Effect of Glutathione on Blood Pressure, Postnatal Lung Growth and Neonatal Outcome In A Rat Model of Pre-Eclampsia

Marwa A. Ahmed, Omayma G. Ahmed*, Asmaa F.Hassan and Tarek Mohamed Mostafa**
Department of Physiology and Anatomy*,
Faculty of Medicine, Assiut University

ABSTRACT

***Background**: Pre-eclampsia is a human pregnancy-specific disorder that adversely affects the mother (by vascular dysfunction)and the fetus (by intrauterine growth restriction). A large portion of the prenatal mortality is consequently due to iatrogenic prematurity. Up to 15% of preterm births is a result of pre-eclampsia . The combination of hypertension plus proteinuria markedly increases the risk of prenatal morbidity and mortality over that of hypertension alone. **Aim of the study:** To elucidate the effect of addition of glutathione on lipid peroxidation products, blood pressure, proteinuria and neonatal outcome in a rat model of pre-eclampsia induced by NG-nitro-L-arginine-methyl ester (L-NAME), an inhibitor of nitric oxide synthase. **Study Design:** Adult pregnant rats (n =30) were allocated into three groups according to medication they received on day 17 from gestation to term. Rats were randomized into control group (n=10) and groups received L-NAME, 50 mg/day orally. (pre-eclampetic group, n = 10) and L-NAME + glutathione, 60mg/kg i.p. (treated group, n= 10) Blood levels of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were assessed .Mean systolic blood pressure (BP) (measured with a tail – cuff device), levels of proteinuria ,total urine output ,pups birth weight and percentages of live and dead pups were recorded. **Results:** Mean systolic BP, plasma levels of SOD, CAT and MDA of pregnant rats were higher in pre-eclamptic group than in control group. In treated group these plasma levels were lower than in pre-eclamptic group but still higher than those of control group. A positive correlation between systolic BPs on day 20 and SOD levels was recordedin both pre-eclamptic and treated groups. Birth weights of pups were higher in the control group than in the other groups. Pups of treated group with glutathione had better survival rates than those of control and pre-eclamptic groups. The mean total body weight, lung weight, internal alveolar wall thickness and internal alveolar surface area of pups of pre-eclamptic group were significantly higher than those of control and treated groups. Histological changes: The lung specimens of pups of pre-eclamptic mothers showed irregular, collapsed, small alveoli. The lumnia of these alveoli contained oedematous fluid rich in erythrocytes. The interalveolar speta were relatively thickened and more cellular than control. The treated group showed more or less the same morphological findings as the control group. **Conclusion:** In this rat model of pre-eclampsia, hypertension, proteinuria, oxidative stress of pregnant rats, a high neonatal death rate and pathological changes of lungs structure of pups, are reversed by exogenous antioxidant use.*

INTRODUCTION

Pre-eclampsia is a condition unique to human pregnancy and is regarded as an endothelial disorder. Studies have successfully demonstrated that nitric oxide inhibition such as NG-nitro-L-arginine-methyl ester (L-NAME), in a rat model results in a condition similar to preeclampsia[(1)]. Although lipid peroxidation and release of reactive oxygen radicals involved in physiological cellular processes, they have been proposed as candidates for such diffusible factors[(2)]. In addition, it has been postulated that deficiency of an endogenous antioxidants or increased demand for it perpetuates endothelial integrity further.

Among several antioxidant defense systems involved in the disease process, intracellular thiol and reduced glutathione have important role in combating the oxidative stress burden in preeclampsia[(3)].

Pre-eclampsia is among the leading causes of intrauterine growth restriction (IUGR).The placental vascular abnormalities associated with preeclampsia impair maternofetal exchanges, thereby restricting fetal growth. Impairments of maternofetal exchanges begin during the third trimester of pregnancy, during which lung development is at the canalicular phase, characterized by formation of the distal airways, blood vessels, and alveolar–capillary barrier[(4)].

Alveolaization of the rat lung begins during the late gestation and continues along the early postal period[(5)]. The transformation of the immature saccular lung to mature alveoli with large surface area entails various developmental and maturational processes. This includes, epithelial cell differentiation, proliferation, fibroblast maturation and surfactant production[(6)].

Intrauterine exposure to various adverse stimuli can disrupt alveolaization and induce lung hypoplasia. Also interfere with surfactant synthesis which precipitate Neonatal Respiratory Distress Syndrome and increase neonatal mortality[(7)]. Among these deleterious stimuli, maternal pre-eclampsia is considered the immanent cause for neonatal deaths and lung immaturity[(8)].

Oxidative stress has been implicated in a wide variety of diseases and degenerative states, there is considerable evidence to suggest that pregnancy leads to the generation of increased oxidative burden, but whether this overwhelms the anti-oxidant capacity within placenta and/or the peripheral circulation remains a point of conjecture, and there is little doubt that oxidative stress is a significant contributor in the pathogenesis of pre-eclampsia[(9)].

Aim of the work:

1. Elucidate how endogenous antioxidants and lipid peroxidation product levels change after the administration of nitric oxide synthase inhibitor to gravid rats.
2. To study the effect of addition of glutathione on blood pressure, degree of urinary protein loss, size, body weight of pups at the end of pregnancy in a rat model of pre-eclampsia.
3. To assess the lung development of rat pups in a rat model of pre-

eclampsia and whether it is impaired or not.

MATERIALS & METHODS

Chemicals:

1. Glutathione supplied from Starchemic.
2. NG-nitro-L-arginine-methyl ester (L-NAME), supplied from MP Biomedicals, Inc.

Animals:

Thirty Adult, non pregnant rats with body weight 250 -300 gm were housed in rooms under condition of controlled temperature (24-26° C), humidity (55-60 %) and 12 hrs light-dark cycle. The animals were acclimated to these conditions for 1 week. They fed on a standard diet with free access to water. For mating, female rats were caged 1:1 with mature males, vaginal smears were checked daily in the early morning for the presence of sperm[10] or presence of vaginal plug[11]. The day at which sperms were detected or the vaginal plug was confirmed, was designated day 0 of gestation.

Daily measurement of systolic blood pressure was done for all groups during period of gestation as follow: Systolic blood pressure was recorded daily with a pneumatic tail–cuff device (NARCO, Biosystems, Inc., Houston, Texas) after animals had been pre warmed for 30 min. in a metabolic chamber maintained at approximately 30^0C. Three consecutive measurements (10-sec intervals) were obtained. All measurements were made at the same time of day.

Mean systolic blood pressure values obtained from three consecutive measurements were recorded as the pressure value for a given rat at each point. Fetal growth in rats accelerated starting at day 17 of gestation [8]. Therefore on postmating day 17 0f the experiment, the rats were divided into 3 groups:

1. Control group: included 10 gravid rats which received pure water orally.
2. Pre-eclamptic group: included 10 gravid rats which were treated with L-NAME daily given orally in drinking water from day 14–21 of gestation 50 mg/Kg/day[12].
3. Treated group: included 10 gravid rats which were treated with L-NAME daily given orally from day 14–21 of gestation 50 mg / Kg / day in addition to glutathione 60 mg/Kg i.p[8].

Starting on day 18 of gestation measurement of urine was done : Urine was collected from gravid rats by applying pressure to the bladder and collecting excreted urine with a capillary tube on day 18,19, 20 and 21 then stored till analysis for urinary protein concentrations.

On day 20 of gestation (before the expected day of normal spontaneous delivery [10]. Blood samples (4 ml) from all the animals were collected via retro- orbital puncture (optic vien) in chilled EDTA-containing microtubes and centrifuged immediately and the plasma was collected and centrifuged at 1500 for 10 min for extraction of plasma and erythrocyte homogenates and the analyzed for plasma malondialdehyde (MDA), ng/ml, erythrocytic superoxide dismutase (erythrocyte SOD), units/Hb and erythrocyte catalase (CAT), unit / Hb. The plasma

levels of MDA were determined as thiobarbituric acid reactivity. The product of reaction between malonic dialdhyde and thiobarbituric acid was measured as described by Ohkawa[13]. Determination of Ery.SOD) activity was done according to Misra and Fridovich[14]. Erythrocyte catalase (CAT) was determined according to Beutler[15]. The day of birth (spontaneous normal delivery) was defined as postnatal day 0. All pregnant female littered spontaneously between day 22 and day 23 of gestation. Pups numbers, weights and their conditions were recorded at the ay of birth.

Lung measurements:

Ten rat pups of each group were killed. Then the lungs were extracted and total lung weight and lung volume of the above used animals were estimated [16]. Then the lungs were fixed in Bouin's fixative solution. The right lungs were used and prepared as paraffin blocks that cut at 10 microns thickness. Several histological techniques were used according to[17].

Haemtoxline and eosin staining technique used for study the morphological and cytoarchitectonic structure of the lung.

Van Gieson's staining technique was used for study collagen fibers contents of interalveolar septa of the lung.

Periodic Acid Shiefe staining technique used for the study glycogen utilization by epithelial alveolar cells.

Estimation of alveolar wall thickness and internal alveolar surface area of rat pups using an image analysis system (Leica Q500 Mc)[18].

Statistical Analysis:

All values were expressed as mean ± SE. Differences between groups were determined by Student's Newman Kelus t- test. Values were considered insignificant if P value is >0.05 and significant if P value was < 0.05. Prism computer program (graph Pab version 3.0) was used for statistical analysis[19].

RESULTS

Biochemical results:

A condition similar to pre-eclampsia developed in all gravid rats in pre-eclamptic and treated groups.

This study confirmed that a successful model for pre-eclampsia was obtained by the administration of L-NAME. On day 20 pre-eclamptic group had higher systolic BP than those of the control group, (151.2 ± 0.388 mmHg vs. 114.7 ± 0.448mmHg, P<0.001). The high BP values of treated group were significantly higher than those of the control group (145.3 ±0.931 mmHg vs.114.7 ± 0.448 mmHg, P<0.001) and those of pre-eclamptic group were statistically higher than treated group (151.2± 0.388 mmHg vs. 145.3 ±0.931 mmHg, P<0.001) table (1). All data concerned of BP values are shown in figure (1).

Plasma levels of SOD in the pre-eclamptic group were significantly higher than those of the control group and the treated group (1506 ± 1.652 units/Hb versus 1352 ± 0.468 units/Hb and 1379±0.603units/Hb respectively , P <0.001) and dropped to lower levels in the treated group in which glutathione was administrated along with L-NAME, Table (2).

Figure (2) shows a positive correlation was observed between systolic BPs recorded on day 20 of pregnancy and plasma levels of SOD ($r = 0.890$, $n = 10$ with $P < 0.001$) in the pre-eclamptic group. This correlation was also observed in the treated groups ($r = 0.755$, $n = 10$, $P < 0.05$) (Figure 3).

Plasma levels of MDA were higher in the pre-eclamptic group than the control group (7.58 ± 0.035 ng/ml versus 5.59±0.06 ng/ml, P <0.001) and was significantly higher than those of treated group (7.58 ± 0.035ng/ml versus 6.28 ± 0.1 ng/ml, P <0.001) Table (2).

Table (2) shows that plasma levels of CAT were higher in the pre-eclamptic group than those of the control and treated groups (3149 ± 1.57 versus 2755± 2.56 and 2956 ± 1.97, respectively, P <0.001) .Blood levels of CAT of treated group were still higher than those of the control group (2956 ± 1.97 versus 2755± 2.56, P< 0.001).

Table (3) shows that values of proteinuria of pre-eclamptic group were significantly higher than those of control and treated group (1.38 ± 0.032 mg versus 0.86±0.452 mg and 0.95± 0.341mg, P< 0.001respectively) and those of treated group was significantly higher than those of control group (0.95± 0.341 mg vs. 0.86 ± 0.452 mg P< 0.01) as shown in table 2. The mean urine volume did not differ among the groups.

Neonatal survival of pups was found to be the lowest in the pre-eclamptic group (75 %).On the other hand , the neonatal survival of pups was highest in the control group (94%) and was elevated in the treated group (89 %) but still lower than that of the control group. The percentage of dead born pups (25 %) was highest in pre-eclamptic group and lowest in control group (6%). The percentage of treated groups was higher than the control group but still lower than the pre-eclampetic group (11%).Table (3).

Lung measurements results:

There was a highly significant difference in the mean total body weight of pups between the control group and pre-eclamptic group (6.04 ± 0.037 gm versus 4.99± 0.098 gm, P<0.001), but no significant differences were noticed between control group and treated group (6.04± 0.037 gm vs. 5.96 ± 0.066 gm) Table (4).

There was a significant difference in the mean total lung weight of pups between the control group and pre-eclamptic group (26.45± 0.26 mg versus 24.48 ± 0.21 mg, P <0.001), but no significant differences were noticed between control group and treated group (26.45±0.26 mg vs. 25.92 ± 0.13 mg) (Table 4).

The mean of the alveolar wall thickening showed a highly significant difference between control group and pre-eclamptic group (15.7±0.068 um vs16.51±0.074 um, P<0.001).No significant differences were detected among the control and the treated groups (15.7±0.068 vs. 15.72±0.057um) (Table 4).

The mean value of internal alveolar surface area showed highly significant difference between control group and pre-eclamptic group (190.04±0.45 vs. 180.5± 0.4 P <0.001 and slightly significant difference between group the control and the

treated groups (190.04±0.45 vs. 189±0.33 cm^2, P<0.05**).**

Histological results:

Control group:

The histological examination showed regularly expanded alveoli with an empty lumen. The interalveolar septa were thin, well formed and with a relatively less cellular content (Plate 1, Fig. A).

The specimens stained with Van Gieson's stain showed well formed alveoli with relatively thin interalveolar speta and minimal amount of collagen fibers. The lumina of the alveoli were empty (Plate 2, Fig.A).

The specimens stained with Periodic Acid Shiefe showed complete disappearance of glycogen content from the alveolar epithelium (Plate 3, Fig. A).

Pre-eclamptic group:

The lung specimens of newborn rats of pre-eclamptic mothers showed irregular, collapsed, small alveoli. The lumnia of these alveoli contained oedematous fluid rich in erythrocytes. The interalveolar speta were relatively thickened and more cellular (Plate 1, Fig. B).

By Van Gieson's stain the alveoli appeared irregular, collapsed with thickened interalveolar septa that had many collagen fibers. The lumen was filled with oedematous fluid and erythrocyte (Plate 2, Fig. B).

The specimen stained with Periods Acid Shiefe showed residual glycogen content within the alveolar epithelium (Plate 3, Fig. C).

Treated Group:

The specimen showed more or less the same morphological findings as the control group (Plate 1, Fig.C).

The specimen stained with Van Gieson's stain showed the same pattern of the control group (Plate 2, Fig.C).

The specimen stained with periodic Acid Shiefe stain appeared more or less similar to the control group (Plate 3, Fig. C).

Table (1): Blood pressure (BP) (mmHg) during pregnancy from day 18 to day 22 (BP18, BP19, BP20, BP21, BP22) in control, pre-eclamptic and treated groups

Variables	Control group	Pre-eclamptic group	Treated group
BP18	108.3 ± 0.538	147 ± 0.6 [a ' 'b]	140.4 ± 0.884 [a]
BP19	109.4 ± 0.763	148.6 ± 0.371 [a ' 'b]	137 ± 0.471 [a]
BP20	114.7 ± 0.448	151.2 ± 0.388 [a ' 'b]	145.3 ± 0.931 [a]
BP21	116.1 ± 0.378	156.5 ± 0.428 [a ' 'b]	149.1 ± 0.348 [a]
BP22	117.1 ± 0.276	159.7 ± 0.300 [a ' 'b]	150.3 ± 0.715 [a]

a : P < 0.001as compared to the control group .

b: P < 0.001 as compared to the treated group.

Table (2): Levels of plasma MDA (ng/ml), SOD (units/Hb), and CAT (units/Hb) in the studied groups

Variables	Control group	Pre-eclamptic group	Treated group
MDA (ng/ml)	5.59±0.06	7.58 ± 0.035 [a ' b]	6.28 ± 0.1 [a]
SOD (units/Hb)	1352 ± 0.468	1506 ± 1.652 [a ' b]	1379 ± 0.603 [a]
CAT (units/ Hb)	2755± 2.56	3149 ± 1.57 [a ' b]	2956 ± 1.97 [a]

a : P < 0.001as compared to the control group
b: P < 0.001 as compared to the treated group

Table (3): Proteinuria , urine volume , birth weight of pups and dead / live ratio in the studied groups

Variables	Control group	Pre-eclamptic group	Treated group
Proteinuria (mg)	0.86 ± 0.452	1.38 ± 0.032 [a' c]	0.95 ± 0.341 [b]
Urine volume (ml)	8.4 ± 0.042	8.71 ± 0.056	8.66 ± 0.339
Pups live (%)	94	75	89
Pups dead (%)	6	25	11

a : P < 0.001as compared to the control group. c: P < 0.001 as compared to the treated group.
b: P < 0.01 as compared to the control group.

Table (4): Total body weight, lung weight, volume, internal alveolar wall thickness and internal alveolar surface area of pups of all studied group

Variables	Control group	Pre-eclamptic group	Treated group
Total body weight (gm)	6.04± 0.037	4.99± 0.098 [a]	5.96 ± 0.066 [c]
Total lung weight (mg)	26.45± 0.26	24.48± 0.21 [a]	25.92 ± 0.13 [c]
Total lung volume (ml)	0.332± 0.001	0.298± 0.004 [a]	0.328± 0.003 [c]
Internal alveolar wall thickness (um)	15.7± 0.068	16.51± 0.074 [a]	15.72± 0.057 [c]
Internal alveolar surface area (cm^2)	190.04± 0.45	180.5± 0.4 [a]	189± 0.33 [b]

a : P < 0.001 as compared to the control group.
b : P< 0.05 as compared to the control group .
c: Non significant as compared to the control group

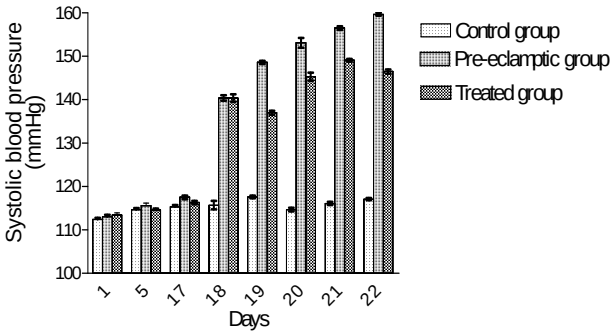

Fig. (1): Systolic blood pressure changes from the first day of gestation to term in all studied groups

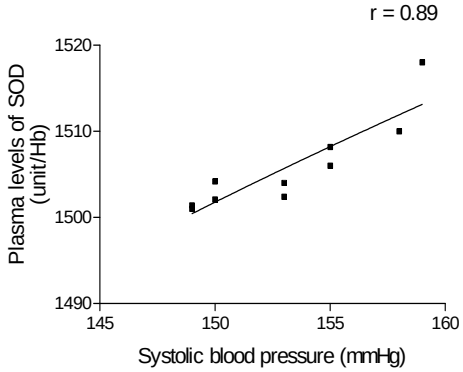

Fig. (2): Correlation between systolic blood pressure (mmHg) and plasma levels of SOD (unit/Hb) on day 20 in pre-eclamptic group

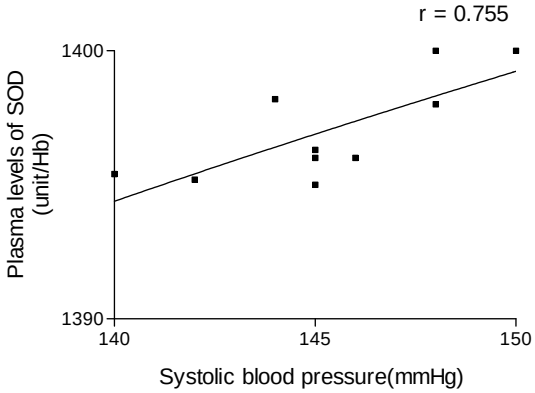

Fig. (3): Correlation between systolic blood pressure (mmHg) and plasma levels of SOD (units/Hb) on day 20 in the treated group

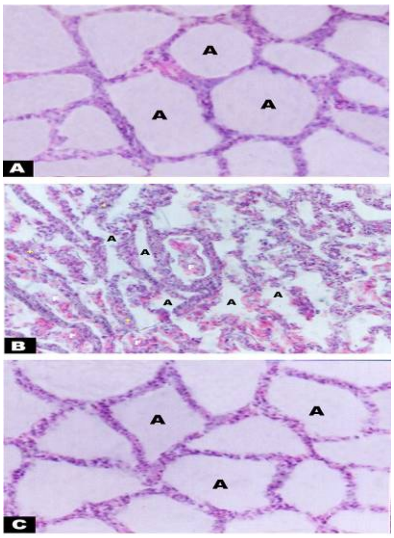

Plate (1): Photomicrograph of newly borne rat lung:
(A)control group showing regularly expanded alveoli(A)
(B)pre-eclamptic group showing irregularly collapsed alveoli(A)with thickened interalveolar septa. The lumen contains oedamatous fluid rich in erythrocytes (F)
(C) Glutathione treated group showing regularly expanded alveoli (A);
(Haematoyline&EosineX250)

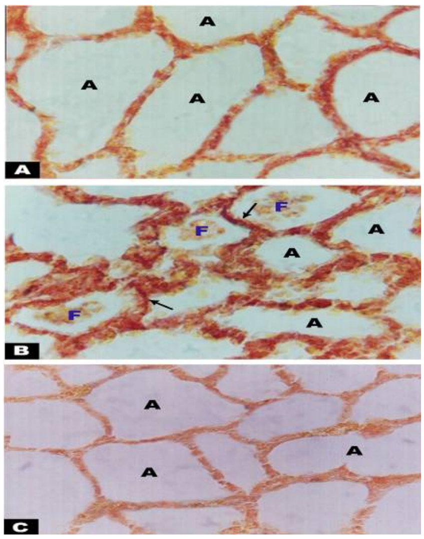

Plate (2): Photomicrograph of newly borne rat lung:
(A)Control group showing regularly expanded alveoli(A)
(B)Pre-eclamptic group showing irregularly collapsed alveoli(A)with many collagen fibers within interalveolar septa. The lumen contains oedamatous fluid rich in erythrocytes (F)
(C)Treated group showing regularly expanded alveoli (A);
(Van Gieson'sX250)

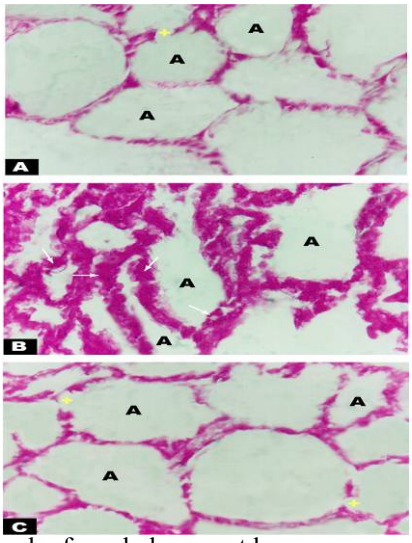

Plate (3): Photomicrograph of newly borne rat lung:
(A)Control group showing regularly expanded alveoli (A) with complete disappearance of glycogen content from alveolar epithelium(+)
(B)Pre-eclamptic group showing irregularly collapsed alveoli (A) with residual glycogen content within alveolar epithelium.
(C) Treated group showing regularly expanded alveoli (A) with disappearance of glycogen content from alveolar epithelium(+)

(Periodic Acid Shief X250)

DISCUSSION

The present study was designed to explore the effect of administration of L-NAME and glutathione on the levels of endogenous antioxidants, lipid peroxidation, systolic BP, degree of urinary protein loss, fetal lung development and neonatal outcome in a rat model of pre-eclampsia.

The systolic BP and proteinuria of the pre-eclamptic group were significantly higher than those of the other groups which confirm that administration of L-NAME during the last 7 days of pregnancy in rats leads to symptoms look like those of pre-eclampsia.

The lipid peroxidation products, primarily measured as thiobarbituric acid-reactive substances (which include MDA), are increased in plasma/sera of women with pre-eclampsia[20]. Furthermore, the pathogenesis of pre-eclampsia is related to an imbalance of increased oxidative stress and lipid peroxidation coupled with a deficiency of antioxidant protection[21].

In this study oxidative stress induced in gravid rats by administration of NOS inhibitor was characterized by high systolic BP and MDA levels. Blood levels of the antioxidants SOD and CAT were significantly increased in the pre-eclamptic group than those of other

groups. There was a positive correlation between systolic BP and plasma levels of SOD on day 20 of gestation in the pre-eclamptic group This was explained by that blood levels of the antioxidants SOD and CAT increased in response to the oxidative stress to prevent or decrease the severity of any further lipid peroxidation- induced cellular injury[21].

These results were explained by Tanir et al.[10] who stated that the high BP created by the infusion of nitric oxide inhibitor results in a hypoxic environment that potentiates an increase in serum lipid peroxidation products and MDA, an example of these products, leads to fluid loss in the cellular membranes, enzymatic dysfunction and impairs the protein synthesis and DNA integrity, which result in endothelial cell dysfunction.

The present study demonstrated that pre-eclampsia could impair lung development and maturation,as the current histological study showed extensive collapsed areas within the lung tissues of the pups of pre-eclamptic mothers compared with those of control group.

Significant morphological changes were noticed among the pups of pre-eclamptic group than control group manifested by thickening of the interalveolar septa, collapsing of the alveoli, filling of luminae with fluid rich in cellularity.

In this study, the histological fluid showed delayed glycogen utilization by the epithelial cells of pups of pre-eclamptic group which was an index of retarded cellular maturation. This is explained by that pre-eclampsia could impair lung maturation though fetal growth retardation, ischemia -hypoxia effect and cellular enzymatic effect[22]. The effect of hypoxia on the alveolar epithelial cells is via impairment of transepithelial sodium ion transport, disruption of cytoskeleton integrity, impairment of oxidative phosphorylation in alveolar epithelial cells and interference with surfactant synthesis[23].

This was supported by Ischioka et al.[24] postulated that pre-eclampsia is accompanied with hypoxic stress that affects palcenta and fetal organs. It inhibits activity and transport mechanism of alveolar sodium ions.

Vascular endothelial growth factor (VEGF) is recognized as a key factor in lung angiogenesis. In new born rats, inhibition of VEGF receptors decreases lung vascular growth factor and alveolization and down-regulates endothelial NOS expression(25). Inhalation of NO improves lung vascular and alveolar growth in newborn rats that are treated with VEGF receptor inhibitor[26]. In this study impairment of fetal lung development may be due to inhibition of VEGF receptors caused by inhibition of fetal NO synthesis by L-NAME having crossed the placenta (27).

In this study, neonatal survival of pups was found to be the lowest in the pre-eclamptic group .This was explained by kassab et al (28) who reported that there were significant reductions in regional blood flow to the heart, lungs, liver, diaphragm, and skeletal muscles in pregnant rats treated with L-NAME.

Glutathione, is essential as a defense against oxidative stress. Both

higher consumption and disturbed synthesis of glutathione have been implicated in the disease process. The total (reduced, oxidised and protein-bound) blood levels of glutathione were found to be lower in women with pre-eclampsia than in pregnancies not characterized by hypertension[29].

In this study, exogenous glutathione treatment in rats yielded better outcomes of attempts to decrease the systolic BP and the lipid peroxidation product, MDA, which emphasizes the role of the plasma thiol status. A substantial number of human studies have shown that in pre-eclamptic cases, as opposed to normotensive controls, there was an inverse correlation between increased free oxygen radical formation and endogenous antioxidant levels[30].

The use of antioxidants in the treatment of pre-eclampsia is a promising light in preventing or slowing down the disease process[31]. However, in this study, following antioxidant use, plasma MDA levels decreased significantly in parallel with a decrease in the activity of the antioxidants SOD and CAT.

In addition to changes in the oxidative stress versus circulating antioxidant levels, systolic BP reduced throughout this experiment when glutathione was given and was positively correlated with plasma levels of SOD. Galley et al.[32] stated that in human trials short-term oral antioxidants (high-dose Vitamin C and Vitamin E) could be used as an adjunctive therapy to reduce BP. However, most studies performed to test the impact of antioxidants on BP in hypertensive disorders address the question of whether antioxidants can prevent or treat pre-eclampsia in clinical trials. In this study, following the antioxidant treatment improvements in renal function (proteinuria) were observed and neonatal survival of the pups was significantly improved, reversing the adverse consequences of pre-eclampsia to some degree.

Conclusion:

In a rat model of pre-eclampsia, hypertension, proteinuria , changes of oxidative stress of pregnant rats, a high neonatal death rate and pathological changes of pups lung appeared to be prevented by exogenous administration of glutathione which provides a valuable protective effect L-NAME induced preeclampsia.

Recommendations:

1-More studies must be carried out for the effect of routine use of antioxidants against pre-eclampsia in human

2-Future studies are warranted to find effective ways of preventing the disease course at an early stage and to document the impact of antioxidant combinations.

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تأثيرمادة الجلوتاثيون على ضغط الدم و نمو الرئة ونتاج حديثى الولادة فى نموذج تسمم الحمل لدى الفئران

*مروه عبد العزيز أحمد - أميمة جلال أحمد- أسماء فرغلى سيد- طارق محمد مصطفى**
قسم الفسيولوجيا- قسم التشريح*- كلية الطب – جامعة أسيوط

يعتبر تسمم الحمل من الحالات الفريدة . كما يعتبر خلل فى الطبقة المبطنة للأنسجة .وقد وجد أن نقص مضادات الأكسدة الداخلية أو زيادة الأحتياج اليها يسيء إلى استقامة الطبقة المبطنة للأنسجة . و يعتبر تسمم الحمل من الأسباب المؤدية إلى النمو المحدود داخل الرحم .

ويهدف البحث إلى معرفة كيفية تغير مستويات مضادات الأكسدة الداخلية و نواتج عملية أكسدة الدهون بعد إعطاء مادة مثبط مصنع النيتيرك أوكسيد وإلى معرفة تأثير إضافة مادة الجلوتاثيون على ضغط الدم و درجة فقد البروتينات فى البول و حجم ووزن صغار الفئران عند نهاية الحمل ولتقييم نموحجم الرئة فى صغار الفئران .

ولقد تم التزواج بين أنثى فئران بالغين وعددهم ٣٠ مع ذكور فئران بالغين تحت ظروف مناسبة . تم قياس ضغط الدم لفئران المجموعات الثلاثة منذ اليوم الأول من الحمل حتى نهاية فترة الحمل .

عند اليوم السابع عشر من التجربة تم تقسيم الفئران الحوامل إلى ثلاثة مجموعات:

١- مجموعة ضابطة: وتشتمل على ١٠ فئران وتتغذى على غذاء معيارى.
٢- مجموعة مصابة بتسمم الحمل : وتشتمل على ١٠ فئران يتم إعطائهم مادة مثبط مصنع أوكسيد النيتيرك بتركيز ٥٠ مج/ كجم مذاب فى ماء الشرب.من يوم ١٧- ٢١ من الحمل .
٣- مجموعة معالجة : وتشتمل على ١٠ فئران يتم إعطائهم مادة مثبط مصنع النيتيرك أوكسيد بتركيز ٥٠ مج/ كجم بالأضافة إلى مادة الجلوتاثيون بتركيز ٦٠ مج / كجم عن طريق الحفن داخل البروتون من يوم ١٧- ٢١ من الحمل.

تم جمع عينات بول يوم ١٨,١٩,٢٠,٢١ و تم قياس حجم البول وتقييم درجة فقد البروتينات فى البول.. وتم سحب عينات دم (٤ مل) من كل الفئران الحوامل و أ ضيفت مادة الأديتا إلى كل عينة دم وتم فصل البلازما بالطرد المركزى وحفظت مجمدة عند درجة حرارة قدرها –٧٠ درجة حرارية لحين قياسها .

وتم قياس الآتى : ١

١- مستوى ألدهيدات حمض المالونيل والسوبر أوكسيد دسميوتيزو الكتالاز فى البلازما بعد الولادة تم تسجيل أوزان و حالة صغار الفئران .
٢- و تم تعيين حجم الرئة لدى١٠ من صغار فئران حديثى الولادة لكل مجموعة بالأضافة إلى أخذ عينات من الرئة لفحص التغيرات النسيجية .

و لقد أوضحت نتائج هذه الدراسة إن إعطاء مادة مثبط مصنع أوكسيد النيتيرك لفئران مجموعة تسمم الحمل أدى إلى إصابة أمهات الفئران بتسمم الحمل و كانت مستويات أ لدهيدات حمض المالونيل والسوبر

أوكسيد دسميوتيزو الكتالاز فى بلازما أمهات الفئران عالية و ذو دلالة إحصائية عن هذه المستويات فى المجموعة الضابطة .كما كان هناك إرتفاع فى مستويات ضغط الدم و إرتفاع فى كمية البروتينات المفقودة فى البول لدى أمهات فئران المجموعة المصابة بتسمم الحمل عن مثيلاتها لدى أمهات فئران المجموعة الضابطة و هذا الإرتفاع ذو دلالة إحصائية .

كما أوضحت نتائج هذه الدراسة إن إعطاء مادة الجلوتاثيون لفئران المجموعة المعالجة أدت إلى إنخفاض مستويات ألدهيدات حمض المالونيل والسوبر أوكسيد دسميوتيزو الكتالاز فى بلازما أمهات فئران المجموعة المعالجة عن مثيلاتها فى مجموعة تسمم الحمل إنخفاضا ذو دلالة إحصائية ولكن ظلت هذه المستويات اعلى من مثيلاتها فىالمجموعة الضابطة .

كما كان هناك إنخفاض فى مستويات ضغط الدم و فى كمية البروتينات المفقودة فى البول لدى أمهات فئران هذه المجموعة عن مستويات بلازما أمهات فئران مجموعة تسمم الحمل إنخفاضا ذو دلالة إحصائية ولكن ظلت هذه المستويات اعلى من مثيلاتها فىالمجموعة الضابطة .

ولقد كان معدل الأحياء لصغار فئران مجموعة تسمم الحمل منخفض عن معدل الأحياء لصغار فئران المجموعة الضابطة والمجموعة المعالجة إنخفاضا ذو دلالة إحصائية .

ولقد كانت أوزان أجسام ورئات صغار فئران مجموعة تسمم الحمل منخفضة إنخفاضا ذو دلالة إحصائية عن مثيلاتها لدى لصغار فئران المجموعة الضابطة والمجموعة المعالجة.ولم يكن هناك أى فرق ذو دلالة إحصائية بين المجموعة الضابطة والمجموعة المعالجة.

وكانت المساحة الحويصلية الداخلية لرئات صغار فئران مجموعة تسمم الحمل منخفضة إنخفاضا ذو دلالة إحصائية عن مثيلاتها لدى صغار فئران المجموعة الضابطة والمجموعة المعالجة.وكانت المساحة الحويصلية الداخلية لرئات صغار فئران المجموعة الضابطة اعلى من مثيلتها فى المجموعة المعالجة.

وكان سمك الجدار الحويصلي الداخلي لرئات صغار فئران مجموعة تسمم الحمل مرتفع إرتفاعا ذو دلالة إحصائية عن مثيله لدى صغار فئران المجموعة الضابطة والمجموعة المعالجة.ولم يكن هناك أى فرق ذو دلالة إحصائية بين المجموعة الضابطة والمجموعة المعالجة.

وعند فحص أنسجة الرئة لصغار فئران مجموعة تسمم الحمل وجدت تغييرات نسيجية مرضية .

ونستنتج من هذه الدراسة أن إعطاء مادة الجلوتاثيون فنموذج تسمم الحمل لدى الفئران يؤدى إلى تقليل درجة الإصابة بمرض تسمم الحمل ، حيث أدى إلى إنخفاض مستويات ضغط الدم و كمية البروتينات المفقودة فى البول و إلى إنخفاض مستويات أ لدهيدات حمض المالونيل والسوبر أوكسيد دسميوتيزو الكتالاز. وإلى تحسن ملحوظ فى التغييرات النسيجية للرئة.