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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 effcets. 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 respectively, 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). Amelia decreased 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 pregnancy. 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 malonydialdehyde (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 of 1, 1, 3, 3 tetraethoxypropane (TEP). Standard curve was made using serial dilutions of TEP (0, 1, 2, 2.5, 5, 10 µM). 0.5 mL of supernatant or standard sulotions were taken in a test tube and 2 mL of the TBA-TCA [TBA-TCA reagent: 0.375 % w/v TBA (thiobarbituric acid); 15 % w/v TCA (trichloroacetic acid) and 0.25 N HCl] solution were added. The mixture was heated in a water bath (90-100 °C) for 15 min, cooled in a cold water bath for 10 min, and then centrifuged at 2000 g for 15 min. The absorbance of solution was read spectrophotometrically at 535 nm.

Fetal limb hemorrhage, malformations and histological study

The external surface of fetal limbs were examined for hemorrhagic and abnormalities. The forlimb and hindlimb mlformations including anomalia, 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 100µm^2 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.

RESULTS

Fetal outcome and heart tissue malondialdehyde

The maternal death or preterm parturition has not seen before day 20 of the pregnancy during the study. As shown in table. 1, the MDA level of fetal heart tissue as indices of oxidative stress was higher in the L-NAME rats compare to the normal group (P<0.01), L-R10 and L-R20 (P<0.05). A significant difference was seen in the body weight of the Pregnant rats and their fetuses in the normal, L-R10 and L-R20 groups compared to the L-NAME group on day 20 of gestation (P <0.01), (table. 1). The placental weight reduced significantly in L-NAME group compared to the L-R10 and L-R20 groups (P <0.05), (table. 1).

Limbs hemorrhage and malformation

All live or dead fetuses from different groups were examined for limb malformations. The number of total fetus increased in L-NAME group but there was no significant difference between the numbers of total fetus among the groups (table. 2). the total number of limbs anomaly were specified in each group. A fetus may has more than one defect and therefore was represented more than once (data has not shown). As shown in table. 2, Amelia decreased in L-R10 and L-R20 groups with %6.9 and %7.7 respectively, compared to the L-NAME group (%27). Similarly, the limbs hemorrhage reduced in L-R10 and L-R20 groups with %17.8 and %27.2 respectively, compare to the L-NAME group (%71.1) (table. 2). in the normal group limb abnormality was not seen (figure.1). The distal limb reduction defect or digits missing has seen the common limb anomalies and in some instances, hematomas were seen with sever defective structures (figure.1). The hindlimb was more affected than the forelimb, left and right limbs were equally affected (the results not shown).

After treatment with resveratrol at 10 and 20 mg/kg, frequencies of brachydactyly malformations Reduced from %7.2 in L-NAME group to %2.9 and %.99 in L-R10 and L-R20 groups respectively. (Figure1).

Percentage is compare to the total fetus number (100%) in each group. The number of amelia in L-NAME group increased more than fourfold versus to the L-R20 rats. Results showed a significant reduction of micromilia from twice to fourfold in L-R10 and L-R20 groups compared to L-NAME group. Abnormality has not seen in the normal group.

Table1. Fetal outcome and heart tissue MDA, pregnant rat weight, fetal weight, fetal heart weight and placental weight in different groups at day 20 of gestation

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

$^{¥}$p<0.01, *p<0.05 compare to the L-NAME group

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 sever 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.

Table 2. Limbs hemorrhage and malformations in different groups at 20 days of gestation

Groups	Number of fetuses	Amelia	Brachydactyly	Hemorrhage
Normal	107	0	0	0
L-NAME	111	30 (%27)	8(%7.2)	79(%71.1)
L-R10	103	8(%7.7)	3(%2.9)	28(%27.2)
L-R20	101	7(6.9)	1(0.99)	18(%17.8)

Table3. The thickness of the myocardium, endocardium, epicardium and outer diameter of the arteries in Left ventricle in different groups (mean± SD)

Groups	Myocardium (mm)	Endocardium (µm)	Epicardium (µm)	Number of arteries	diameter of the arteries (µm)
Normal	0.750±0.31*	20.1±0.014	29.7±3.01	7.04±0.06	58.07±11.1
L-name	0.972±0.026	19.9±0.3	27.6±2	7.2±0.04	81.02±17.3
L-R10	0.801±0.023	19.8±0.6	28.04±1.4	7.01±0.01	70.09±8.04
L-R20	0.785±0.017¥	20±0.11	28.82.3	7.05±0.04	69.06±10.5¥

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

Fetal histological study

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 rats. Similarly, a significant difference was in mean diameter of the arteries in the L-R20 Group compared to L-NAME group($p<0.05$). There was no statistical difference in the mean thickness of the endocardium, epicedium and the number of the artery among different groups (table. 3).

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 teratogenecity that could be prevented by some antioxidants (4 and 18). Singh et al shown 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 teratogenecity 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 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, frurther works are necessary to determine the safety and the optimum doses of resveratrol in the preeclampsia disease.

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CONFLICT OF INTEREST

There is no conflict of interest in this article.

REFERENCES

[1] Henderson, J.T., Whitlock, E.P., Connor, E.O., Senger, C.A., Thompson, J.H., Rowland, M.G., Low-dose aspirin for prevention of morbidity and mortality from preeclampsia: a systematic evidence review for the U.S. preventive services task force. *Ann Intern Med.* 2014, 160, 695-703.

[2] Wong, F., Cox, B., Proteomics Analysis of Preeclampsia, a Systematic Review of Maternal and Fetal Compartments. *J Proteomics Bioinform.*2014, 10, 2-7.

[3] McMaster-Fay, R.A., Pre-eclampsia e a disease of oxidative stress resulting from the catabolism of DNA (primarily fetal) to uric acid by xanthine oxidase in the maternal liver: A hypothesis. *Bioscience Hypotheses.* 2008, 1, 35-43.

[4] Fantel, P.R., Further evidence for the role of free radicals in the limb teratogenicity of L-name.*Teratology.*2002, 66, 24-32.

[5] Buhimschi, I.A., Saade, G.R., Chwalisz, K., Garfield, R.E., The nitric oxide pathway in pre-eclampsia: pathophysiological implications. *Hum. Reprod Update.* 1998, 4, 25-42.

[6] Tanirhm, S.T., 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.* 2005, 118, 190-195.

[7] Tiboni, G.M., Giampietro, F., Digiulio, C., The Nitric Oxide Synthesis Inhibitor N-Nitro-L-Arginine Methyl Ester (L-name) Causes Limb Defects in Mouse Fetuses: Protective Effect of Acute Hyperoxia. *Pediatrics Review.*2003, 54, 69-76.

[8] Cooper, W.O., Hernandez-Diaz, S., Arbogast, P.G., Dudley, J.A., Dyer, S., Gideon, P.S., Hall, R.K., Ray WA, Major congenital malformations after first-trimester exposure to ACE inhibitors. *N Engl J Med.* 2006, 354, 2443-51.

[9] Magee, L.A., Pels, A., Helewa, M., Rey, E., Dadelszen, P.V., Diagnosis, Evaluation, and Management of the Hypertensive Disorders of Pregnancy: Executive Summary. *J Obstet Gynaecol Can*.2014, 36, 575-576.

[10] Jang, J.Y., Park, D., Shin, S., Jeon, J.H., Choi, B.I., Joo, S.S., Hwang, S.Y., Nahm, S.S., Kim, Y.B., Antiteratogenic effect of resveratrol in mice exposed in utero to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *Eur J Pharmacol.* 2008, 591, 280-283.

[11] Chander, V., Chopra, K., Protective effect of nitric oxide pathway in resveratrol renal ischemia-reperfusion injury in rats. *Arch Med Res. 2006, 37*, 19-26.

[12] Edwards, J., Beck, M., Riegger, C., Bausch, J., Safety of resveratrol withexamples for high purity, trans-resveratrol, resVida. *Ann N Y Acad Sci*. 2011, 1215, 131-137.

[13] Williams, L.D., Burdock, G.A., Edwards, J.A., Beck, M., Bausch, J., Safety studies conducted on high-purity trans-resveratrol in experimental animals. *Food Chem Toxicol.* 2009, 47, 2170-82.

[14] Schmatz, R., Perreira, L.B., Stefanello, N., Mazzanti, C., Spanevello, R., Gutierres, J., Effects of resveratrol on biomarkers of oxidative stress and on the activity of delta aminolevulinic acid dehydratase in liver and kidney of streptozotocin-induced diabetic rats. *Biochimie*. 2012, 94, 374-83.

[15] Hoyland, D.V., Taylor, A.J., A review of the methodology of the 2-thiobarbituric acid test. *Food Chem*. 1991, 40, 271-291.

[16] Noris, M.N., Remuzzi, G., Mechanisms of disease: Pre-eclampsia. *Nat Clin Pract Nephrol*. 2005, 1, 98-114.

[17] Ornaghi, S., Hsieh, L.S., Bordey, A., Vergani, P., Paidas, M.J., van den Pol, A,N., Valnoctamide Inhibits Cytomegalovirus Infection in Developing Brain and Attenuates Neurobehavioral Dysfunctions and Brain Abnormalities. *J Neurosci*. 2017, 19, 6877-6893.

[18] Tanir, H.T., 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 [19] Singh, C.K., Kumar, A., Hitchcock, D.B., Fan, D., Goodwin, R., Lavoie, H.A., Resveratrol prevents embryonic oxidative stress and apoptosis associated with diabetic embryopathy and improves glucose and lipid profile of diabetic dam. *Mol Nutr Food Res*. 2011, 55, 1186-96.

[20] Dolinsky, V.W., Soltys, C.L., Rogan, K.J., Chan, A.Y., Nagendran, J., Wang, S., Dyck, J.R., Resveratrol prevents pathological but not physiological cardiac hypertrophy, *J Mol Med (Berl)*. 2015, 93, 413-425.

[21] Rebecca, K.V., Candice, P., Fiona RC oulson& Andrew SF, Resveratrol Prevents Cardiovascular Complications in the SHR/STZ Rat by Reductions in Oxidative Stress and Inflammation. *BioMed Research International*. 2015, 2015, 1-8 .

[22] Dong, Q., Wu, Z., Li, X., Yan, J., Zhao, L., Yang, C., Lu, J., Deng, J., Chen, M., Resveratrol ameliorates cardiac dysfunction induced by pressure overload in rats via structural protection and modulation of Ca (2+) cycling proteins. *J Transl Med*. 2014, 26, 2-12.

[23] Arnal, J.F,, el Amrani, A.I., Chatelliwe, G., Menard, J., Michel, J.B., Cardiac weight in hypertension induced by nitric oxide synthetase blockade. *Hypertension*. 1993, 22, 380-387.

[24] Takemoto, M., Egashira, K., Tomita, H., Usui, M., Okamoto, H., Kitabatake, A., Shimokawa, H., Sueishi, K., Takeshita, A., Chronic angiotensin-converting enzyme inhibition andangiotensin II type 1 receptor blockade: effects on cardiovascularremodeling in rats induced by the long-term blockade of nitricoxide synthesis. *Hypertension. 1997,* 30, 1621-7.

[26] Takemoto, M., Egashira, K., Usui, M., Numaguchi, K., Tomita, H., Tsutsui, H., Shimokawa, H., Sueishi, K., Takeshita, A., Important roleof tissue angiotensin-converting enzyme activity in thepathogenesis of coronary vascular and myocardial structure changes induced by long-term blockade of nitric oxide synthesis in rats. *J Clin Invest*. 1997, 99, 278-287.

[27] Fabricio, V., Oishi, J.C., Biffe, B.G., Ruffoni, L.D., Silva, K.A., Nonaka, K.O., Rodrigues, G.J., Resveratrol Treatment Normalizes the Endothelial Function and Blood Pressure in Ovariectomized Rats. *Arq Bras Cardiol*. 2017, 108, 116-121

[28] Phyu, H.E., Irwin, J.C., Vella, R.K., Fenning, A.S., Resveratrol shows neuronal and vascular-protective effects in older, obese, streptozotocin-induced diabetic rats. *Br J Nutr.* 2016,115, 1911-8

[29] Rivera, L., Moron, R., Zarzuelo, A., Galisteo, M., Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochem Pharmacol.* 2009, 77, 1053-63.