Resveratrol attenuates fetal limb malformation and cardiac hypertrophy after preeclampsia induced by L-NAME in pregnant rats

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Abstract

Resveratrol is a natural phenolic antioxidant with pleiotropic effects that produced by several plants and thought to has cardioprotective and anti-teratogenic effects. This study conducted to determine the effect of resveratrol on Fetal limb Hemorrhage, malformations and cardiac hypertrophy in an L-NAME-induced preeclamptic rat model. Forty-four pregnant Sprague-Dawley rats (5 months, 200-220g) were divided into 4 equal groups; the normal and L-name groups received respective, 1 cc of distilled water and L-name 70mg/kg (IP) from 9 to 20 days of the pregnancy, two other preeclamptic groups received L-name 70mg/kg and 10 and 20 mg/kg of resveratrol, respectively as L-R10 and L-R20 groups. Fetal left ventricular wall thickness, Concentration of MDA in fetal heart tissue, the limbs defects, number and weight of the fetuses were measured at twenty days of the pregnancy. The left ventricular myocardium thickness in the L-R20 group decreased significantly compared to the L-NAME group (p<0.05). The limbs Hemorrhage reduced in L-R10 and L-R20 groups compared to the L-NAME group (27.2 and 17.8 percent versus 71.1 percent). Ameliorated in L-R10 and L-R20 groups, compared to the L-NAME group (6.9 and 7.7 percent versus 27 percent). Resveratrol with Reduction of oxidative stress has beneficial effect to improve fetal outcomes in a pregnant rat model of preeclampsia induced by L-name.

Key words: limbs; L-name, Oxidative stress ; Preeclampsia; Rat; Resveratrol.

INTRODUCTION

Preeclampsia is one of the common diseases that causes of health problems in pregnancy and characterized by hypertension, proteinuria and edema (1). This clinical syndrome is accompanied with vascular disorder and could lead to fetal growth retardation, malformations and premature delivery (2). In the most cases, the etiology of preeclampsia is not clear but, it seems that nitric oxide (NO) has critical role in pathogenesis of this disease (3). L-NG-Nitroarginine Methyl Ester (L-name), as an inhibitor of nitric oxide synthetases, is one of the most popular compounds in experimental study to induce the preeclampsia (4). L-name created hypertension and proteinuria in gravid rats with glomerular endotheliosis, limb and cardiac malformations (5,6). Reactive oxygen species (ROS) formation by L-name could induce hemorrhages, oxidative stress and limb reduction defects (7). In this regard, several radical scavengers such as Alpha-phenyl-N-t-butyl nitrone (PBN) and quercetin have protective effect on fetal development by inhibiting of free radicals such as superoxide anion and hydroxyl (4 and 6). Some antihypertensive drugs with renoprotective properties especially angiotensin-converting-enzyme (ACE) inhibitors use for management of proteinuria, but this treatment are contraindicated in pregnant women (8). Other drugs including Methyldopa, hydralazine and labetalol are prescribed to pregnant women to control the complications of the preeclampsia but, these drugs have many side effects (9). Therefore introducing a new compound that affectively manages all complications of preeclampsia is necessary. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is natural polyphenolic antioxidant that produced in grapes and other plants has different properties such as anti-tumor , antihypertensive and renoprotective activity in diabetic nephropathy (10). It has been reported that resveratrol produces protective effect in diabetic-induced embryonic damage (11). In addition, animal models have shown no teratogenic effects associated with resveratrol (12, 13).

Therefore, the aim of this study was to evaluate whether resveratrol with dose 10 and 20 mg/kg body weight attenuates teratogenicity and complications of hypertension in a pregnant rat model of preeclampsia induced by L-NAME or not.

MATERIALS AND METHODS

Animals

Forty four Female Sprague Dawley rats (5 months, 200-220g) were procured from the animal house of the Yasuj University of Medical Sciences (YUMS). All experiments were performed according to the guidelines of Yasuj University of Medical Sciences Syndicate for application and care of animals (Ethical code: 91011020). The animals were maintained under the standard conditions based on ad libitum at room temperature 20±5°C with a regular 12: 12 h L/D cycle. Each Female rat mated overnight with a single fertile male of the same strain and the observed of the vaginal plugs were considered the day zero of the gestation. The Forty four pregnant rats were randomly divided equally into four groups: normal group received only distilled water (1 cc, IP) daily from 9th until 20th days of the gestation. L-NAME group was treated with L-NAME, L-NG-nitroarginine Methyl Ester (Alexis Biochemical USA, 70 mg/kg, IP) daily from 9th until 20th days of the gestation (Bryant, Allcock, & Warner, 1995). Two other groups including L-R10 and L-R20 were treated with L-NAME (70 mg/kg) plus resveratrol (Sigma chemical, IP) 10 and 20 mg/kg respectively, daily from 9th until 20th days of the gestation (14).

Fetal outcome and heart tissue malondialdehyde

The body weight (BW) of the pregnant rat determined on day 20 of gestation before normal delivery. Then, pregnant rats were anesthetized by sodium pentobarbital 100 mg/kg administered intraperitoneally and laparotomized. The fetuses, placentas and fetal heart were removed, washed in phosphate-buffered saline
(PBS) and weighed. Twenty two heart from each group (two sections from each pregnant rat) homogenized in ice cold isotonic saline (1:10 ratio) for malondialdehyde (MDA) assay. They were centrifuged at 10000 g for 10 min at 4°C and the MDA content was measured by the thiobarbituric acid method (15). The results were normalized to gram heart weight. Heart tissue MDA concentrations were determined by comparison to a standard curve

Fetal limb hemorrhage, malformations and histological study

The external surface of fetal limbs were examined for hemorrhagic and abnormalities. The forlimb and hindlimb malformations including anamalia, and brachydactyly were recorded in different groups at day 11 of pregnancy. For histological study, the fetal heart removed and placed in 10% neutral formalin solution for 48 hours. The fetal hearts (n=11 for each group) were cut 5µm-thick sections with using of a freezing microtome (Leica cryostat, CM 3000). Each section mounted onto gelatin-coated glass slides, stained with H&E dehydrated and cover slipped. The images of the sections were taken with using of a digital camera (DP 11) attached to the microscope (Olympus AX70). As previously described (Kawel N) the thickness of the endocardium, myocardium, epicardium were measured in five different area of the posterior wall of the left ventricle. Additionally, in each section the number and the outer diameters of the arteries were measured in dimension of 100µm² in posterior wall of the left myocardium.

Statistical analysis

All data are expressed as mean ± SD, One-way ANOVA was used for data analysis, followed by the Tukey test for post hoc analysis. A P-value<0.05 was considered to be statistically significant.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pregnant rat weight (g)</th>
<th>Fetal weight (g)</th>
<th>Fetal Heart weight (mg)</th>
<th>Placental weight (g)</th>
<th>Fetal heart MDA nmol/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>344.6±21.56*</td>
<td>6.30±0.38*</td>
<td>25.6±0.8</td>
<td>0.39±0.03*</td>
<td>32.37±6.18*</td>
</tr>
<tr>
<td>L-name</td>
<td>300.6±19.55</td>
<td>3.95±1.00</td>
<td>22.2±0.3</td>
<td>0.29±0.01*</td>
<td>48.67±10.28</td>
</tr>
<tr>
<td>L-R10</td>
<td>326.3±21.6*</td>
<td>5.24±0.48a</td>
<td>21.1±0.2</td>
<td>0.33±0.02*</td>
<td>36.5±7.59</td>
</tr>
<tr>
<td>L-R20</td>
<td>339.8±22.6*</td>
<td>6.70±0.59*</td>
<td>24.3±0.1</td>
<td>0.33±0.03*</td>
<td>33±8.48*</td>
</tr>
</tbody>
</table>

*p<0.01, p<0.05 compare to the L-NAME group

<table>
<thead>
<tr>
<th>Normal</th>
<th>L-NAME</th>
<th>L-R10</th>
<th>L-R20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1: The external hindlimb morphology of rat fetuses on 20th day of gestation. Proximal, distal regions of hind limbs and digits of them are normal in normal rat. L-NAME (70mg/kg) induced severe limb reduction defects. Hemorrhagic lesions with short phalanx are seen in left hindlimb of L-R10 rat fetus. Brachydactyly is seen in Right hindlimb of fetus from L-R20 group.</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
As shown in table 3, one-way ANOVA revealed a significant difference in mean thickness of the myocardium in the normal and L-R20 groups compared to the L-NAME group. Similarly, a significant difference was in mean diameter of the arteries in the L-R20 groups compared to the L-NAME rats. Similarly, a no statistical difference in the mean thickness of the endocardium, significant difference was in mean diameter of the arteries in the L-R20 groups compared to the L-NAME rats. Therefore, the obtained results of the effect of resveratrol on L-NAME rats were beneficial effects to diminish teratogenic effects of L-NAME in the rat model of preeclampsia during the embryonic and fetal period is a significant promise. Due to it was effective to inhibit oxidative stress in embryos, reduced the fetal limbs malformation and has beneficial effect to decrease the myocardial hypertrophy. However, further works are necessary to determine the safety and the optimum doses of resveratrol in the preeclampsia disease.

### DISCUSSION

Preeclampsia is an important disorder during pregnancy and is a leading cause of maternal, fetal morbidity and mortality. This disorder is characterized by hypertension, proteinuria and edema (16). Fetal growth retardation, fetal malformations and premature delivery are other outcomes of preeclampsia (17). In the present work, the effect of resveratrol on fetal complications was investigated in experimental preeclampsia rat model. Preeclampsia was induced by intrapritoneal administration of L-NAME from the Day 9 of gestation till the 20th day of pregnancy. Administration of L-NAME resulted in limb defects, limb hemorrhage, fetal weight reduction and increased thickness of fetal myocardium (Table 1, 2 and 3). It was also associated with increased levels of fetus heart MDA, an indicator of oxidative stress (Table 1). Resveratrol at doses of 10 and 20 mg/kg/day have shown beneficial effects to diminish teratogenic effects of L-NAME in embryonic period of rat fetuses (Table 2). In both preeclamptic groups under treatments of resveratrol (10 and 20 mg/kg/day), limb anomaly, limb hemorrhage and fetal oxidative stress decreased significantly. In the present study, Resveratrol decreased fetus complications in a dose-dependent manner. Indeed, increase and decrease of MDA was in parallel with teratogenicity in different groups (Table 1 and 2). A numerous studies have proposed that oxidative stress has a pivotal role in the L-NAME-induced teratogenesis that could be prevented by some antioxidants (4 and 18). Singh et al showed that resveratrol improve lipid metabolism and prevented oxidative stress in embryos with diabetic embryopathy. In addition, it is reported that resveratrol improve fetal weight (19). Therefore, the obtained results of the effect of resveratrol on L-NAME-induced teratogenesis in our study are plausible and consistent with other reports. The same pattern of dose-dependent action of resveratrol has been seen on ventricular hypertrophy of animal models (20-22). L-NAME as an inhibitor of nitric oxide synthesis contributes to the pathogenesis of cardiac and vascular changes that damage endothelial function which increases blood pressure and cardiac hypertrophy (23-25). It seems that increased endothelial formation of NO by resveratrol at high dose (20 mg/kg) causes cardioprotective effects (26). Also, Joyce critina de oliveira et al showed that resveratrol is a potent antioxidant could improve hypertension and cardiac hypertrophy in renal hypertensive rats (27). So, another possibility is that resveratrol prevented fetal malformations because of it’s antihypertensive effect in preeclamptic rats. Similar results have reported by Rivera (28). Moreover, the thickness of epicardium, endocardium and number of arteries did not show any significant differences among studied groups (Table 3). We have no explanation for this discrepancy.

### CONCLUSION

These findings indicated that the use of resveratrol against L-NAME in the rat model of preeclampsia during the embryonic and fetal periods is a significant promise. Due to it was effective to inhibit oxidative stress in embryos, reduced the fetal limbs malformation and has beneficial effect to decrease the myocardial hypertrophy. However, further works are necessary to determine the safety and the optimum doses of resveratrol in the preeclampsia disease.

### ACKNOWLEDGEMENTS

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### CONFLICT OF INTEREST

There is no conflict of interest in this article.

### REFERENCES


### Table 1. Limbs hemorrhage and malformations in different groups at 20 days of gestation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of fetuses</th>
<th>Amelia</th>
<th>Brachydactyly</th>
<th>Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>107</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-NAME</td>
<td>111</td>
<td>30(72%)</td>
<td>8(7.2)</td>
<td>79(71.1)</td>
</tr>
<tr>
<td>L-R10</td>
<td>103</td>
<td>8(7.2)</td>
<td>3(5.2)</td>
<td>28(8.27)</td>
</tr>
<tr>
<td>L-R20</td>
<td>101</td>
<td>7(6.9)</td>
<td>10(9.9)</td>
<td>18(17.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Myocardium (mm)</th>
<th>Endocardium (µm)</th>
<th>Epicardium (µm)</th>
<th>Number of arteries</th>
<th>Diameter of the arteries (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.750±0.31</td>
<td>20.1±0.014</td>
<td>29.7±3.01</td>
<td>7.04±0.06</td>
<td>58.07±11.1</td>
</tr>
<tr>
<td>L-name</td>
<td>0.972±0.026</td>
<td>19.9±0.3</td>
<td>27.6±2</td>
<td>7.2±0.04</td>
<td>81.02±17.3</td>
</tr>
<tr>
<td>L-R10</td>
<td>0.801±0.023</td>
<td>19.8±0.6</td>
<td>28.04±1.4</td>
<td>7.01±0.01</td>
<td>70.99±8.04</td>
</tr>
<tr>
<td>L-R20</td>
<td>0.785±0.017</td>
<td>20±0.11</td>
<td>28.82±0.3</td>
<td>7.05±0.04</td>
<td>69.06±10.5</td>
</tr>
</tbody>
</table>

p<0.01, p<0.05 compare to the L-NAME group


