Evaluation of the effect of vitamin D on the placenta, kidney, and growth of developing fetuses in preeclamptic mice

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Background
Preeclampsia (PE) is a complicated syndrome that leads to maternal and fetal morbidity and mortality. PE is defined by the elevation of the mother's blood pressure (hypertension) and the presence of proteinuria.
Objective
This study aimed to evaluate the effect of vitamin D on 18-day-old mice fetuses in which the PE syndrome was induced in the mother by L-NG-nitro arginine methyl ester (L-NAME).
Materials and methods
The mice grouping was divided as: (a) the control group (group I), (b) the female pregnant mice of the second group intraperitoneally injected with 50 mg/kg/day of L-NAME (group II), (c) the female pregnant mice of the third group were administered orally with 50 IU/kg/day of vitamin D (group III), and (d) the female pregnant mice of the fourth group were intraperitoneally injected with 50 mg/kg/day L-NAME and then orally, with 50 IU/kg/day vitamin D (group IV). All groups were treated daily from 7 to 14 days of gestation.
Results and conclusion
The placenta of mice injected with L-NAME showed different phases of histopathological changes in the basal and labyrinth zone. Meanwhile, the kidney in 18-day-old fetuses maternally injected with L-NAME showed an apparent enlargement in the glomerular area and the presence of hemorrhages among the tubules. However, the 18-day-old fetuses maternally treated with L-NAME and vitamin D (group IV) showed mild injury. This study concluded that induced PE-like symptoms in pregnant mice by L-NAME caused increased fetal growth restriction, impairment of placental histology, and histopathology of the kidneys of fetuses. On the other hand, vitamin D ameliorated the effect of L-NAME and reduced the risk of PE.

Keywords:
blood pressure, L-NG-nitro arginine methyl ester, preeclampsia, vitamin D

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Introduction

Hypertensive disorders and complications of pregnancy can lead to maternal and infant mortality and morbidity. The main characteristic of preeclampsia (PE) is abnormal maternal vascular physiology [1]. There were many risk factors for PE, such as primigravida, twin pregnancy, having a history of PE, and drinking alcohol during pregnancy [2]. Moreover, placental maternal vascular malperfusion was more common in PE with significant proteinuria [3]. Weak neurological outcomes may be related to the mother's placental malperfusion in early gestational age [4].

On the other hand, PE was defined as a complex, multiorgan disease associated with pregnancy [5]. Moreover, PE, especially early-onset PE, was a medical condition in the placenta that caused some complications for the mother and the fetus, such as fetal growth restriction [6]. Meanwhile, Motta *et al.* [7] suggested that sildenafil treatment can be used without causing harmful effects.

On the PE mouse model by L-NG-nitro arginine methyl ester (L-NAME), which had a vasodilator effect and reduced the nitric oxide (NO). Moreover, the pathological effects of L-NAME administered in rats, such as high blood pressure, proteinuria, and podocyturia were very similar to PE syndrome [8].

The PE mice model produced by L-NAME injection was confirmed by the high systolic blood pressure, proteinuria, and the PE placenta suffered excessive

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autophagy due to long-term anoxic circumstances [9]. Autophagy is a factor in the symptoms of PE. Also, they suggested that esomeprazole inhibits excessive placental autophagy in PE, which reduces PE-like symptoms. However, Schulz *et al*. [10] demonstrated that vitamin D3 supplementation improves placental health for pregnant women at risk of potentially developing vascular diseases. Also, at 23–28 weeks of gestation, a vitamin D deficiency was linked to a higher chance of developing PE [11]. Whereas women diagnosed with PE have been found to have an imbalance in proangiogenic and antiangiogenic factors [12]. Studies using cell cultures have shown that vitamin D can influence angiogenesis. However, it is unclear how maternal vitamin D influences placental angiogenesis in PE; further research is necessary [13]. However, there is a statistically significant association between vitamin D insufficiency and PE, suggesting that vitamin D deficiency could be a risk factor for PE [13].

Maternal vitamin D insufficiency is associated with an increased risk of preterm birth [14,15]. In addition, the higher vitamin D concentration during pregnancy could be linked with a reduced risk of PE and prematurity [16]. However, research was inconclusive regarding the link between vitamin D insufficiency and the risk of developing PE [17]. So, the present study aimed to evaluate the effect of vitamin D on 18-day-old mice fetuses, where PE syndrome was induced in pregnant mice by L-NAME.

Materials and methods

Drugs

L-NAME (Alfa Aesar; Thermo Fisher Scientific Chemicals Inc., Waltham, Massachusetts, USA) was prepared in distal water and stored in a freezer at −20°C. Vitamin D tablets (Jamieson Wellness Inc., Canada) are freshly prepared daily as aqueous dispersions in distilled water and stored in an amber-colored glass container at room temperature. The Research Ethics Committee at the Faculty of Science, Ain Shams University was accepted to conduct and approve this study. The conduct of this study adhered to the code of ethics and animal rights standards ASU-SCI/ZOOL/2023/9/2.

Animal breeding and grouping

This experimental study was performed in the laboratory of the Department of Zoology, Faculty of Science, Ain-Shams University, Cairo, Egypt. The CD-1 mice with an average body weight of 20–30 g were obtained from the breeding unit of Theodor Bilharz Research Institute (TBRI, Giza). Virgin males and females were housed separately in wire cages, keeping light–dark cycles of 12/12 h. In addition to tap water, the mice were given daily cubes containing crude protein, fiber, and minerals.

Pregnancy was obtained by housing one adult male with two adult virgin females in the proestrus stage overnight, from 5.00 pm till 9.00 am of the next day. Fertilization was demonstrated either by the formation of vaginal plugs or by the presence of spermatozoa in the vaginal smears. The assured pregnant females were divided into four groups, five females per each. The control group (group I) served as a control group; the individuals of second group (group II) were injected intraperitoneally with L-NAME (50 mg/kg/day) [18] from day 7 to day 14 of gestation. The pregnant females of the third group (group III) were given daily oral vitamin D (50 IU/kg/day) [19] from day 7 to day 14 of gestation. The fourth group (group IV) was injected with L-NAME (50 mg/kg/day) and simultaneously given oral vitamin D (50 IU/kg/day) from day 7 to day 14 of gestation.

Examination of mothers and fetuses

The gestation period of mice was 20–21 days. The control and the experimental groups' maternal body weight were reported daily during the gestation period. Females of both control and experimental groups were dissected on the 18th day of pregnancy. The number, length, and body weight of fetuses were recorded. Moreover, the placental weight was recorded.

Histological examination

The paraffin sections of the placenta and kidney from fetuses of all groups were stained with Harris's hematoxylin and eosin. Sections of the placenta and kidneys from each group were thoroughly examined by light microscope.

Statistical analysis

Numerical data were expressed as mean±SD. The significance of the interrelation of the treated groups to the control group was tested using a one-way analysis of variance and Student *t* test. All statistics were processed by using Origin Pro 6.1 software (One Roundhouse Plaza, Northampton, MA 01060, USA).

Results

Effect on mother parameter

The data recorded indicate that the pregnant females of both control and experimental groups showed a regular increase in the mother's body weight during the first

Table 1 Mother weight (g), fetus length (cm), fetus weight (g), placental weight (g), and placenta–fetus index in all experimental groups

Groups	Mother weight			Fetuses no.	Fetus length (cm)	Fetus weight (g)	Placenta weight (g)	Placenta–fetus index
	0 day of gestation	18th day of gestation	Mean increase(g)					
Group I	26.6±0.89	45.4±1.81	18.8±1.92	8.8±0.91894	2.58±0.19	1.32±0.19	0.146±0.00548	0.11±0.02
Group II	26.8±1.30	44.8±4.14	18±4.84	5±4.34613*	2.32±0.13*	1.22±0.04	0.097±0.01718*	0.07±0.01*
Group III	26±1.41	41.4±1.94*	15.4±0.89*	8.3±0.82327	2.44±0.27	1.30±0.10	0.138±0.03962	0.09±0.02
Group IV	25.8±1.30	38.8±3.11*	13±2.91*	6.5±2.71825*	2.38±0.14	1.20±0.15	0.116±0.02966	0.8±0.02

*A significant difference at *P* value ($P<0.05$).

week of gestation. After 18 days of pregnancy, the least increase in the maternal body weight was noticed in group IV (13±2.91) in which the pregnant mice were injected with L-NAME (50 mg/kg/day) from day 7 to day 14 of gestation simultaneously with vitamin D (50 IU/kg/day) from day 7 to day 14 of gestation (Table 1).

Effect on the body length and body weight of developing fetuses

The mean body length of 18-day-old fetuses showed a significant decrease in group II (2.32±0.13) as compared with that of the control (2.58±0.19). However, fetuses of groups II, III, and IV showed a nonsignificant decrease of mean fetal body weight (1.22±0.04, 1.30±0.10, and 1.20±0.15, respectively), as compared with that of the control group (1.32 ±0.19) (Table 1 and Fig. 1).

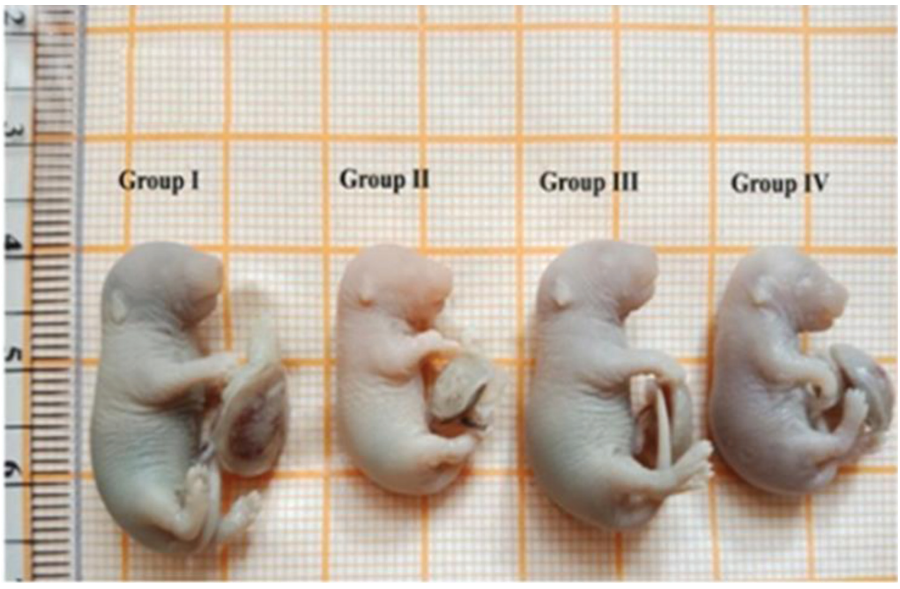
Effect on the placental–fetuses index

The current work has studied placental weight and its relationship to fetus size at birth. A highly significant decrease in the placenta–fetus index (ratio) was recorded in group II (0.07±0.01) as compared with that of control (0.11±0.02) (Table 1).

Histological results of the placenta

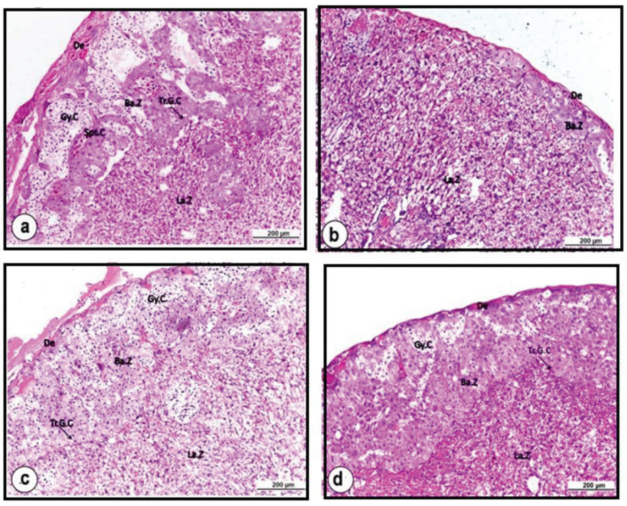
The mice have a discoid type of placenta. Histologically, as shown in section of the control group, mice's placenta is composed of the decidua, the basal zone, and the labyrinth zone (Fig. 2a). The histological results revealed that the placenta of mice injected with L-NAME (50 mg/kg/day) (group II) showed a marked decrease in basal zone length with a reduction, cytolysis in glycogen cell islands with marked dilatation of maternal sinusoids. Congested blood vessels were observed in the labyrinth zone, as degeneration and necrosis of the trophoblasts. Also, maternal blood vessels showed irregular dilatation, necrotic cells, and the corruption of epithelial cells lining the blood vessels. On the other hand, the section of the placenta of the mice injected with L-NAME (50 mg/kg/day) synchronously with oral vitamin D (50 IU/kg/day) (group IV) showed ameliorative effects of placental tissue with mild degenerative changes and mild cytolysis of glycogen cells. However, the labyrinth zone showed some hemorrhage in the fetal blood vessels, degeneration of epithelial cells lining blood vessels, and some trophoblastic cells showed necrosis. The placenta of

Figure 1



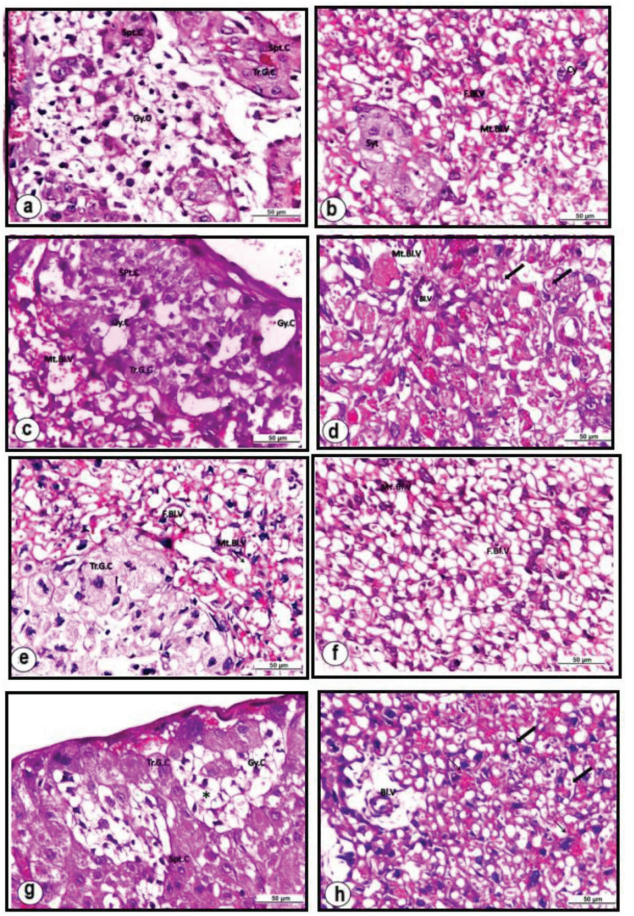
Photograph of 18-day-old fetuses in all experimental groups.

Figure 2



Photomicrograph of sections of the placenta (a) normal placenta of the control group (group I) showing decidua (De), basal zone (Ba.Z), and labyrinth zone (La.Z), spongiotrophoblast (Spt. C), glycogen cell (Gy. C), and trophoblastic giant cell (Tr. G.C). (b) Placenta maternally injected by L-NAME (group II) showing a marked decrease in basal zone length. (c) Placenta maternally administered orally by vitamin D (group III). While (d) placenta maternally treated by L-NAME and vitamin D (group IV) showed a marked increase in basal zone length compared with that in group II. Scale bar=200 μm (hematoxylin and eosin stain).

Figure 3



Photomicrograph of a section of placenta of the control group (group I) (a and b); (c and d) section of the placenta maternally injected by L-NAME (group II), showing cytolysis of glycogen cells (Gy. C) in the basal zone and the maternal blood vessels (Mt. Bl.V) showing irregular dilatation, necrotic cell (thick arrow) and degeneration of epithelial cells lining blood vessel (Bl. V). (e and f) placenta maternally administered orally by vitamin D (group III). (g and h) Placenta maternally treated by L-NAME and vitamin D (group IV) shows congestion in the fetal blood vessels (F. Bl. V) (thin arrows), degeneration of epithelial cells lining the blood vessel (Bl. V), and necrotic cells (thick arrow). Scale bar=50 μm (hematoxylin and eosin stain).

mice given oral vitamin D (50 IU/kg/day) during gestation more or less resembles the normal placenta in group I (Figs 2 and 3).

Histological results of the fetus's kidney

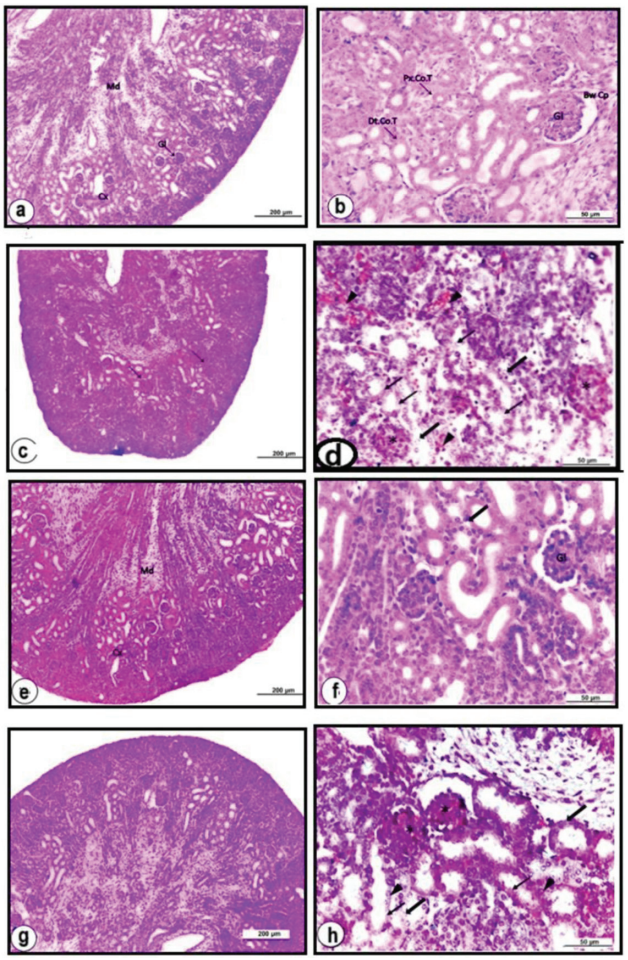
The histological results of the present study of the kidney in fetuses (18 days old) maternally injected by L-NAME showed an apparent enlargement in the glomerular area. In addition, hemorrhages were present among the tubules. The epithelial cells of convoluted tubules exhibited degenerative changes, including nuclear pyknosis; cells lacked distinct boundaries and unrecognized nuclei of some proximal tubules compared to the control group. However, fetuses (18 days old) maternally treated by L-NAME and vitamin D (group IV) showed mild injury, whereas some glomeruli showed standard structure with ordinarily Bowman's space. On the other hand, some glomeruli appeared small with wider Bowman's space. However, the epithelial lining of proximal and distal convoluted tubules was distorted, and there was a complete absence of some nuclei. The medullary region exhibited some pathological changes in collecting tubules, including signs of necrosis, and most of the nuclei were utterly absent (Fig. 4).

Discussion

One of the most common causes of pregnancy-related maternal and fetal morbidity and mortality around the world is hypertensive disorders of pregnancy. PE is the most severe hypertensive disorder of pregnancy and represents one of the causes of maternal death [20]. Then, L-NAME has been used to generate PE-like symptoms in mice, causing elevated blood pressure and proteinuria in mothers. Also, placental morphology disruption, fetal growth reduction, and other significant organ and vascular changes [21].

L-NAME is one of the nitric oxide syntheses inhibitors and is used to produce a PE rat model

Figure 4



Photomicrograph of sections of the kidney of 18-day-old fetuses; (a and b) control fetus showing the normal structure of the two regions of the kidney; the cortex (Cx) with glomerulus (Gl), the medulla (Md) proximal and distal convoluted tubules (Px. Co. T), and (Dt. Co. T), respectively. While (c and d) sections of the kidney of group II show hypertrophy of the glomeruli (arrows) and complete absence of some of the nuclei of convoluted tubules (thin arrows). Moreover, the presence of pyknotic nuclei in the collecting tubules (thick arrows) and the presence of hemorrhage among the tubules (arrowheads); (e and f) sections of the kidney of vitamin D (group III) show the normal structure of the cortical (Cx) and medullary (Md) regions with normal glomerulus (Gl) and convoluted tubules (thick arrow); (g and h) sections of the kidney of group IV showing some ameliorative effect of some glomeruli however, other glomeruli with a wide Bowman's space (asterisks). The complete absence of some nuclei in the cells of the convoluted tubules (thick arrows), signs of necrosis in the collecting tubules (arrowheads), and most of the nuclei completely disappeared (thin arrows) are observed. Scale bar=200 μm & 50 μm (hematoxylin and eosin stain).

due to the similarities in clinical characteristics between PE women and L-NAME-treated rats, such as hypertension and renal abnormalities [22]. L-NAME, a known inhibitor of nitric oxide generation, can cause a syndrome that mimics PE in humans when it is given to pregnant animals during their middle to late gestational period, which was demonstrated by decreased placental perfusion, increased generation of reactive oxygen species), hypertension, and proteinuria [23]. According to clinical and experimental results, reduced placental perfusion is a distinctive characteristic that may cause PE. PE is frequently discovered in conditions with microvascular problems [24]. Higher vitamin D during pregnancy was linked to a lower risk of PE, according to a recent review and meta-analysis [25]. In the present study a steady increase in maternal body weight in all groups during the gestation period was observed. However, on the 18th day of gestation, groups III and IV recorded a significantly lower body weight than other groups. This is in agreement with Fernández Celadilla *et al.* [26] who reported a lower body weight and BMI in the preeclamptic cases. In contrast to Soares de Moura *et al.* [27] and Zamzam *et al.* [28], the L-NAME injection into pregnant rats was found to induce a significant increase in body weight and BMI. Vitamin D may improve fat reduction and aid with weight loss [29]. On the other hand, some research mentioned that vitamin D supplementation did not significantly change final body weight gain [30]. In the present study, 18-day-old fetuses of group II showed a slightly significant decrease in the mean body length compared with other groups. However, members of groups II, III, and IV showed nonsignificant differences in mean fetal body weight compared to the control group.

The placental weight and structure have a strong relationship with the fetus size at birth, so, in the present work, a highly significant decrease in the placenta–fetus index (ratio) was recorded in group II compared with that of the control. This may be due to L-NAME inhibiting nitric oxide synthase, which could end in fetal intrauterine growth restriction and maternal hypertension. L-NAME is also used in experiments to mimic PE. Accompanied by decreased nutrition transfer in the syncytial trophoblast and increased free radicals in the spongiotrophoblast, the placenta is hypoxic. Additionally, e-nitric oxide syntheses/-mice have shorter capillary lobules and a lower capillary density [31]. The current findings revealed that the placenta of mice injected with L-NAME (50 mg/kg/day) from day 7 to day 14 of gestation (group II) showed a marked decrease in basal zone length with a reduction in glycogen cell islands. The basal zone showed cytolysis of glycogen cells with marked dilatation of maternal sinusoids, and bleeding was observed in the labyrinth zone. The labyrinth zone showed congestion of maternal and fetal blood vessels, degeneration, and necrosis of the trophoblasts. The maternal blood vessels showed irregular dilatation, necrotic cells, and the corruption of epithelial cells lining the blood

vessels. In the study of de Alwis *et al.* [32], mice with L-NAME administration showed a significant reduction in placental weight and fetal crown-to-rump length. However, the cross-sectional area and the areas of the blood space, junctional zone, and labyrinth zone were not significantly changed in the placentas taken from mice that were given L-NAME. Moreover in the present study, the section of the placenta of the mice injected with L-NAME (50 mg/kg/day) synchronously with oral vitamin D (50 IU/kg/day) from day 7 to day 14 of gestation (group IV) showed ameliorative effects with mild degenerative changes and mild cytolysis of glycogen cells. On the other hand, the labyrinth zone showed marked hemorrhage in fetal blood vessels, distortion of epithelial cells lining the blood vessels, and some trophoblastic cells showed necrosis. Vitamin D supplementation is a safe and well-tolerated treatment [33]. Vitamin D promotes decidualization and extravillous trophoblast invasion in the placenta [34]. Recently, treating a PE rat model with vitamin D improved placental angiogenesis and decreased blood pressure [35]. PE is a multiorgan disorder characterized by serious dysfunctions, including severe liver and kidney damage, vascular injury, and placental ischemia [36]. The whole kidney tissue might be damaged, resulting in acute kidney injury and other pregnancy-related complications like shock and sepsis [37]. The histological results in this study on the kidney of fetuses (18 days old) maternally injected by L-NAME showed an apparent enlargement in the glomerular area. In addition, the presence of hemorrhages among the tubules. On the other hand, fetuses (18 days old) maternally treated with L-NAME and vitamin D showed some ameliorative effects. These findings were similar to the histopathological changes that other studies have shown [38,39]. Inappropriate placentation and trophoblast invasion, which produce placental ischemia and hypoxic conditions during pregnancy, are contributing factors to PE. The disproportionate release of proangiogenic and antiangiogenic agents, such as soluble FMS-like tyrosine kinase 1, soluble endoglin, and placental growth factor and vascular endothelial growth factor, as well as the stimulation of inflammatory cells that release inflammatory cytokines and autoantibodies, are the results of placental ischemia. This, in turn, leads to a significant increase in oxidative stress and the release of inflammatory cytokines and autoantibodies, which in turn causes hypertension, renal endothelin, and blood coagulation [40,41]. PE is characterized by endothelial dysfunction, decreased vasodilation, high peripheral resistance, and renal abnormalities (proteinuria, decreased glomeruli filtration, and glomeruloendotheliosis) [42].

Vitamin D treatment reduces the production of certain placental vascular genes (soluble FMS-like tyrosine kinase-1, vascular endothelial growth factor), whose increased levels were linked to PE, and increases T cell helper 2 dominance. These elements could contribute to vitamin D's protective effects [43,44]. Finally, those potentially beneficial effects of vitamin D supplementation in L-NAME in the present work are supported by the marked improvement in results of the histopathological studies as regards both the placenta and the fetal kidney.

Conclusion

This study concluded that L-NAME promotes pregnant mice to exhibit symptoms resembling PE, causing increased fetal growth restriction, impairment of the placenta, and histopathology of the kidney of fetuses. On the other hand, vitamin D administration showed an ameliorative effect in placental tissue and kidneys. These findings support the treatment with vitamin D supplementation during pregnancy as a protective agent against PE symptoms.

Authors' contributions

Authors' contributions: Sarah H. Abd El Rahman completed laboratory tasks, data analysis, and manuscript writing. Hamza El Shabaka assisted in creating the work plan, manuscript writing, and revision. Mervat El Ansary assisted in manuscript revision. Hend A. Mohammed assisted with work environments, work plan formulation, data analysis, and manuscript writing and revision.

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Conflicts of interest

There are no conflicts of interest.

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