the basal zone showed marked invasion by degenerating trophoblasts and abundant giant cells which were more evident at midterm. In agreement with this observation, *Pijnenborg et al. (1981)* reported that giant cells were most numerous during mid-gestation, but may be seen in small number near term. The present results could be attributed to the stimulation of the giant cells by degenerated trophoblasts to phagocytose and remove damaged cells in order to achieve normal placental function *(Kosif et al., 2008)*.

De Visser et al. (2008) studied the lungs of neonatal rats and observed that rats were born at the saccular stage of lung development. The sacculi were transformed into alveoli in the second week after birth with secondary septation and thinning of the interalveolar septa. Drugs that improve preterm lung in pre-term infants act through thinning of septa, reducing inflammation and decreasing edema and thus resulting in enlarged alveoli. In the current study, the lungs of both the sildenafil- treated midterm and full term fetuses showed dilatation of the bronchial and bronchiolar lumina, decrease in interstitial parenchyma, and an increase in the pulmonary interstitial vascularity. *De Visser et al. (2009)* reported the use of sildenafil in the treatment of bronchopulmonary dysplasia (BPD) in pre- term infants as it improves alveolarization and restores pulmonary angiogenesis in experimentally-induced BPD.

Several life- threatening diseases during pregnancy, such as hypertension and eclampsia, are closely associated with placental dysfunction. Genetic susceptibilities, secreted pro-inflammatory substances into maternal circulation and poor placentation explain in part

maternal predisposition to such diseases *(Furuya et al., 2008)*. Pre- eclampsia is also a pregnancy specific disorder associated with insufficient adaptations of spiral arteries. Such changes could release factors which induce maternal endothelial dysfunction *(Hutchinson et al., 2009)*. It was suggested that its underlying etiology is abnormal maternal immunological response to fetally expressed paternal antigens. This leads to poor trophoblasts invasion early in pregnancy and development of a relatively hypoperfused placenta *(Kenny et al., 2002)*. *Hirano et al. (2002)* added that the wall of spiral artery undergoes gradual degeneration as the pregnancy advances. In normal late pregnancy, the wall of spiral artery was thin while that of pre-eclampsia was thick. It was suggested that the vascular invasion by trophoblasts was related to thinning of the wall and decrease of elasticity of spiral arteries.

Sildenafil citrate promotes relaxation of myometrial smooth muscles and improves endothelial function of myometrial vessels. It was used in the treatment of intrauterine growth retardation and premature delivery with no evidence of teratogenicity *(Wareing et al., 2005; Villanueva-Garcia et al., 2007)*. Scanning electron microscopic examination of full term fetuses in the present study also showed no apparent external or internal congenital malformations. In conclusion, sildenafil proved to increase the placental blood flow without any apparent fetal teratogenicity. It also increased the fetal lung vascularity. So, it could be used safely in the conditions associated with placental vascular dysfunctions and fetal lung hypoperfusion.

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دراسة تركيبية على تأثير سيترات السيلدينافيل (الفياجرا) على المشيمة والتدفق الدموى لرئة أجنة الجرذ الأبيض، مع التركيز بصفة خاصة على سلامة الجنين أثناء الحمل: دراسة باستخدام كل من المجهرالضوئى والمجهرالماسح الالكترونى

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ملخص البحث

إن تدفق الدم فى المشيمة ونموالأوعية الدموية يعتبران أمرا حاسما وضروريا لنمو الجنين وتطوره. ولقد إستخدمت سيترات السيلدينافيل (الفياجرا) على نطاق واسع في السنوات الأخيرة في علاج ضعف الانتصاب، كما إستخدم في الآونة الأخيرة لعلاج كثير من أمراض الأوعية الدموية مثل ارتفاع ضغط الدم الشرياني الرئوي وقصور القلب الاحتقاني ومرض رينولدز. وفى الوقت الحاضر أعطى فى علاج طائفة واسعة من الأمراض المرتبطة بالحمل.

وكان الهدف من هذا البحث توضيح التركيب الهستولوجى للمشيمة فى منتصف الحمل ونهايته بالإضافة إلى دراسة تأثير السيلدينافيل عليها و التحقق من إحتمال أن يكون له أى تأثير مسخى على أجنة الجرذان البيضاء.

و قد إستخدم فى هذه الدراسة أربعة وعشرون من الجرذان الحوامل قسمت إلى أربع مجموعات يتكون كل منها من ستة جرذان:

مجموعة ضابطة فى منتصف الحمل (من بداية الحمل وحتى اليوم الثالث عشرمن الحمل)، مجموعة ضابطة فى آخر الحمل (من بداية الحمل وحتى اليوم الواحد والعشرين من الحمل)، المجموعة المعالجة فى منتصف الحمل (والتى تلقت الفياجرا من بداية الحمل وحتى اليوم الثالث عشرمن الحمل)، ومجموعة معالجة فى آخر الحمل (والتى أعطيت الفياجرا من بداية الحمل وحتى اليوم الواحد والعشرين من الحمل. ولقد أعطى الفياجرا في جرعة واحدة يوميا عن طريق الفم (٢,٥مج/ كج من وزن الجسم والمذاب في ٥ مل من المياه المالحة). أخذت عينات المشيمة والأجنة من كل المجموعات، وحضرت لدراستها وفحصها بكل من المجهر الضوئى و المجهر الماسح الإلكتروني.

وقد أظهر فحص مشيمة الجرذان فى آخر الحمل زيادة فى تكون الأوعية مع نقص فى سمك حاجز المشيمة مقارنة بمشيمة الجرذان فى منتصف الحمل. أما المجموعات المعالجة فوضح بها زيادة فى عدد الأوعية الدموية مع إحتقانها. كما أظهر الفحص بالميكروسكوب الماسح الإلكترونى أيضا إحتقانا فى داخل خملات المشيمة ورؤية الشرايين اللولبية عابرة للمسافة بين هذه الخملات كما لوحظ وجود براعم متعددة لهذه الشرايين، كما لوحظ فى آخرالحمل عدم وجود أى تشوهات داخلية أو خارجية واضحة للأجنة. أما فحص الرئتين فى أجنة الجرذان المعالجة فقد أظهر وجود زيادة واضحة فى أقطار الشعب والشعيبات الرئوية بالإضافة إلى نقص فى كثافة النسيج الأساسى الخلالى مع إتساع وإحتقان الأوعية الدموية البينية. و بصفة عامة فقد ثبت وجود زيادة فى تدفق الدم في المشيمة و فى رئتى الأجنة مع عدم ظهور أى تشوهات خلقية واضحة.