

**Research Article** 

# Effect of Hydroxyprogesterone (17-OHPC) on Placenta in a Rat Model of Preeclampsia: Histological and Immunohistochemical Study

Nada Amgad Mohammed, Azza Hussein Ali, Nashwa Fathy Gamal El-Tahawy\* and Rehab Ahmed Rifaai

Department of Histology and Cell Biology, Faculty of medicine, Minia University, Egypt

### Abstract

**Background:** Preeclampsia (PE) is a leading cause of maternal and fetal morbidity and mortality. Patients with severe PE exhibit significantly lower serum progesterone concentrations. There is limited information about the use of progesterone to manage or treat PE.

Aim of the work: To investigate the possible protective and/or therapeutic effects of 17-hydroxyprogesterone caproate (17-OHPC) therapy in a rat model of PE and the possibly involved mechanisms that monitored biochemically, histologically, and immunohistochemically.

**Methods:** Twenty-four pregnant female albino rats were randomly divided into 4 groups (6 rats/each): The control group, preeclampsia group (PE-group), prophylactic group, and treated group. The mean arterial blood pressure (MAP) and 24-hour protein in urine were determined. Rats were sacrificed at day 22 of gestation and placentae were processed for paraffin.

**Results:** The MAP and proteinuria in the PE-group were significantly higher compared to the control group. The prophylactic and treated groups showed significant decrease in MAP and proteinuria as compared to PE-group. In the treated group, they nearly returned to the normal levels. The histological examination of the PE-group showed dilated maternal blood sinuses, depositions of hemosiderin granules and numerous phagocytic trophoblastic cells containing cytoplasmic hemosiderin granules. Fetal blood vessels showed homogenous acidophilic material occluding their lumen, edema of the extra-embryonic fetal membranes and intra-villous tissue, and numerous nucleated RBCs. The prophylactic group showed some improvement while the treated group showed more or less normal maternal blood sinuses and interhemal membrane with few hemosiderin granules and few nucleated RBCs. There was a significant increase in caspase-3 expression and a significant decrease in the eNOS expression in PE-group compared to the control group. While the prophylactic and the treated groups had a significant decrease in caspase-3 expression compared to PE-group.

**Conclusion:** The biochemical, morphological and morphometric findings suggested that the administration of 17-OHPC to preeclamptic rat decreased blood pressure, proteinuria, inflammation, apoptosis, and improved vascular eNOS expression in placenta. The 17-OHPC possessed curative effect on L-NAME induced PE changes in rat placenta which was more obvious than its protective effect.

**Keywords:** Placenta; Preeclampsia; 17-Hydroxyprogesterone caproate; Caspase-3; eNOS

## Introduction

Preeclampsia (PE) is a syndrome of human pregnancy characterized by hypertension and proteinuria after 20 weeks of gestation in previously normotensive non-proteinuric pregnant women. It is a leading cause of maternal and fetal morbidity and mortality [1]. The etiology and pathogenesis of this disorder are not fully understood yet [2]. Poor placental perfusion, the endothelial cell dysfunction, and a disturbed balance of angiogenic factors may all contribute to this disorder. Poor placental perfusion is a stimulus of reactive oxygen species product that has a critical role in the manifestations and complications of PE [3]. In contrast to normal pregnancy, increased systemic vascular resistance and wide spreaded vascular endothelial damage characterize PE [4].

To study various aspects of PE, several animal models have been proposed. Administration of Nitro.L.arginine methyl ester (L-NAME); as a nitric oxide synthase (NOS) inhibitor, during mid to late period of gestation of the animal is one of the most popular models of PE [5]. Patients with severe PE exhibit significantly lower serum progesterone concentrations than gestational age- and race-matched non-preeclamptic pregnant women [6]. Additionally, there is evidence that progesterone have anti-inflammatory and vasodilator effects. It also can improve NO bioavailability and increases placental NO, thus improving hypertension [6,7]. Therefore, the aim of this study was to investigate the possible protective and/or therapeutic effects of 17-OHPC therapy in a rat model of PE and the possibly involved mechanisms that monitored by biochemical, histological, and immunohistochemical studies.

## **Materials and Methods**

The experiment was approved by the ethical committee for animal handling for research work in Faculty of Medicine, Minia University.

### Animals

Twenty-four female albino rats (12 weeks old, 200-220 gm) were

\*Corresponding author: El-Tahawy NF, Assistant professor at: Department of Histology and Cell Biology, Faculty of medicine, Minia University, Minia, Egypt, Tel: 02/01145435777; E-mail: nashogo@yahoo.com

Received November 20, 2017; Accepted January 06, 2018; Published January 15, 2018

**Citation:** Mohammed NA, Ali AH, Gamal El-Tahawy NF, Rifaai RA (2018) Effect of Hydroxyprogesterone (17-OHPC) on Placenta in a Rat Model of Preeclampsia: Histological and Immunohistochemical Study. J Cytol Histol 9: 496. doi: 10.4172/2157-7099.1000496

**Copyright:** © 2018 Mohammed NA, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Page 2 of 10

purchased from the animal house of faculty of Agriculture of Minia University. Animals were housed in clean plastic cages that were open to the room environment with normal light/dark cycles. Rats were fed standard laboratory diet and water ad libitum.

After one week of adaptation, female rats were mated overnight (each male rat was mated with 3 females). Day 0 of pregnancy was defined as the day when the spermatozoa were found in the vaginal smear.

## **Experimental design**

Animals were randomly divided into four groups (6 rats in each group) as following:

- 1. The control group: Pregnant rats were received standard rat chow diet and water.
- 2. The preeclampsia group (PE-group): Pregnant rats were received L-NAME dissolved in distilled water (50 mg/kg/day) by gavage from day 14 to day 19 of gestation [8].
- 3. The prophylactic group: Pregnant rats were received a single intraperitoneal dose of 17-OHPC diluted in normal saline (6.6 mg/kg) on day 7 of gestation [5] followed by induction of PE by L-NAME as in PE-group.
- 4. The treated group: Pregnant rats were received L-NAME (the same as PE-group) and a single intra-peritoneal dose of 17-OHPC (6.6 mg/kg) on day 18 of gestation which followed by the last dose of L-NAME on day 19 of gestation [5].

### Determination of 24-hour protein in urine

On day 19 of pregnancy, rats were placed separately in metabolic cages for 24-hour urine collection. Urine proteins were measured by the principle of turbidimetry by adding 5% trichloroacetic acid [9].

### Blood pressure measurement

Systolic and diastolic blood pressures were measured using a tail-cuff method on day 19 of gestation for all pregnant rats. Three measurements with 30 second intervals were recorded and the average of these readings was calculated followed by calculation of the mean arterial blood pressure (MAP).

### Sample collection

Rats from all groups were sacrificed at day 22 of gestation by decapitation under light halothane anesthesia. The gravid uterine horns were exposed by lower midline abdominal cavity incision. There were about 3 sacs on each horn. Each sac was opened, the fetus and the umbilical cord were exposed after the uterine wall and fetal membranes had been carefully incised far enough from the attachment site of the placenta. The placentae were detached from the fetus immediately. The specimens of the placentae were rapidly fixed in 10% formal saline for 48 hours and then washed by tap water.

## Methods

## Histological procedures

Placental specimens then were processed for paraffin sections. Some sections were cut, mounted on glass slides and stained with hematoxylin and eosin (H&E) for the histological examination [10].

### Immunohistochemistry

The polyclonal rabbit antibodies; cleaved caspase-3 and endothelial

nitric oxide synthase (eNOS) antibodies (Sigma Aldrich, Egypt) were used. Sections were deparaffinized, hydrated and washed. Endogenous peroxidases were quenched by treatment with  $H_2O_2$  in methanol followed by washing in tris buffer saline. Sections were incubated with the diluted 1<sup>ry</sup> antibodies for cleaved caspase-3 (1:200) and anti-eNOS antibody (1:100). Sections subsequently incubated in a biotinylated goat anti-rabbit 2<sup>ry</sup> antibodies (1:1000). Following further 30 minutes incubation with Vectastain ABC kits (Avidin, Biotinylated horse radish peroxidase Complex), diaminobenzidine tetra hydrochloride (DAB) was added for 5-10 min. Tonsils were used as positive control for activated caspase-3. Endothelium of the placental tissue used as the positive control for eNOS and the negative control sections were performed by removal of the 1<sup>ry</sup> antibody [11,12]. The result is immuno-positivity appeared as brown reaction which could be:

- For caspase 3: cytoplasmic, nuclear or both.
- For eNOS antibody: cytoplasmic reaction.

### Photography

Olympus light microscope and its digital camera (Olympus, Japan) was used for examining and capturing images from the histological and immunohistochemical sections. Images were saved as jpg and processed using Adobe Photoshop 7.

# Measuring area fraction of activated caspase-3 and eNOS immune-positivity)

Image J 22 software (open source Java image processing program) was used for area fraction measurement of the activated caspase-3 and eNOS immune-positivity. Area fraction was measured in a standard measuring frame per 5 photomicrographs in each group using a magnification X 400 by light microscope transferred to the monitored screen. Areas containing positively immunostained tissues were used for evaluation regardless the intensity of staining [13].

## Statistical analysis

Statistical analysis for numerical data was done by SPSS (IBM corp., Version 20). The mean number (MN) and standard deviation (SD) were determined for parameter in each group. The significance of differences observed in these groups were assessed by Kruskal-Wallis test and post HOC test. Significance was determined as probability factor (p-value) <0.05.

## Results

## The mean arterial blood pressure (MAP)

There was a significant increase in the MAP and the 24-h protein in urine in PE-group compared to the control group. In contrast, administration of 17-OHPC in the prophylactic group and the treated group, significantly lowered the MAP and the 24-h protein in urine if compared to the PE-group. Comparing the prophylactic group to the treated group, the MAP and the 24-h protein in urine were significantly decreased (all p<0.001) (Table 1).

## **Histological Results**

## H&E results

In the control group, the mature rat placenta at the level of labyrinth zone was composed of a web-like vascular system of sinuses for maternal blood and capillaries for the fetal blood circulation. Both circulations were strongly interdigitated with each other but separated

### Page 3 of 10

by the interhemal membrane. The interhemal membrane composed of cytotrophoblast cells lining the maternal sinuses and facing the maternal blood, 2 layers of syncytiotrophoblast cells (their elongated nuclei were located in-between the cytotrophoblast layer), and the fetal capillaries which recognized by their characteristic endothelial nuclei (Figure 1a). The chorionic projections composed of outer trophoblastic cells, middle mesenchymal connective tissue, and inner allantoic vasculature (fetal blood vessels) containing non-nucleated red blood corpuscles (RBCs) (Figure 1b). In the PE-group, the labyrinth zone showed dilated maternal blood sinuses, depositions of hemosiderin granules and numerous phagocytic trophoblastic cells containing cytoplasmic hemosiderin granules (Figure 2a). Some fetal blood vessels within the chorionic projections showed homogenous acidophilic material occluding their lumen. Some sections showed edema of the extra-embryonic fetal membranes and intra-villous tissue and numerous nucleated RBCs (Figure 2b-2d). Syncytial knots appeared as multinucleated aggregates of syncytial nuclei protruded from the surface of chorionic projections (Figure 2d).

Groups	Mean arterial blood pressure (mmHg) Mean ± S.D	Mean 24-hour proteins (g) in urine Mean ± S.D
1. Control Group	96.333 ± 1.211	0.45± 0.026
2. PE-group	155.666 ± 1.366 ª	$1.40 \pm 0.025^{a}$
3. Prophylactic group	127.5 ± 1.378 <sup>b</sup>	$0.94 \pm 0.018^{b}$
4. Treated group	119.5± 2.167 <sup>bc</sup>	0.71± 0.017 <sup>bc</sup>

a; vs the control group. b; vs the PE-group. c; vs the prophylactic group. All p< 0.001

Table 1: The mean arterial blood pressure (mmHg) and 24-hour proteins (g) in urine in the studied groups.









Page 4 of 10

In the prophylactic group, the labyrinth zone showed edema of the interhemal membrane, dilated maternal blood sinuses, hemosiderin deposits and activated phagocytic trophoblastic cells with cytoplasmic hemosiderin granules (Figure 3a). The chorionic projections showed minimal edema of the extra-embryonic fetal membrane. Some intravillous fetal blood vessels showed few nucleated RBCs (Figure 3b).

In the treated group, the labyrinth zone showed more or less normal maternal blood sinuses and interhemal membrane. However, few hemosiderin granules were noticed within the cytoplasm of the trophoblastic phagocytic cells (Figure 4a). Chorionic projections appeared normal with outer trophoblastic cells, middle mesynchymal connective tissue and inner fetal blood vessels. Nevertheless, some fetal blood vessels showed few nucleated RBCs (Figure 4b).

### Immunohistochemical results

**Immunohistochemical results using activated caspase-3 antibody:** The positive control sections of the tonsil showed positive immune reaction (Figure 5a). Placental tissue was used as a negative control (Figure 5b).

In the control group, no detectable immune labeling for activated caspase-3 in the placental sections (Figure 6a and 6b). In the PE-group, high immune labeling was noticed in the labyrinth zone and in the chorionic projections (Figure 7a). The chorionic projection showed positive cytoplasmic or nuclear immune labeling in the trophoblastic cells, endothelial cells lining the fetal blood vessels, and in the cells lining of the extra-embryonic membranes (Figure 7b). Sections of the



Figure 3: Photomicrographs of full term rat placenta of the prophylactic group at the level of labyrinth zone showing: (a) Dilated maternal blood sinuses (thin arrow), edema in the labyrinth interhemal membrane (thick arrows). Notice the trophoblastic cells with its cytoplasmic hemosiderin granules (\*). (b) Chorionic projection fetal blood capillary with some nucleated RBCs (arrow) and edematous extra-embryonic membranes. H&E 400×.



Figure 4: Photomicrographs of full term rat placenta of the treated group at the level of labyrinth showing: (a) intact interhemal membrane and its component (double headed arrow) fetal capillaries (FC), syncytiotrophoblas (S), cytotrophoblast (C) and normal maternal sinus (M) and sporadic hemosiderin within trophoblastic cell (arrow). (b) Chorionic projection fetal blood capillary (FC) with few nucleated RBCs (arrow) H&E 400×.



Figure 5: photomicrographs from sections immunostained for activated caspase-3 showing: (a) A positive control slide from the tonsil showing fine brown cytoplasmic granules (arrows). (b) A negative control slide from full term rat placenta at the level of labyrinth zone. Immunohistochemistry, counter stained with H, a) 400×, b) 100×.





Figure 6: Photomicrographs of full term rat placenta of the control group immunostained for activated caspase-3 showing: (a) No immune reaction in the labyrinth zone (double headed arrow) or the chorionic projection (arrow). (b) A higher magnification of the chorionic projection showing negative immune reaction in trophoblastic cells (long arrow), mesenchymal tissue (arrowhead) and endothelial lining of fetal blood capillary (short arrow) Immunohistochemistry, counter stained with H, a) 100×, b) 400×.



**Figure 7:** Photomicrographs of full term rat placenta of the PE-group immunostained for activated caspase-3 showing: (a) High reactivity in the labyrinth zone (double headed arrows) and chorionic projections (arrows). (a) A higher magnification of the chorionic projection showing positive reaction in the cells lining extraembryonic membrane (thick arrows), trophoblast cells (thin arrows) and endothelial cells lining fetal blood capillary (\*). Immunohistochemistry, counter stained with H, a) 100×, b) 400×.



Figure 8: Photomicrographs of full term rat placenta of the prophylactic group immunostained for activated caspase-3 showing: (a) Faint reactivity in the labyrinth zone (double headed arrow) and chorionic projections (black arrows). (a) A higher magnification of chorionic projection showing positive cytoplasmic immunoreaction in scattered cells within the mesenchyme (thin arrows) and cells lining extra-embryonic membrane (thick arrow). Immunohistochemistry, counter stained with H, a) 100×, b) 400×.

prophylactic group showed apparent decrease in the immune labeling in the labyrinth zone and the chorionic projections compared to the PE-group (Figure 8a). The chorionic projection showed scattered positive immunolabeled cells within the mesenchymal tissue and in the cells lining extra-embryonic membranes (Figure 8b). No detectable immune labeling was noticed in the labyrinth zone or chorionic projections of the treated group (Figure 9a and 9b).

**Immunohistochemical study using anti eNOS antibody:** Placental tissue was used as negative control for eNOS (Figure 10). Sections of the control group displayed high positive eNOS immune labeling in the labyrinth zone and in the endothelial cells lining fetal blood vessels within the chorionic projection (Figure 11a and 11b). In the PE-group, the labyrinth zone and the chorionic projections showed negative immune reaction for eNOS (Figure 12a and 12b). While in the prophylactic group, positive reaction for eNOS was noticed in the labyrinth zone and in some endothelial cells of the fetal blood vessels within the chorionic projections (Figure 13a and 13b). While the treated group showed high reactivity for eNOS in the endothelial cells of blood capillaries within the labyrinth zone and in the endothelial cell of the fetal blood vessels within the chorionic projections (Figure 14a and 14b).

Morphometric analysis of area fraction of activated caspase-3 and eNOS immune-positive cells: There was a significant increase in the area fraction of activated caspase-3 expression and a significant decrease in the area fraction of eNOS expression in the PE-group compared to the control group, the prophylactic group and the treated

Page 6 of 10





Figure 12: Photomicrographs of full term rat placenta of the PE-group immunostained for eNOS showing: (a) Negative reaction in the labyrinth zone (double headed arrow) and the chorionic projection (arrow). (a) Higher magnification of chorionic projection showing negative reaction of in the endothelial cells of fetal blood capillary (arrow). Immunohistochemistry, counter stained with H, a) 100×, b) 400×.

### Page 7 of 10



Figure 13: Photomicrographs of full term rat placenta of the prophylactic group immunostained for eNOS showing: (a) Positive immune reaction in the labyrinth zone (double headed arrows) and the fetal blood capillaries within the chorionic projections (thin arrows). (a) Higher magnification of chorionic projection showing positive cytoplasmic immune reaction in the endothelial cells of fetal blood capillaries (thin arrows). Immunohistochemistry, counter stained with H, a) 100×, b) 400×.



Figure 14: Photomicrographs of full term rat placenta of the treated group immunostained for eNOS showing: a. high reactivity of endothelial lining of fetal blood capillaries within labyrinth zone (double headed arrow) and the chorionic projection (thin arrows). b. Higher magnification of chorionic projection showing strong positive immune reaction of endothelial cells of fetal blood capillary (arrow). Immunohistochemistry, counter stained with H, a) 100×, b) 400×.



**Graph 1:** The mean area fraction of caspase-3 immunoreactivity in different groups. <sup>a</sup>Significant (p<0.001) difference from the control group, <sup>b</sup>Significant (p<0.001) difference from the PE-group, <sup>c</sup>Significant (p<0.001) difference from the prophylactic group.

group (all p<0.001). Comparing the prophylactic group with the PEgroup, there was a significant decrease in the area fraction of caspase-3 expression and a significant increase in the area fraction of eNOS expression (all p<0.001). Comparing the treated group with the PEgroup and the prophylactic group, there was a significant decrease in the area fraction of caspase-3 expression and a significant increase in the area fraction of eNOS expression (all p<0.001) (Graphs 1 and 2).

### Discussion

Preeclampsia (PE) remains one of the most common causes of maternal and fetal morbidity and mortality in the world [14]. Although the pathogenesis of PE is still not fully understood, a multi-stage





model is generally accepted [15]. The utero-placental syndrome with impaired placental development in the first stage of the disease causes generalized maternal endothelial dysfunction as a main clinical feature of PE in the second stage [16]. An array of placenta-derived factors is candidate contributors to endothelial dysfunction in PE [17].

In the reproductive and developmental toxicity studies, careful attention should be paid to the histological structure of the interhemal area when extrapolating information concerning placental transfer characteristics to different animal species [18]. Therefore, the present work was done to describe the structural changes in the fetal part of placenta (labyrinth zone) of preeclamptic rat, as it is functionally analogous for human fetal part of the placenta in which materno-fetal exchange take place. In addition, it was done to study the possible protective and/or the curative effect of 17-OHPC on the placenta of a model of PE induced by L-NAME to shed a light on the possibly involved mechanisms.

The term "animal model of PE" was commonly used when L-NAME was administered from day 14 of gestation to induce PE-like syndrome in rats [19]. It has been reported that L-NAME produces virtually all the pathophysiologies of PE in the animal model [20].

In this study, the mean arterial blood pressure (MAP) and the 24-hour protein in urine of the PE-group were increased after administration of L-NAME compared to the control group, which was in agreement with several studies [21-25]. Whereas, 17-OHPC had beneficial effect in decreasing the severity of PE by decreasing the MAP and mean 24-hour protein in urine of the prophylactic and treated groups when compared with the PE-group. This result was supported by other studies [26,27]. They demonstrated that 17-OHPC is a drug of treatment of hypertension in response to elevated TNF-alpha during pregnancy and increased local production of endothelin-1 (ET-1) in the kidney, placenta, and vasculature of rats. They added that administration of 17-OHPC on day 18 of gestation decreased renal cortex prepro-ET-1 mRNA levels and significantly decreased MAP.

In the current study, a histological study was done to demonstrate the structural changes in the placenta in the L-NAME model of PE using the 17-OHPC. H&E sections of the placentae showed that L-NAME administration in the PE-group caused marked morphological changes in the labyrinth zone including dilated maternal blood sinuses and dilated fetal blood capillaries.

Vasodilatation is a fast response that increases perfusion of blood to the hypoxic tissues. The decreased placental perfusion is thought to lead to fetoplacental ischemia [28]. This ischemic placenta may produce certain circulating agents which cause the wide spread dysfunction of the maternal vascular endothelium. Endothelial dysfunction could be originated from oxidative stress in placenta which is likely to arise from hypoxia which may occur due to failure of myometrial small arteries remodeling [29].

Congestion and hemosiderin deposits were also observed. These observations could be due to ischemia and the subsequent reaction of the placental tissue to ischemia after interruption of maternal blood flow. Hemosiderin deposits occurred after rupture of the congested sinuses [30].

Intra-villous deposition of acidophilic material occluding the lumen of fetal blood vessels was another finding in this study. Akhilesh et al. [31] demonstrated ruptured villi with extravasation of fetal RBCs into the maternal space, thickened wall of fetal blood capillaries, intravillous and peri-villous fibrinoid deposition in their model of PE. Deposition of fibrinoid prevents normal gas and nutritive exchange between the maternal and fetal circulations. This consequently can lead to fetal growth restriction [32]. The fibrinoid deposits are structurally and chemically related to fibrin. It is formed by the activation of fibrinogen in the blood vessels, and is present in all normal placentae. The functional importance of the fibrinoid besides their sealing effects, they also have a role in the immunologic barrier between feto-maternal tissues [33]. Increased fibrinoid formation may be related to microlesions of the syncytiotrophoblast. Therefore, fibrinoid could envelope all necrotic material that results from placental degeneration. These injuries make mesenchymal tissue comes into contact with maternal blood, leading to altered function of hemostatic factors interacting with activated protein C in the placenta. The increased activated protein C resistance lead to ineffective anticoagulant response [34].

Syncytial knots are normally present and increasing in number with increasing gestational age, so it can be used to evaluate villous maturity. Increased syncytial knots are also associated with other abnormal conditions like PE [35]. Increased syncytial knots in the chorionic projections of the PE-group, in this study, was in agreement with consistent with Stark et al. [36].

The extra-embryonic tissue such as the trophoblast gives rise to the placenta and provides the epithelial cover of the placental villous trees. They show high rates of proliferation as well as high rates of apoptosis. This villous trophoblast comes into direct contact with maternal blood displaying a continuous turnover of its layers [37]. The villous trophoblast displays proliferation differentiation with a final apoptotic shedding event, releasing apoptotic syncytial knots into the maternal circulation. As a normal process of trophoblast turnover apoptosis, the release of apoptotic material does not induce any inflammatory response of the mother [38].

The PE is characterized by alteration in the balance between proliferation and apoptosis of villous trophoblast. This results in a dysregulated release of material from the syncytiotrophoblast into maternal blood. There is also an increasing release by necrosis [39]. Therefore, due to ongoing apoptosis within the syncytiotrophoblast, the necrotic release of apoptotic material leads to aponecrotic shedding. Cell-free components of the syncytiotrophoblast like G-actin and DNA may be able to damage the maternal endothelium and hence trigger PE. Therefore, PE might be a result rather than a cause of this altered balance [40].

Other important finding, in this study, was the presence of nucleated RBCs in the fetal blood capillaries of the PE-group at this age. Nucleated RBCs are common in very early gestations, but as gestation proceeds, and especially at term, nucleated RBCs should not be present. When present, fetal anemia with increased erythroid production should be suspected. Additionally recent investigations have addressed the presence of nucleated RBCs in advanced-gestation placentas, correlating such findings with erythropoietin secretion by the fetus and concomitant fetal hypoxia [41].

In the prophylactic group, only minimal improvement in the histological changes that induced by L-NAME was noticed. Dilated maternal blood vessels, congestion and hemosiderin granules deposition were still observed. Another important finding in this group was the marked edema in the labyrinth and extra-embryonic membrane. The chorionic projections showed dilated fetal blood vessels containing some nucleated RBCs. This was in agreement with Phillips et al. [42], Smith and Critchely [43] and Guttinger [44]. They studied the histological effects of progestins on the endometrium and reported increased dilated thin-walled fragile blood vessels and hemorrhagic infarction, with other stromal findings including myxoid changes and hemosiderin pigment deposition.

Other studies demonstrated a profound effect of progesterone to inhibit endothelin-1 (ET-1) secretion from human umbilical venous endothelial cells stimulated with PE serum within a short 6 hours period of progesterone treatment [6,26].

In the treated group, the histological assessment showed improvement in the placental labyrinth zone and the chorionic projections. This was enforced by the biochemical study of Amaral and LaMarca [45] who studied the effect of 17-OHPC on late gestation of rat model of PE. They found that 17-OHPC was significantly attenuated the renal and placental endothelin-1 (ET-1), decreased circulating IL-6 and TNF-alpha. They added that 17-OHPC improved hypertension, inflammation and NO bioavailability in response to placental ischemia during pregnancy. Other data demonstrated from cultured placental explants in presence of hypoxia and 1 u Mole progesterone that IL-6, TNF-alpha and IL-17 were all reduced when compared with hypoxic cultures alone, this indicated an important anti-inflammatory role for progesterone that could play at the level of placenta. Caspases play a key role in apoptosis [46]. In this study, the area fraction of caspase 3-immune-positive cells in control rats was very low. In the other hand, there was a significant increase in caspase 3-immuno-positive cells in PE-group, which showed a decrease in the prophylactic group and more decrease in the treated group when compared to the PEand prophylactic groups. L-NAME treatment was reported to cause apoptosis in rat placenta [47]. This was in line with Afroze et al. [48], who observed the over expression of active caspase 9 in human preeclamptic placenta and umbilical cord.

Progesterone protected cardiomyocytes [49] and hypoxic ischemic brain damage [50] from apoptosis. The anti-apoptotic effect of progesterone was dose dependent and time dependent causing decrease in caspase-3 activity [48]. Bcl-2 is an important molecule that regulate apoptosis. PE caused ischemia and hypoxia resulted in change the mitochondrial permeability to induce the release of cytochrome C and down regulate Bcl-2 result in cell apoptosis but suggest that progesterone exerted its anti-apoptotic effect by up-regulating Bcl-2 expression [51].

Nitric oxide (NO) is synthesized in a variety of tissues, including rat uterus, from L-arginine by NO synthase (NOS), of which there are three isoforms, one of them is endothelial NOS (eNOS). eNOS is primarily responsible for the generation of NO in the vascular endothelium [52]. NO produced by eNOS in the vascular endothelium plays crucial roles in regulating vascular tone, cellular proliferation [53]. In the present work, there was a significant increase in eNOS expression in the prophylactic and treated groups when compared with the PE-group. A significant increase in the eNOS-immune positive cells in the treated group compared with the prophylactic group. This was inconsistent with Haung et al. [54] who revealed reduced eNOS expression in compromised pregnancies like PE. Another study of Kulanavelu, et al. [55] demonstrated reduced fetal growth and fetoplacental blood flow at mid and late gestation in eNOS knockout mice fetuses. On contrary, another study [56] demonstrated immunolocalization of eNOS in syncytiotrophoblasts and endothelium of fetal blood capillaries in normal and preeclamptic placentae. Therefore, they excluded a possible pathogenic role of eNOS in this disease.

Zoltan and Sandor [57] suggested that decreasing eNOS expression in the PE was due to plasma asymmetric dimethyl arginine (ADMA) which was recognized as a biomarker of many disorders including endothelial disorders. Maternal plasma ADMA levels were reduced in a normal pregnancy but increased in compromised pregnancy such as those with PE. This substance may decrease NO production by different ways such as reduced affinity of eNOS to the cofactor tetrahydrobiopetrin (BH4). Thus, manipulation of the ADMA-NO pathways may have a therapeutic potential to improve placental insufficiencies.

Progesterone affects function of eNOS by both genomic and nongenomic mechanisms [58], the latter perhaps involving activation of a membrane bound receptor and subsequent activation of PI3K/AKt leading to eNOS activation [59]. Non-genomic actions of progesterone on endothelial production of NO are also mediated by activation of

Page 9 of 10

In spite of great debate about the role of eNOS in the pathogenesis of PE, NO produced by eNOS, was the main vasodilator in the placenta. It is involved in the regulation of fetoplacental vascular reactivity, placental bed vascular resistance, trophoblast invasion, apoptosis, and platelet adhesion and aggregation in the intervillous space [61].

tyrosine kinase and PI-3 kinase pathways [60].

### Conclusion

The biochemical, morphological and morphometric findings suggested that the administration of 17-OHPC to preeclamptic rat induced beneficial effects in term of decreasing blood pressure, proteinuria, inflammation, apoptosis, and improved vascular eNOS expression in placenta. The17-OHPC possessed curative effect on L-NAME induced PE changes in rat placenta which was more obvious than its protective effect.

### References

- Noris M, Perico N, Remuzzi G (2005) Mechanisms of disease: Preeclampsia. Nature clinical practice. Nephrology 1: 98-114.
- Acikgoz S, Bayar UO, Can M, Guven B, Mungan G, et al. (2013) Levels of oxidized LDL estrogens and progesterone in placenta tissues and serum paraoxonase activity in preeclampsia. Mediators Inflamm 2013: 862-982.
- Sedek M, Gillbert JS, Lamarca B (2008) Role of reactive oxygen species in hypertension produced by reduced uterine perfusion in pregnant rats. American Journal of Hypertension 21: 152-1156.
- Khalil RA, Granger JP (2002) Vascular mechanisms of increased arterial pressure in preeclampsia: lessons from animal models. American Journal of Physiology: Regulatory Intergrative and Comparative Physiology 28: 29-45.
- Podjarny E, Losonczy G, Baylis C (2004) Animal models of Preeclampsia. Seminars in Nephrology 24: 596-606.
- Kiprono LV, Wallace K, Moseley J, Martin Jr J, Lamarca B (2013) Progesterone blunts vascular endothelial cell secretion of endothelin-1in response to placental ischemia. Am J Obstet Gynecol 209: 441-446.
- Amaral LM, Kiprono L, Cornelius DC, Shoemaker C, Wallace K, et al. (2014) Progesterone supplementation attenuates hypertension and the autoantibody to the angiotensin II type I receptor in response to elevated interleukin-6 during pregnancy. Am J Obstet Gynecol 211: 1581-1586.
- Kemse NG, Kale AA, Joshi SR (2014) Acombined supplementation of Omega-3 fatty acids and micronutrients (folic acid vitamin B<sub>12</sub>) reduses oxidative stress markers in a rat model of pregnancy induced hypertension. PLoS One 9: e111902.
- Abuelo JG (1983) Proteinuria: diagnostic principles and procedures. Ann Intern Med 98: 186-91.
- Bancroft J, Gamble M (2008) Theory and practice of histological techniques (6thedn), Harcourt: Churchill Livingstone 126: 422-425.
- Krajewska M, Wang HG, Krajewska S, Shabik A, Reed JC (1997) Immunohistochemical analysis of in vivo patterns of expression of CPP32 (Caspase-3) a cell death protease. National Institutes of Health 57: 1605-1613.
- Zhu XY, Li P, Yang YB, Liu ML (2013) Xuezhikang extract of red yeast rice improved abnormal hemorheology suppressed caveolin-1 and increased eNOS expression in atherosclerotic rats. PLoS 1: 62731.
- Ibrahim NA, Khalid DM (2014) Histological and immunohistochemical study on human placental tissue in normal pregnancy and preeclampsia. Cell Biology 2: 72-80.
- Ilekis JV, Reddy UM, Roberts JM (2007) Preeclampsia a pressing problem: an excutive summary of a National Institute of Child Health and Human Development workshop. Reprod Sci 14: 508-523.
- 15. Erlandsson L, Navva A, Hennessy A, Vaiman D, Gram M, et al. (2016) Inventory of novel animal models addressing etiology of preeclampsia in the development of new therapeutic/intervention opportunities. American Journal of Reproductive Immunology 75: 402-410.

Page 10 of 10

- Redman CW, Sargent IL (2005) Latest Advances in Understanding preeclampsia. Science 308: 1592-1594.
- Mynard SE, Min JY, Merchan J, Lim KH, Li J (2003) Excess placental soluble fms-like tyrosin kinase 1(sFlt 1) may contribute to endothelial dysfunction hypertension and proteinuria in preeclampsia. J Clin Invest 111: 649-658.
- Furukawa S, Kuroda Y, Sugiyama A (2014) A Comparison of the Histological Structure of the Placenta in Experimental Animals. J Toxicol Pathol 27: 11-18.
- Brown C, McFarlane-Anderson N, Alexender-Lindo R, Bishop K, Dasgupta T (2013) The effects of S-nitrosoglutathione and S-nitroso-N-accetyl-D Lpenicillaminein in a rat model of preeclampsia. J Nat SciBiol Med 4: 330-335.
- 20. Takei H, Lizuka S, Yamamoto M, Takeda S (2007) The herbal medicine Tokishakuyakusan increases fetal blood glucose concentrations and growth hormone levels and improves intrauterine growth retardation induced by N (omega)-nitro-L-arginine methyl ester. J Pharmacol Sci 104: 319-328.
- LaMarca B, Bennett W, Cockrell K, Fournier L, Granger JP (2010) The role of immune activation in contributing to vascular dysfunction and pathophysiology of hypertension during preeclampsia. Minerva Gynecol 62: 105-120.
- Adamcova M, Ruzickova S, Simko F (2013) Multiplexed immunoassays for simultaneous quantification of cardiovascular biomarkers in the model of H (G)nitro-L-arginine methyl ester (L-NAME) hypertensive rat. J Physiol Pharmacol 64: 211-217.
- Gad HI (2013) The potential role of tumor necrosis factor- alpha antibodies on some renal functions and vasoregulatory factors in preeclamptic pregnant Wister rat. Saudi Med. J 34: 490-496.
- Sung JH, Jo YH, Kim SJ, Ryu JS, Kim MC (2013) Effects of lutin on L-NAME induced hypertensive rat. Korean J Physiol Pharmacol 17: 339-345.
- 25. Zhou Q, Shen J, Zhou G, Shen L, Zhou S (2013) Effects of magnesium sulfate on heart rate blood pressure variability and baroreflex sensitivity in preeclamptic rats treated with L-NAME. Hypertens Pregnancy 32: 422-431.
- Keiser SD, Veillon EW, Parrish MR, Bennett W, Cockrell K, et al. (2009) Effects of 17-hydroxyprogesterone on tumor necrosis factor-alpha induced hypertension during pregnancy. Am. J. Hypertens 22: 1120-1125.
- Lorena M, Amaral DC, Cornelius HA, Moseley J, LaMarca B (2015) 17-Hydroxyprogesterone caproate significantly improves clinical characteristics of preeclampsia in the RUPP rat model. Hypertens 65: 225-231.
- Yang S, Hu Y, Zhang Y, Wang J (2010) Effects of danshensu on maternal syndrome in phosphatidyleserine/phosphatidylecholine microvesicle inducedmouse model: is it a candidate for preeclampsia remedy. Chine Med J 123: 895-900.
- 29. Raijmakers MTM, Dechend R, Poston L (2004) Oxidative stress and preeclampsia rationale for antioxidant clinical trials. J Hyper 44: 374-380.
- Kroger C, Vijayaraj P, Reuter U, Reinhard W, David S, et al. (2011) Placental vasculogenesis is regulated by keratin-mediated hyperoxia in murine decidual tissues. Am J Pathol 178: 1578-1590.
- 31. Akhilesh M, Mahalingam V, Nalliah S, Ali RM, Ganesalingam M, et al. (2013) Participation of hypoxia-inducible factor-1 alpha in the pathogenesis of preeclampsia related placental ischemia and its potential as a marker of preeclampsia. Biomarkers and Genomic Med 66: 121-125.
- Romero R, Whitten A, Korzeniewski SJ, Than NG, Chaemsaithong P, et al. (2013) Maternal floor infarction/massive perivillous fibrin deposition: a manifestation of maternal antifetal rejection. Am J Reprod Immunol 70: 285-298.
- Kaufmann P, Huppertz P, Frank HG (1996) Origin composition and functional relevance. Anat Anz 178: 485-501.
- 34. Sedano-Balbes S, Lyons M, Cleary B, Murray M, Gaffney G, et al. (2014) Placental prothrombin mRNA levels in APC resistance (APCR) women with increased placental fibrin deposition. Ir J Med Sci 183: 477-80.
- Loukeris K, Sela R, Baergen RN (2010) Syncytial knots as a reflection of placental maturity: reference values for 20 to 40 weeks gestational age. Pediatr Dev Pathol 13: 305-309.
- Stark MW, Clarck L, Craver RD (2014) Histologic differences in placentas of preeclamptic/eclamptic gestations by birthweight placental weight and time of onset. Pediatr Dev Pathol 17: 181-189.
- Huppertz B (2006) Placental villous trophoblast: the altered balance between proliferation and apoptosis triggers preeclampsia. J Repro Med Endo 6: 103-108.

- Huppertz B, Gauster M (2011) Trophoblast fusion. Adv Exp Med Biol 713: 81-95.
- Gauster M, Moser G, Orendi K, Huppertz B (2009) Factors involved in regulating trophoblast fusion: potential role in the development of preeclampsia. Placenta 30: 49-54.
- 40. Dechend R, Staff AC (2012) Placenta messages to the mother: not just debris. Hypertension 59: 191-193.
- Benirschke K (2004) The placenta: structure and function. J. Am. Acad Pedi 5: 252-261.
- Phillips V, Graham CT, Manek S, McCluggage WG (2003) The effects of levenorgestrel intrauterine system (Mirena coil) on endometrial morphology. J Clin Pathol 56: 305-307.
- Smith OP, Critchely HO (2005) Progesterone only contraception and endometrial break through bleeding. Angiogenesis 8: 117-126.
- Guttinger A (2007) Endometrial effects of intrauterine levonorgestrel. Contraception 75: 93-98.
- Amaral LM, LaMarca B (2015) 17-hydroxyprogesterone caproate as a potential therapeutic to add to the management of preeclampsia. J Pharmacol Clin Toxicol 3: 1059.
- Shi Y (2004) Caspase activation: revisiting the induced proximity model. Cell 117: 855-858.
- Xuan R, Niu T, Chen H (2016) Astaxanthin blocks preeclampsia progression by suppressing oxidative stress and inflammation. Mol Med Rep 14: 697-704.
- Afroze SH, Kalagiri RR, Reyes M, Zimmerman JD, Beeram MR, et al. (2016) Apoptotic and stress signaling markers are augmented in preeclamptic placenta and umblical cord. BBA Clin 6: 25-30.
- Morrisy S, Xu B, Aguilar D, Zhang J, Chen QM (2010) Inhibition of apoptosis by progesterone in cardiomyocytes. PMC 9: 799-809.
- 50. Li X, Zhang J, Zhu X, Wang P, Wang X, et al. (2015) Progesterone reduces inflammation and apoptosis in neonatal rats with hypoxic ischemic brain damage through the PI3K/AKt pathway. Int J Clin Exp Med 8: 8197-8203.
- 51. Ishrat T, Sayeed I, Atif F, Hua F, Stein DG (2012) Progesterone is neuroprotective against ischemic brain injury through its effects on the phosphoinositide-3kinase/protein kinase B signaling pathway. Neuroscience 210: 442-450.
- 52. Fish JE, Marsden PA (2006) Endothelial nitric oxide synthase: insight into cellspecific gene regulation in the vascular endothelium. Cellular and Molecular Life Sciences 63: 144-162.
- Forstermann U, Munzel T (2006) Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 113: 1708-1714.
- Haung LT, Hsieh CS, Chang KA, Tain YL (2012) Roles of nitric oxide and asymmetric dimethylarginine in pregnancy and fetal programming. Int J Mol Sci 13: 14606-14622.
- Kulanavelu S, Whiteley KJ, Bainbridge SA, Qu D, Adamson SL (2013) Endothelial NO synthase augments fetoplacental blood flow placental vascularization and fetal growth in mice. Hypertension 61: 259-266.
- 56. Corthorn J, Germain AA, Chacon C, Rey S, Soto GX, et al. (2006) Expression of kallikrein bradykinin b2 receptor and endothelial nitric oxide synthase in placenta in normal gestation preeclampsia and placenta accrete. Endocrine 29: 491-499.
- 57. Zoltan K, Sandor V (2010) Nitric oxide and preeclampsia. Orv Hetil 151: 2125-2135.
- Duckles SP, Miller VM (2010) Hormonal modulation of endothelial NO production. Euro J Physiol 459: 841-851.
- Ellmann S, Sticht H, Thiel F (2009) Estrogen and progesterone receptors: from molecular structures to clinical targets. Cell Mol Life Sci 66: 2405-2426.
- Mendiberri J, Rauschemberger MB, Steles J (2006) Involvement of phosphoinositide-3-kinase and phospholipase C transduction systems in the non-genomic action of progesterone in vascular tissue. Int J Biochem Cell Biol 38: 288-296.
- Reynolds LP, Borowicz PP, Caton JS, Vonnahme KA, Luther JS (2010) Uteroplacental vascular development and placental function: an update. Int J Dev Biol 54: 355-366.