Physiology and Pharmacology 27 (2023) 254-260
Original Article

# The effect of tempol, a potent synthetic antioxidant, on the limb teratogenicity in an experimental model of preeclampsia in rats

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## ABSTRACT

**Introduction:** Preeclampsia is the principal cause of maternal morbidity and is characterized by hypertension, proteinuria, and edema. It is believed that oxidative stress plays an essential role in the pathophysiology of preeclampsia. This study was conducted to determine the effect of tempol on fetal limb hemorrhage, malformations, and oxidative stress in an N-nitro-L-arginine methyl ester (L-NAME)-induced preeclamptic rat model.

**Methods:** To induce preeclampsia, L-NAME (50 mg/kg/day, oral) was administered from day 11 of pregnancy to day 22. Four preeclamptic groups received L-NAME alone, L-NAME+tempol (20, 60, 180 mg/kg/day; L-NAME, L-T20, L-T60, L-T180 groups, respectively). The control group (normal pregnant) received only tap water, and the T60 group received tempol (60 mg/Kg) alone (without L-NAME). The concentration of 8-isoprostane in plasma and placenta, number and weight of the fetuses, and the limb defects were measured on the 22nd day of the pregnancy.

**Results:** L-NAME administration caused placental oxidative stress, limb defects and hemorrhage, and low fetal weight. Administration of tempol at 20 and 60 mg/kg/day reduced limb defects (10.5 and 7.2 percent versus 24.7 percent) and limb hemorrhage (14.5 and 9.5 percent versus 23 percent) induced by L-NAME. After administration of tempol (20 and 60 mg/kg), fetal weight increased (5.14±0.08 and 5.44±0.15 versus 4.27± 0.11 g). Administration of tempol at the high dose (180 mg/kg/day) did not produce any significant effects on the measured parameters.

**Conclusion:** Tempol with certain doses improves fetal outcomes in an experimental rat model of preeclampsia. These may be the results of its antioxidant action.

**Keywords:**
Limb malformation
L-NAME
Oxidative stress
Preeclampsia
Rat
Tempol

## Introduction

Preeclampsia is the chief cause of maternal morbidity and is characterized by hypertension, proteinuria, and edema (Noris, 2005; Ramos et al, 2017). The etiology of preeclampsia is not very well understood. A current hypothesis is that reactive oxygen species (ROS) have an essential role in the development of maternal complications (i.e., hypertension and proteinuria) (Burton and Jauniaux, 2004; Gupta et al, 2005; Ramos et al, 2017; Roggensack et al, 1999; Sedeek et al, 2008) and

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Received 13 September 2021; Revised from 30 July 2022; Accepted 27 August 2022

Citation: Namavar M, Sharif Talebianpoor M, Nazari S, Mirkhani H. The effect of tempol, a potent synthetic antioxidant, on the limb teratogenicity in an experimental model of preeclampsia in rats. Physiology and Pharmacology 2023; 27: 254-260. http://dx.doi.org/10.61186/phypha.27.3.254

fetal complications (i.e., hemorrhage and Limb defects) of preeclampsia (Tenório et al, 2019). A variety of animal models, such as reduced uterine perfusion pressure (RUPP), administration of endothelin, single-dose injection of suramine, and administration of L-NAME, have been introduced to recognize the pathogenesis of the disease (Cushen and Goulopoulou, 2017; Podjarny, 1999). The L- NAME rat model, because of its similarity to human preeclampsia, has become increasingly popular. Administration of L-NAME, a well-known inhibitor of NO production, to experimental animals during late gestation induces a syndrome resembling preeclampsia in humans and is associated with increased ROS production, hypertension, proteinuria, and limb malformations (Amaral et al, 2018; Sedeek et al, 2008; Tiboni et al, 2003).

It has been suggested that ROS generation is an essential factor in the L-NAME-induced teratogenicity model. several radical scavengers such as Alpha-phenyl-N-t-butyl nitrone (PBN) and quercetin, significantly restored fetal malformations and fetal weight reduction induced by L-NAME in rats (Fantel, 2002; Tanir et al, 2005). Moreover, in our previous study, we showed that tempol, as a potent antioxidant, prevented hypertension (HTN) and proteinuria induced by L-NAME in pregnant rats (Talebianpoor et al, 2012). Tempol is a superoxide dismutase (SOD)–mimetic agent with significant antioxidant activity. It has excellent cell permeability because of its low molecular weight (Beckman, 1996). It has been reported that tempol produces a protective effect in diabetic-induced embryonic damage (Ryu et al, 2007). So, it is possible that tempol may have beneficial effects on fetal complications of preeclampsia, such as embryonic damage induced by L-NAME. In addition, to our knowledge, no report about the effect of tempol on the fetuses of normal animals has been published. Therefore, this study aimed to evaluate whether the administration of tempol during the second half of pregnancy ameliorates L-NAME–induced teratogenicity or not. Also, the effect of tempol on normal pregnant rats was studied.

## Materials and methods

*Animals*

Female Sprague-Dawley rats (250-300 g) were purchased from the animal house of the Shiraz University of Medical Sciences (SUMS). Animal procedures were approved by the institutional ethics committee of Shiraz University of Medical Sciences (Ethical code: IR.SUMS.REC.1388.S4895). All animals were kept in cages at a temperature-controlled room (23±1 $^{o}$C) with a 12-h artificial light and dark cycle. The female rats were mated at night with male Sprague-Dawley rats. Day 0 of pregnancy was defined as the day when vaginal plugs were found (8). On day 10 of pregnancy, the rats were divided into five groups: in group I, normal animals received only distilled water (normal group); group II was treated with L-NAME (Alexis Biochemical, USA, 50 mg/kg/day, through drinking water; L-NAME group); groups III, IV and V were treated with L-NAME (50 mg/kg/day) plus tempol (Sigma chemical, UK, 20, 60, 180 mg/kg/day, respectively, through drinking water; L-T20, L-T60, L-T180 groups). To study the potential teratogenic effect of tempol; group VI received only tempol (60 mg/kg/day; T60 group).

*Measurement of plasma and placental 8-isoprostane*

On gestational day 20, a blood sample was taken via the tail vein, then centrifuged at 3000 rpm, and plasma was collected and immediately frozen to -80 °C until analysis. Plasma 8-isoprostane concentration was measured by an enzyme immunoassay kit. The amount of placenta isoprostane was determined as a ratio to protein in the sample, determined by the Bradford method (Bradford, 1976).

*Fetal limb malformations and hemorrhage*

On the 22$^{nd}$ day of gestation, pregnant rats were sacrificed and subjected to laparotomy under deep ether anesthesia. Uteri were removed and fetal weights were recorded.

The external surfaces of fetal limbs were examined for hemorrhage and abnormalities. For hemorrhage evaluation, we reported both the incidence (percentage) and severity of hemorrhage. In order to compare the severity of limb hemorrhage, the following scale was developed: 0, normal; 1, minimal hemorrhage in distal digit(s); 2, moderate hemorrhage in foot and/or hand; 3, hemorrhage, edema in entire limb(s); and 4, extensive hemorrhage and edema with necrotic limbs separating from the body and/or hemorrhage extending into adjacent flank (Fantel et al, 1999).

The forelimb and hindlimb malformations have also been evaluated for their incidence (percentage). The

**TABLE 1:** Fetal outcome, pregnant rat weight, fetal weight, placental weight, plasma and placenta isoprostane in different groups at the 22nd day of gestation

| Groups | Number of fetuses | Pregnant rat weight (g) | Fetal weight (g) | Placental weight (g) | Placenta isoprostane (Pg/mg per tissue) | Plasma isoprostane (Pg/ml) |
|---|---|---|---|---|---|---|
| Normal | 10.1±0.4 | 270±4** | 5.70±0.11*** | 0.35±0.01 | 99.4±12.8** | 25.9±4.4*** |
| L-NAME | 10.0±0.6 | 250±4 | 4.27± 0.11 | 0.31± 0.01 | 214.2±25.2 | 123.5±22.7 |
| L-T20 | 10.0±0.7 | 269±4* | 5.14±0.08** | 0.32±0.01 | 66.8±12.0*** | 27.5±4.3** |
| L-T60 | 8.7±0.7 | 269±3* | 5.44±0.15** | 0.33±0.01 | 52.0±9.5*** | 22.2±5.1** |
| L-T180 | 10.1±0.7 | 243±4 | 4.02±0.10 | 0.31±0.01 | 200.2±43.4 | 156.8±34.2 |
| T60 | 8.8±0.9 | 267±3 | 5.71±0.09 | 0.35±0.01 | 74.5±9.8 | 23.2±6.9 |

The values are expressed as mean±SEM of 11 pregnant rats in each group. Normal: pregnant rats that received no treatment; L-NAME: rats that received L-NAME (50 mg/Kg) alone; L-T20, L-T60 and L-T180 are rats that received tempol at doses of 20, 60 and 180 mg/Kg, respectively, in addition to L-NAME. T60: rats that received tempol (60 mg/Kg) alone (without L-NAME). The treatments were started from day 11 of gestation. *$P$<0.05, **$P$<0.01, ***$P$<0.001 compared to the L-NAME group. The T60 group has been compared with normal rats.

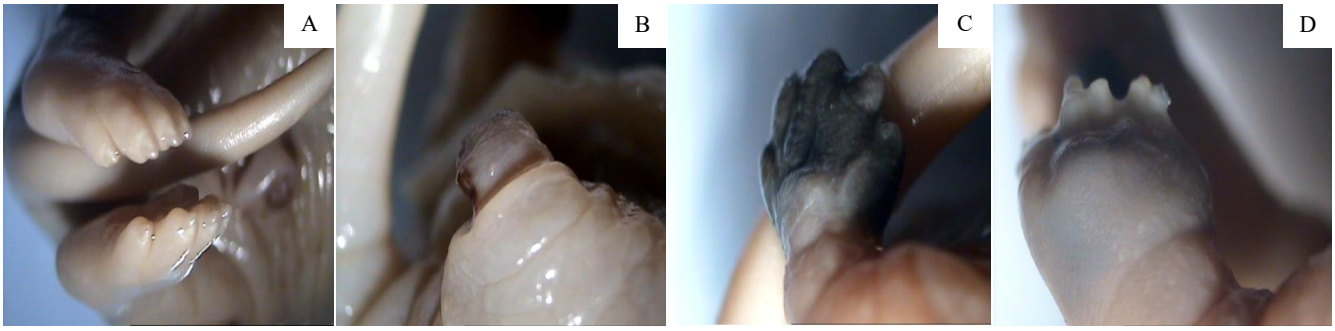


**FIGURE 1.** External limb morphology of rat fetus on the 22nd day of gestation. A) Normal hindlimb from normal group. B) Hindlimb from L-NAME group with amelia and hemorrhage. C) Hemorrhagic lesions with fused digit and micromelia are seen in hindlimb of the fetus from L-T180 group. D) Fused digit and hemorrhagic lesion from the L+T60 group.

type of malformations [fused digits, micromelia (partial absence of a limb or limbs), amelia (lacking one or more limbs)] was also considered (Makris et al, 2009).

*Statistical analysis*

Continuous data were compared using one-way analysis of variance (ANOVA) and the Tukey post hoc test. The chi-square test was used to evaluate the differences in the percentage of limb malformations and hemorrhage among groups. The severity of hemorrhage between groups was analyzed by the Kruskal-Wallis test. A $p$<0.05 was considered to be statistically significant.

## Results

*Plasma and placenta concentration of isoprostane*

The isoprostane level in plasma and placenta as indices of oxidative stress, was higher in the L-NAME group compare to the normal pregnant rats ($p$<0.001; Table 1). Tempol at doses of 20 and 60 mg/kg reduced plasma ($p$<0.01) and placenta ($p$<0.001) isoprostane levels in preeclamptic rats, while the dose of 180 mg/kg had no meaningful effect on this parameter (Table 1). Administration of tempol (60 mg/kg) to normal pregnant rats had no significant effect on plasma and placenta isoprostane levels (Table 1).

*Pregnancy and fetal outcomes*

Maternal death or preterm parturition was not observed before day 22 of the pregnancy during the study. The body weight of the pregnant rats and their fetuses was significantly reduced in the L-NAME group compared to the normal pregnant rats ($p$<0.001, Table 1). Tempol at doses of 20 and 60 mg/kg increased the body weight of preeclamptic rats and their fetuses ($p$<0.01), while the dose of 180 mg/kg had no meaningful effect on these parameters (Table 1). Administration of tempol (60 mg/kg) to normal pregnant rats had no significant effect on placental weight and their fetuses (Table 1)

*Limbs malformation and hemorrhage*

L-NAME caused limb defects, digits missing, and in some instances, subcutaneous hematomas (Figure1). The

**TABLE 2:** Limbs malformations in different groups at the 22nd day of gestation

| Groups | Number of studied fetuses | Amelia | Micromelia | Fused digit | Fused digit + micromelia | Total malformations |
|---|---|---|---|---|---|---|
| Normal | 62 | 1(1.6) | 0 | 0 | 0 | 1(1.6%)*** |
| L-NAME | 61 | 4(6.6) | 3(5%) | 1(1.6) | 7(11.5%) | 15(24.7%) |
| L-T20 | 48 | 2(4.2) | 0 | 2(4.2) | 1(2.1%) | 5(10.5%)* |
| L-T60 | 42 | 1(2.4) | 1(2.4%) | 0 | 1(2.4%) | 3(7.2%)* |
| L-T180 | 63 | 1(1.6) | 2(3.2%) | 3(4.8) | 12(19%) | 18(28.6%) |
| T60 | 28 | 0 | 1(3.6%) | 0 | 0 | 1(3.6%) |

Incidence (total number and percentage) of amelia, micromelia, fused digit, fused digit+micromelia and total malformations in studied groups. Normal: pregnant rats that received no treatment; L-NAME: rats that received L-NAME (50 mg/Kg) alone; L-T20, L-T60 and L-T180 are rats that received tempol at doses of 20, 60 and 180 mg/Kg, respectively, in addition to L-NAME. T60: rats that received tempol (60 mg/Kg) alone (without L-NAME). The treatments were started from day 11 of gestation. *$P$<0.05, **$P$<0.01, ***$P$<0.001 compared to the L-NAME group. The T60 group has been compared with normal rats.

**TABLE 3:** Limbs hemorrhage in different groups at the 22nd day of gestation

| Groups | Number of studied fetuses | Hemorrhage (%) | Average severity score (mean±SEM) |
|---|---|---|---|
| Normal | 62 | 3(4.8%)** | 0.06±0.04*** |
| L-NAME | 61 | 14(23%) | 0.34±0.09 |
| L-T20 | 48 | 7(14.5%) | 0.17±0.06 |
| L-T60 | 42 | 4(9.5%) | 0.10±0.05 |
| L-T180 | 63 | 20(31.7%) | 0.46±0.09 |
| T60 | 28 | 2(7.1%) | 0.11±0.08 |

Incidence (percentage) of hemorrhage and average severity score in studied groups. Normal: pregnant rats that received no treatment; L-NAME: rats that received L-NAME (50 mg/Kg) alone; L-T20, L-T60 and L-T180 are rats that received tempol at doses of 20, 60 and 180 mg/Kg, respectively, in addition to L-NAME. T60: rats that received tempol (60 mg/Kg) alone (without L-NAME). The treatments were started from day 11 of gestation. *$P$<0.05, **$P$<0.01, ***$P$<0.001 compared to the L-NAME group. The T60 group has been compared with normal rats.

incidence (total number and percentage) of the different types of malformations is shown in table 2. The percentage is compared to the total fetus number (100%) in each group. As shown in table 2, the L-NAME group had a higher frequency of total malformations compared to the normal group (24.7% versus 1.6%, respectively, $p$<0.001). The L-T60 group had a statistically lower frequency of total malformations compared to the L-NAME group (7.2% versus 24.7%, respectively, $p$<0.05) (Table2). Also, tempol at a dose of 20 mg/kg reduced the frequency of total malformations (10.5%versus 24.7%, $p$=0.05, Table 2). While, tempol at a high dose (180 mg/kg/day) had no significant effect on the teratogenic effects of L-NAME on fetuses (Table 2 and Table 3). Rare limb abnormalities and hemorrhage were seen in the normal and T60 groups with no significant difference between these two groups (Table 2 and Table 3). There were no significant sex-related differences in the occurrence of limb anomalies (not shown). Limb malformations were more frequent on the left side ($p$<0.001), while hemorrhage was observed more frequently on the right side ($p$<0.001; not shown).

Table 3 shows the limb hemorrhage in different groups. Results of hemorrhage in groups were almost similar to malformations as shown in table 3. Similarly, the limb hemorrhage and average severity score in the L-NAME group were higher than the normal group ($p$<0.01-0.001, Table3). Tempol at dose 60 reduced the percentage of hemorrhage non-significantly and also had an average severity score with $p$=0.053.

## Discussion

Preterm delivery, intrauterine growth restriction, placental abruption, and prenatal death are the most critical fetal complications of preeclampsia (Ornaghi et al, 2017; Sedeek et al, 2008). Vast attempts have been

carried out to define the pathogenesis of this syndrome and discover effective treatments. The aim of the present work was to determine the effect of tempol on limb teratogenicity in a rat model of preeclampsia.

Experimental preeclampsia was induced by the administration of L-NAME. It is well-known that administration of L-NAME to the pregnant rat result in maternal and fetal complications resembles preeclampsia. In our experiments, we observed some limb abnormalities (discussed below) but no considerable changes in pregnancy outcomes, other than reduced fetal weight (Table 1). In contrast to our results, some researchers have reported that treatment of pregnant rats with L-NAME produces considerable effects on fetus weight, the number of fetal resorption, and alive fetuses (Altoama et al, 2016; Helmbrecht et al, 1996; Podjarny et al, 2001). This discrepancy may be related to the route, dose, and duration of L-NAME administration.

L-NAME, also caused fetal malformations and limb hemorrhage, findings consistent with other reports (Talebianpoor et al, 2018).

Although the exact mechanisms of the L-NAME-induced fetal malformations are not clear very well, it is proposed that a combination of maternal and placental factors are involved. As mentioned above, oxidative stress plays a vital role in the L-NAME model of preeclampsia. Our results showed that L-NAME elevated plasma and placenta 8-isoprostane. Also, a previous study on pregnant rats showed that it increased maternal plasma MDA. (Gupta et al, 2005). These are markers of oxidative stress. By reduction of NO synthesis and increase of oxidative stress, it seems that L-NAME causes vascular insult in both mothers and fetuses (maternal blood pressure, placenta perfusion, myometrium contraction and perfusion, fetal limb vasculature). Vascular insults are known to give rise to limb abnormalities. Vascular insults may be a reduction in blood flow secondary to prolonged or intense vasoconstriction, thromboembolic events, or impaired angiogenesis (Gregg et al, 1998).

Tempol significantly reduced fetal malformation at doses of 20 and 60 mg/kg/day. Tempol at dose 60 mg/kg/day also reduced hemorrhage ($p$=0.053). Also, tempol (20 and 60 mg/kg/day) significantly reduced plasma and placenta 8-isoprostane and improved fetal weight reduction. In our previous study, reduction of plasma MDA by tempol was observed (Talebianpoor et al, 2012). Our results show that there is a good correlation between oxidative stress and fetal weight reduction, malformation, and hemorrhage. This finding has also been reported by other researchers (Fantel, 2002; Tanir et al, 2005). It is rational to assume that tempol as a potent antioxidant potentiates body antioxidant defense and prevents malformation induced by L-NAME. In our previous studies, we demonstrated the beneficial effects of tempol on hypertension, proteinuria, and renal injuries in the preeclamptic rats. These effects can be attributed to its antioxidant action or other possible properties such as direct vasodilation action. So, another possibility is that tempol prevented fetal malformations because of the reduction of maternal complications of preeclampsia (Talebianpoor et al, 2012).

The high dose of tempol (180 mg/kg/day) had no practical effects on L-NAME-induced fetal malformation. These results are consistent with our previous finding that showed tempol at a dose of 180 mg/kg/day did not reduce hypertension, proteinuria, and renal injuries in the preeclamptic rats (Talebianpoor et al, 2017). Interestingly, tempol at this dose did not reduce placenta 8-isoprostane; it underlines again the importance of the balance between oxidative stress and antioxidant defense in the pathogenesis of experimental preeclampsia and its complications. A similar result was observed with this dose on maternal MDA level in a previous study (Talebianpoor et al, 2012). Tempol, as a SOD mimetic, converts superoxide radicals to $H_2O_2$. After that, $H_2O_2$ is detoxified by glutathione peroxidase and catalase (Talebianpoor et al, 2017). Tempol at the high dose may generate an excessive level of $H_2O_2$ that cannot be detoxified efficiently. These increased levels of $H_2O_2$ can exacerbate the complications of L-NAME (Preti et al. 2005). This proposed mechanism can justify our observed results with a high dose of tempol. Similar results are reported by others (Ardanaz and Pagano, 2008; García-Redondo et al, 2009; Preti et al, 2005; Rafikova and Tofovic, 2008). Preti *et al*, observed that tempol at 200 mg/kg/day not only cannot reduce hypertension but also worsen that (Preti et al, 2005).

Since, we observed a desirable protective effect of tempol 60 mg/kg on limb teratogenicity in preeclamptic rats; we also administered this dose to normal pregnant rats to evaluate its possible teratogenic effect. Although, an event was observed in this group, a similar situation also occurred in normal pregnant rats without any treat-

ment. So, it seems that tempol, at the applied dose, has no deleterious effect on pregnancy.

## Conclusions

Tempol can decrease the occurrence of fetal limb malformations and hemorrhage in an experimental model of preeclampsia. This effect is dose-dependent and is probably the result of its antioxidant action.

## Conflict of interest

The authors report no conflict of interest.

## Acknowledgements

The authors are very grateful to those who contributed to the study design and implementation. We also acknowledge the efforts of all laboratory technicians in this study. This study was funded by Shiraz University of Medical Sciences, Shiraz, Iran (Grant number: 4895).

## References

Altoama K, Mallem MY, Thorin C, Betti E, Desfontis JC. Effect of nebivolol treatment during pregnancy on the intrauterine fetal growth, mortality and pup postnatal development in the l-NAME-induced hypertensive rats. Eur J Pharmacol 2016; 791: 465-72. https://doi.org/10.1016/j.ejphar.2016.09.028

Amaral TA, Ognibene DT, Carvalho LC, Rocha APM, Costa CA, Moura RS, Resende AC. Differential responses of mesenteric arterial bed to vasoactive substances in L-NAME-induced preeclampsia: Role of oxidative stress and endothelial dysfunction. Clin Exp Hypertens 2018; 40: 126-35. https://doi.org/10.1080/10641963.2017.1339073

Ardanaz NBW, Pagano PJ. Distinct hydrogen peroxide-induced constriction in multiple mouse arteries: potential influence of vascular polarization. Pharmacol Rep 2008; 60: 61-7.

Beckman JS KW. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol; 1996: 271, C1424-37. https://doi.org/10.1152/ajpcell.1996.271.5.C1424

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-54. https://doi.org/10.1016/0003-2697(76)90527-3

Burton GJ, Jauniaux E. Placental oxidative stress: from miscarriage to preeclampsia. JSGI 2004;11: 342-52. https://doi.org/10.1016/j.jsgi.2004.03.003

Cushen SC, Goulopoulou S. New models of pregnancy-associated hypertension. Am J Hypertens 2017; 30: 1053-62. https://doi.org/10.1093/ajh/hpx063

Fantel A, Stamps L, Tran T, Mackler B, Person R, Nekahi N. Role of free radicals in the limb teratogenicity of L-NAME ($N^G$-nitro-$_L$-arginine methyl ester): a new mechanistic model of vascular disruption. Teratology 1999; 60: 151-60. https://doi.org/10.1002/(SICI)1096-9926(199909)60:3<151::AID-TERA11>3.0.CO;2-E

Fantel AGPR. Further evidence for the role of free radicals in the limb teratogenicity of L-NAME. Teratology 2002; 66: 24-32. https://doi.org/10.1002/tera.10047

García-Redondo ABBA, Beltrán AE, Alonso MJ, Simonsen U, Salaices M. Hypertension increases contractile responses to hydrogen peroxide in resistance arteries through increased thromboxane A2, Ca2+, and superoxide anion levels. J Pharmacol Exp Ther 2009; 328: 19-27. https://doi.org/10.1124/jpet.108.144295

Gregg AR, Schauer A, Shi O, Liu Z, Lee CG, O'Brien WE. Limb reduction defects in endothelial nitric oxide synthase-deficient mice Am J Physiol Heart Circ Physiol 1998; 275: H2319-24. https://doi.org/10.1152/ajpheart.1998.275.6.H2319

Gupta S, Agarwal A, Sharma RK. The role of placental oxidative stress and lipid peroxidation in preeclampsia. Obstet Gynecol Surv 2005; 60: 807-16. https://doi.org/10.1097/01.ogx.0000193879.79268.59

Helmbrecht GD, Farhat MY, Lochbaum L, Brown HE, Yadgarova KT, Eglinton GS, Ramwell PW. L-arginine reverses the adverse pregnancy changes induced by nitric oxide synthase inhibition in the rat. Am J Obstet Gynecol 1996; 175: 800-5. https://doi.org/10.1016/S0002-9378(96)80002-0

Makris SL, Solomon HM, Clark R, Shiota K, Barbellion S, Buschmann J, Hazelden KP. Terminology of developmental abnormalities in common laboratory mammals (version 2). Congenit Anom 2009; 49: 123-246. https://doi.org/10.1111/j.1741-4520.2009.00239.x

Noris MPN, Remuzzi G. Mechanisms of disease: Pre-eclampsia. Nat Clin Pract Nephrol 2005: 1: 98-114. https://doi.org/10.1038/ncpneph0035

Ornaghi S, Hsieh LS, Bordey A, Vergani P, Paidas MJ, van den Pol AN. Valnoctamide inhibits cytomegalovirus infection in developing brain and attenuates neurobehavioral dysfunctions and brain abnormalities J Neurosci 2017; 37: 6877-93. https://doi.org/10.1523/JNEUROSCI.0970-17.2017

Podjarny EBC, Losonczy G. Animal models of preeclampsia. Semin Perinatol 1999; 23: 2-13. https://doi.org/10.1016/S0146-0005(99)80055-X

Podjarny E, Benchetrit S, Katz B, Green J, Bernheim J. Effect of methyldopa on renal function in rats with L-NAME-induced hypertension in pregnancy. Nephron 2001;88: 354-59. https://doi.org/10.1159/000046020

Preti SC, Da Cunha V, Vassallo DV, Stefanon I. The superoxide dismutase mimetic, tempol, reduces the bioavailability of nitric oxide and does not alter l-name-induced hypertension in rats. Basic Clin Pharmacol Toxicol 2005; 97: 29-34. https://doi.org/10.1111/j.1742-7843.2005.pto_97105.x

Rafikova OSE, Tofovic SP. Renal and metabolic effects of tempol in obese ZSF1 rats--distinct role for superoxide and hydrogen peroxide in diabetic renal injury. Metabolism 2008; 57: 1434-44. https://doi.org/10.1016/j.metabol.2008.05.014

Ramos JGL, Sass N, Costa, SHM. Preeclampsia. Rev Bras Ginecol Obstet 2017; 39: 496-512. https://doi.org/10.1055/s-0037-1604471

Roggensack AM, Zhang Y, Davidge ST. Evidence for peroxynitrite formation in the vasculature of women with preeclampsia. Hypertension 1999; 33: 83-89. https://doi.org/10.1161/01.HYP.33.1.83

Ryu SKR, Samuni A, Ornoy A. Nitroxide radicals protect cultured rat embryos and yolk sacs from diabetic-induced damage. Birth Defects Res A Clin Mol Teratol 2007; 79: 604-11. https://doi.org/10.1002/bdra.20383

Sedeek M, Gilbert JS, LaMarca BB, Sholook M, Chandler DL, Wang Y, Granger JP. Role of reactive oxygen species in hypertension produced by reduced uterine perfusion in pregnant rats. Am J Hypertens 2008; 21: 1152-56. https://doi.org/10.1038/ajh.2008.239

Talebianpoor MS, Mirkhani H.. The effect of tempol administration on the aortic contractile responses in rat preeclampsia model. ISRN pharmacol 2012; 2012. https://doi.org/10.5402/2012/187208

Talebianpoor MS, Owji SM, Goharinia M, Mirkhani H. Effect of tempol, a synthetic antioxidant, on renal complications of L-NAME induced preeclampsia in rat. Physiol Pharmacol 2017; 21: 241-50.

Talebianpoor MS, Delaviz H, Rafei R, Mohannadi B, Sadeghi H, Mohammadi J, Rad P. Resveratrol attenuates fetal limb malformation and cardiac hypertrophy after preeclampsia induced by L-NAME in pregnant rats. J Pharm Sci Res 2018; 10: 361-4.

Tanir HM ST, Inal M Akyuz F, Uzuner K, Sivri E. Effect of quercetine and glutathione on the level of superoxide dismutase, catalase, malonyldialdehyde, blood pressure and neonatal outcome in a rat model of pre-eclampsia induced by NG-nitro-L-arginine-methyl ester. Eur J Obstet Gynecol Reprod Biol 2005; 118: 190-5. https://doi.org/10.1016/j.ejogrb.2004.04.033

Tenório MB, Ferreira RC, Moura FA, Bueno NB, de Oliveira ACM, Goulart MOF. Cross-Talk between Oxidative Stress and Inflammation in Preeclampsia. Oxid Med Cell Longev, 2019; 8238727. https://doi.org/10.1155/2019/8238727

Tiboni GM GF, Di Giulio C. The nitric oxide synthesis inhibitor Nomega-nitro-L-arginine methyl ester (L-NAME) causes limb defects in mouse fetuses: protective effect of acute hyperoxia. Pediatr Res 2003; 54: 69-76. https://doi.org/10.1203/01.PDR.0000069840.78984.76