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

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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, deposits 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 and a significant increase in the eNOS 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
Some sections were cut, mounted on glass slides and stained with specimens of the placentae were rapidly fixed in 10% formal saline for placenta. The placentae were detached from the fetus immediately. The had been carefully incised far enough from the attachment site of the umbilical cord were exposed after the uterine wall and fetal membranes were about 3 sacs on each horn. Each sac was opened, the fetus and the horns were exposed by lower midline abdominal cavity incision. There decapitation under light halothane anesthesia. The gravid uterine of these readings was calculated followed by calculation of the mean measurements with 30 second intervals were recorded and the average tail-cuff method on day 19 of gestation for all pregnant rats. Three the principle of turbidimetry by adding 5% trichloroacetic acid [9]. cages for 24-hour urine collection. Urine proteins were measured by

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 intrauterine 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 intrauterine 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 

H2O2 in methanol followed by washing in tris buffer saline. Sections were incubated with the diluted 1st antibodies for cleaved caspase-3 (1:200) and anti-eNOS antibody (1:100). Sections subsequently incubated in a biotinylated goat anti-rabbit 2nd antibodies (1:1,000). 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 1st 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 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...
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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean arterial blood pressure (mmHg)</th>
<th>Mean 24-hour proteins (g) in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D</td>
<td>Mean ± S.D</td>
</tr>
<tr>
<td>1. Control Group</td>
<td>96.333 ± 1.211</td>
<td>0.45 ± 0.026</td>
</tr>
<tr>
<td>2. PE-group</td>
<td>155.666 ± 1.366^a</td>
<td>1.40 ± 0.025^a</td>
</tr>
<tr>
<td>3. Prophylactic group</td>
<td>127.5 ± 1.378^b</td>
<td>0.94 ± 0.018^b</td>
</tr>
<tr>
<td>4. Treated group</td>
<td>119.5 ± 2.167^c</td>
<td>0.71 ± 0.017^c</td>
</tr>
</tbody>
</table>

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.

![Figure 1](image1.png)

**Figure 1:** Photomicrographs of the control group of full term rat placenta at the level of labyrinth showing: a. Normal interhemal membrane and its component (double headed arrow) with normal fetal capillaries (Fc), normal syncytiotrophoblas (S), cytotrophoblast (C) and normal maternal sinus (M). b. Chorionic projection with outer trophoblastic cells (thick arrow), middle mesenchymal connective tissue (arrow head) and inner fetal blood capillary with normal RBCS (thin arrow) H&E 400×.

![Figure 2](image2.png)

**Figure 2:** Photomicrographs of full term rat placenta of the PE-group at the level of labyrinth zone showing: (a) Dilated maternal blood sinuses (thin arrows), hemosiderin granules deposition (thick arrow) and trophoblastic cells with cytoplasmic hemosiderin granules (circle). (b) Chorionic projection with fetal blood capillaries filled with homogenous acidophilic material occluding their lumen (arrows). (c) Intra-villous dilated fetal blood capillary (FC) with numerous nucleated RBCs (arrow). (d) Edematous extra-embryonic membranes (thin arrows), syncytial knots of chorionic projection (thick arrows) and intra-villous edema (arrow head). Notice dilated maternal sinusoid (star) H&E a, b and c 400× d 100×.
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 intra-villous 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 mesenchymal 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

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**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), syncytiotrophoblasts (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×.
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 group.
Figure 9: Photomicrographs of full term rat placenta of the treated group immunostained for activated caspase-3 showing: (a) Negative immune reaction in labyrinth zone (double headed arrow), and chorionic projection (arrow). (b) A higher magnification of chorionic projection showing negative cytoplasmic immune reaction in trophoblastic cells (thick arrow), mesenchymal tissue (arrow head) and endothelial lining of fetal blood capillary (thin arrow). Immunohistochemistry, counter stained with H, a) 100×, b) 400×.

Figure 10: A photomicrograph of rat placenta at the level of labyrinth zone represents negative control for eNOS. Immunohistochemistry, counter stained with H, 100×.

Figure 11: Photomicrographs of full term rat placenta of the control group immunostained for eNOS showing: (a) High immune reaction in the labyrinth zone (double headed arrow) and endothelium of fetal blood capillaries within the chorionic projections (arrows). (b) A higher magnification of the chorionic projection showing strong endothelial immunoreactivity (arrow). Immunohistochemistry, counter stained with H, a) 100×, b) 400×.

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). (b) 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×.
group (all p<0.001). Comparing the prophylactic group with the PE-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). Comparing the treated group with the PE-group 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 placentation 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 the placenta (labyrinth zone) in preeclamptic rat, as it is functionally analogous for human fetal part of the placenta in which materno-fetal

Figure 13: Photomicrographs of full term rat placentas 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 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 placentas 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×.
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 pathophysiologicals 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 propro-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 placenta 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 widespread 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 occcluding the lumen of fetal blood vessels was another finding in this study. Akhilesh et al. [31] demonstrated ruptured vili with extravasation of fetal RBCs into the maternal space, thickened wall of fetal blood capillaries, intra-villous 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 placenta. 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 erythropoietin 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 myoid 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...
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-immune-positive cells in PE-group, which showed a decrease in the proliferative group and more decrease in the treated group when compared to the PE- and prophylactic groups. L-NAME treatment was reported to cause apoptosis in rat placenta [47]. This was in line with Afroz 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 isoforins, 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 proliferative 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 Kulanevelu, et al. [55] demonstrated reduced fetal growth and feto-placental 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 tetrahydrobiopterin (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 non-genomic 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 tyrosine kinase and PI-3 kinase pathways [60].

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 feto-placental vascular reactivity, placental bed vascular resistance, trophoblast invasion, apoptosis, and platelet adhesion and aggregation in the intervillous space [61].

**Conclusion**

The biochemical, morphological and morphometric findings suggested that the administration of 17-OHPC to preeclamptic rat induced beneficial effects in terms of decreasing 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.

**References**


