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Research Article

Evaluation of Hemodynamics and Oxidative Stress in the Pathophysiology of L-NAME Induced Preeclampsia in Rats

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Abstract

Background: Preeclampsia (PE) is a serious pregnancy disorder that accounts for 4-5% of fetal and maternal mortality and morbidity worldwide. It is imperative to understand the pathophysiology of PE to devise an effective therapy or cure for PE. In this study, we aim to characterize an animal model of PE and evaluate the hemodynamic changes and oxidative stress in PE.

Methods: Systolic and diastolic blood pressure, mean arterial pressure, and heart rate were recorded invasively along with proteinuria on the 21st day of gestation in an NG-nitro-L-arginine-methyl-ester (L-NAME) induced animal model and by phenotypic characteristics were accounted for. Alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) levels were measured. Cardiac abnormalities were confirmed by electrocardiography and creatinine-kinase (CK) levels. Plasma concentrations of nitric oxide, reactive oxygen species (ROS), and antioxidants were measured.

Results: The hemodynamic parameters and proteinuria were found to be elevated in the PE group as compared to the other groups. The animal model was validated by phenotype characterization. We observed ST elevation and increased p-wave dispersion (Pd) and CK-MB levels. In PE groups, plasma levels of AST, ALT, and ALP were significantly higher. Significantly increased levels of ROS and decreased levels of antioxidants were observed. The results are significant with a p-value <0.05.

Conclusion: The L-NAME-induced animal replicates most of the clinical symptoms of PE. It may be used to study the pathophysiological aspects of preeclampsia, especially the effects on the maternal heart. Here, a rat model is used for the first time to show Pd, ST elevation, and cardiac hypertrophy.

Keywords: Preeclampsia; L-NAME; Oxidative Stress; Hemodynamics; ECG; Biochemical Tests

Graphical Abstract

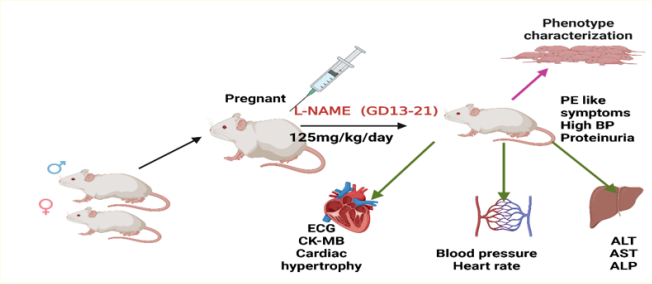

Figure a

Introduction

Preeclampsia (PE), which is generally presented as the most dangerous pregnancy condition, has been documented for millennia. This gestation-specific condition affects 4-5% of births worldwide and is still a leading cause of maternal and neonatal morbidity and mortality [1-3]. Miscarriage, abruptio placenta, foetal growth restriction (FGR), pre-term rupture of the membranes, and preterm birth are all obstetrical sequelae. Long-term problems can affect the health of both the mother and the foetus later in life [4-6]. PE is described as new-onset hypertension with organ injury after 20 weeks of pregnancy and proteinuria (> 300 mg/dl) or other severe characteristics such as renal insufficiency, liver involvement, neurologic or haematological problems, or indications of uteroplacental dysfunction that could manifest themselves as its counterparts [7]. Because of the diverse nature of its clinical presentation and evolution, such as gestational age of start of clinical symptoms and severity of disease progression impacting both mother and fetus, preeclampsia is considered a syndrome rather than a disease. Early-onset disease appearing at less than 34 weeks' gestation and late-onset disease occurring at more than 34 weeks' gestation are the two subtypes of preeclampsia that are clinically identifiable by the period of commencement of clinical disease. Placental disorders since they occur in individuals with hydatidiform moles and the only treatment available so far is the delivery of the placenta [8]. PE is considered to be a state of increased oxidative stress resulting from an imbalance between prooxidants and antioxidants [9]. PE increases the likelihood of the mother acquiring the disease in subsequent pregnancies, as well as the risk of female children developing the condition and male offspring contributing to the development of PE [10]. PE is the subject of intense research, and rats could be a good animal model for studying molecular pathways that might unlock the secrets of PE in women [11]. It has been observed in previous literature that PE-like conditions could be developed in rats by inhibiting nitric oxide [12]. NG-nitro-L-arginine-methyl-ester (L-NAME) is a nitric oxide synthase (NOS) inhibitor that is involved in inducing pre-eclampsia in rats as significant similarities have been observed in the clinical symptoms between L-NAME-treated rats and pre-eclamptic women [13]. It has been known that pre-eclamptic women suffer from cardiovascular (CV) complications in the long term, and their effect on maternal health is quite profound. Previously, it was thought that PE leads to CV diseases in affected women, but recently, many research studies indicate that prior CV complications could be a risk factor for developing PE [14]. PE increases the risk of cardiovascular mortality and morbidity both during pregnancy and long-term after delivery by causing cardiac changes that may lead to atrial and ventricular arrhythmias [15]. In this study, we want to find out what kind of relationship exists between the cardiovascular system and PE by looking at hemodynamic parameters and the electrical activity of the heart in Wistar rats using an L-NAME-based model.

Despite enormous scientific efforts aimed at effectively predicting, diagnosing, and treating PE, a cure remains elusive. There is a dire need for translatable approaches for PE. Therefore, it is imperative to develop animal models of PE for understanding causal pathways and designing and developing effective therapies to thoroughly assess the safety and efficacy of interventions before introducing them in clinical trials. Such approaches would also allow us to consider the future implications for maternal and fetal health. These animal models could allow us to have a more clear understanding of the mechanisms involved in the pathophysiology of PE using *in vivo, in vitro*, and molecular approaches [16].

Materials and Methods

Animals

The Wistar rats (250-300g) were procured from the animal house at Jamia Hamdard, New Delhi, for performing all the experiments after taking permission from the Institutional Animal Ethical Committee (IAEC) of Jamia Hamdard, New Delhi, India. We have followed the standards of the Committee for Control and

Supervision of Experiments on Animals (CPCSEA), Government of India. During the experiment, the rats were kept in a room with a constant temperature, humidity, and light cycle (12 hours of light and 12 hours of darkness). They had free access to tap water and food ad libitum.

Animal model development

Male (aged: 250-300g) and virgin female (180-210g) Wistar rats, aged 12-14 weeks, were acclimatized to laboratory conditions for 7 days. Before breeding, neither parent rat was used in any experiments or given any treatments. Females were mated with males (2:1) overnight and examined the next morning for the vaginal plug. The pregnancy was confirmed after the detection of the presence of sperm in vaginal smears. The day on which sperm was detected was designated as gestational day 0 (GD 0). On the 21st day of gestation, the pregnant and non-pregnant rats of all the groups were anaesthetized using ketamine (87 mg/kg) and xylazine (13 mg/kg) and were euthanized by head decapitation after collecting blood by cardiac puncture in the citrated vials. Blood was further centrifuged, and the supernatant (plasma) was collected and stored at -20°C till further use.

Randomization of animals into groups

After confirmation of pregnancy, the animals were randomized into four groups-I. Control: non-pregnant rats who were given only normal saline; II. Preg ctrl: healthy pregnant controls who were given normal saline; III. Per se: non-pregnant rats who were given L-NAME (Sigma Aldrich) (125mg/kg/day, intraperitoneally (i.p.)) from gestational days 13 to 20; and IV. PE: pregnant rats were given L-NAME (125mg/kg/day dissolved in saline; i.p.) from GD 13 to GD 20, resulting in pregnant rats having persistently elevated blood pressure and other physiological abnormalities along with proteinuria.

Measurement of hemodynamic parameters

Hemodynamic parameters such as systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured by cannulation of the femoral artery in all groups on GD 21 in the anaesthetized animals by a polyethylene catheter attached to a pressure transducer (MLT0699-DC-06A), which is connected to a Power Lab Data Acquisition System (Chart v.8.1.8, AD Instruments) [17].

Twenty-four-hour urinary protein monitoring

Animals were put in metabolic cages to collect 24-hour urine after acclimatization on gestational days 7 and 20. The animals had access to food and water. 24-hour urine output was collected to measure the concentration of protein using a rat urinary protein assay kit assay (Chondrex, inc.) as per the manufacturer's instructions.

Phenotypic characteristics

All the animals were weighed on GD 0 and GD 21 to calculate the percentage weight gain in PE vs. healthy pregnant rats. After the successful delivery of pups, we counted the number of pups in both groups, and the weight, crown to rump length (CRL), and weight of placenta were measured in both groups (groups 2 and 4). We also looked at the pups in both groups to see if they had any obvious developmental anomalies such as hematoma in the limbs, improper growth, undeveloped digits, etc. In the PE group, we have also calculated the percentage of fetal resorption, developmental anomalies, and mortality. After the experiment, the animals were euthanized by decapitation [20].

Cardiac hypertrophy

After being perfused with phosphate-buffered saline (PBS) and rinsed multiple times in normal saline to remove all blood, the hearts of rats were surgically removed. After weighing the heart, the heart-to-body weight ratio was calculated [18].

Measurement of electrical changes in the heart

On the 21st day of gestation, the pregnant and non-pregnant rats of all the groups were anesthetized using ketamine (87 mg/kg) and xylazine (13 mg/kg) [19], and the dermal layer of their fore and hind limbs were connected to the electrocardiography (ECG) leads, which were connected to the Power Lab Data Acquisition System (Chart 5.4.2, AD Instruments, Australia), to record ECG [18].

Biochemical estimations

To assess the plasma levels of myocardial injury marker creatine kinase-Muscle/Brain (CK-MB), liver injury markers (aspartate transaminase (AST), alanine aminotransferase (ALT/), and alkaline phosphate (ALP) were measured in blood plasma by using commercially available kits as per manufacturer's instructions (Erba Diagnostics kits).

Oxidative stress

To evaluate oxidative stress in PE, we performed a nitric oxide (NO) assay, an assessment of lipid peroxidation malondialdehyde (MDA), reduced glutathione (GSH), and a level of superoxide dismutase enzyme (SOD) in the plasma. The level of NO was measured by using the chemiluminescent method using Krebs buffer (pH 7.4) by quantitating the spectral changes in the reaction mixture due to the conversion of oxyhemoglobin to methemoglobin, i.e., a decrease in the absorbance at 575 and 630 nm maxima as described by Singh., *et al.* [21]. Results were expressed as nmol/hr. Thiobarbituric acid reactive substances (TBARS) are the end products of lipid peroxidation, which are measured as MDA according to Ohkawa's method [22]. To measure SOD activity, the auto-oxidation of pyrogallol by SOD at an alkaline pH was measured as described by Marklund and Marklund [23]. The change in absorbance was measured at 420 nm and the activity was expressed as percentage inhibition. The level of reduced GSH content was measured using Ellman's reagent (5,5'-Dithiobis (2-nitrobenzoic acid) [24].

Statistical analysis

Data from three or more separate experiments were reported as mean ± standard deviation (unless otherwise stated). The Student t-test was used to evaluate data, and group data were analyzed using analysis of variance (ANOVA) followed by the Bonferroni post-hoc test in Graph Pad Prism 8 (Graph Pad Prism, CA, USA). P value <0.05 is considered statistically significant.

Results

Hemodynamic parameters

The administration of L-NAME (125kg/day, i.p.) in pregnant rats resulted in significant changes in hemodynamic parameters (PE group) on GD 21 when compared with normal pregnant and non-pregnant rats. Significant changes were found in SBP (177.1 ± 3.9 vs. 124.2 ± 1.7 and 108.3 ± 2.4), DBP (119.32 ± 3.66 vs. 66.1 ± 1.99 and 71.8 ± 2.40), MAP (84.7 ± 1.65 vs. 91.83 ± 0.96 and 138.5 ± 4.04) and HR (387.5 ± 4.7 vs 343.8 ± 6.6 and 302.8 ± 10.2) in the PE group vs control and healthy pregnant groups respectively. There were not any significant differences in these parameters when the control and pregnant control groups were compared (Figure 1. a-d).

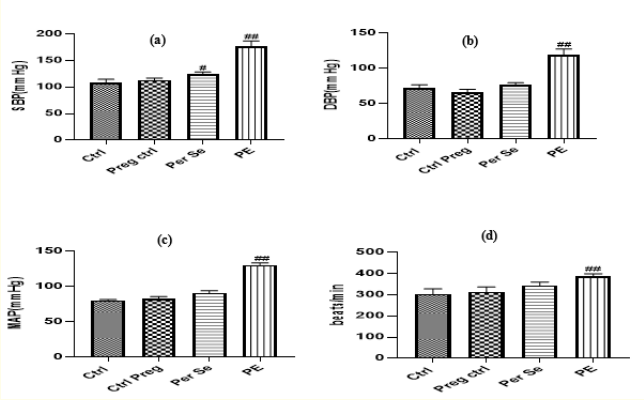

Figure 1: (a-d) The changes in SBP, DBP, MAP and HR have been shown graphically. The levels of these hemodynamic parameters have been altered drastically in all the PE groups. Values are expressed as Mean ± SEM for 8 animals in each group. SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; MAP = Mean Arterial Pressure; and HR = Heart Rate. Control group were administered normal saline, Preg ctrl is normal pregnant, per se is L-NAME administered nonpregnant control and PE were pregnant rats administered with L-NAME.

Determination of proteinuria

24-hour urine was assessed for the presence of protein urine using a commercially available kit. It was observed that the concentration of protein in the PE group was highly increased as compared to normal pregnant (120.8 ± 1.79 mg/ml vs. 59.12 ± 1.15 mg/ ml, *p-value of < 0.05) (Table 1).

Characteristics of L-NAME induced model: Phenotypic characters

In the developed animal model, we have observed a decrease in the birth weight of pups (3.09 ± 0.3 vs. 7.67 ± .12) and weight of placentae (6 placentae per animal; 4.53 ± .39 vs. 2.56 ± 0.8) when compared with healthy pregnant rats (Figure 2a). We have also observed less weight gain (20.65 ± 1.17 vs. 32 ± 2.36), reduced litter size (5.4 ± 0.71 vs. 10.2 ± 0.30), and lower crown to rump length (CRL) (2.68 ± 0.107 vs. 5.36 ± 0.039) in the PE group as compared to healthy pregnant rats. Reduced placenta weight, lower litter size, and CRL are indicative of IUGR. We have also observed mortality and some developmental anomalies such as defects in limb formation in our model (Table 1).

S. No.	Phenotype	Pregnant Control	Preeclampsia
1.	Weight gain (%)	32 ± 2.36	20.65 ± 1.17####
2.	No. of Pups	10.2 ± 0.30	5.4 ± 0.71##
3.	Weight of pups (g)	7.67 ± 0.12	3.1 ± 0.3##
4.	CRL (cm)	5.36 ± 0.039	2.68 ± 0.10#7
5.	Developmental anomaly (%)	-	24 ± 1.68
6.	Mortality (%)	-	2.75 ± 0.54
7.	IUGR (%)	-	40.5 ± 4.2
8.	Proteinuria	59.12 ± 1.15	120.8 ± 1.79

Table 1: Percentage of weight gain in pregnant and PE rats, number of pups, weight of pups, crown to rump length (CRL) of fetus, developmental anomaly, percentage of developmental anomaly, mortality and intra uterine growth reduction (IUGR; intra uterine growth restriction) have been depicted.

Values are expressed as Mean ± SEM for 8 animals in each group. The results are significant with ## p value < 0.01, # p value < 0.05 as compared to pregnant control. IUGR=intra uterine growth restriction. Control group were administered normal saline, Preg ctrl is normal pregnant, per se is L-NAME administered non pregnant control and Preeclamptic were pregnant rats administered with L-NAME.

The heart weight to body weight ratio (HW/BW, mg/g) in control, pregnant control, and PE rats were 2.82 ± 0.11, 3.69 ± 0.14, and 4.63 ± 0.16 respectively. The increase in the heart-to-body weight ratio in the PE model is indicative of myocardial hypertrophy (Figure 2b).

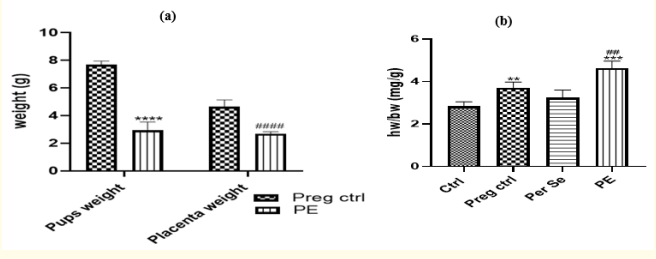

Figure 2: (a-b) The above figure represents a.) weight of pups and placenta in the control pregnant and preeclamptic groups and b.) Cardiac hypertrophy in all the groups. Values are expressed as Mean ± SEM for 8 animals in each group. The result is significant with * p value < 0.05, **p value < 0.01 as compared to control and #p value <0.05, # #p value < 0.01 as compared to pregnant control. Control pregnant were sadministered normal saline, Preeclamptic were pregnant rats administered with L-NAME.

Electrical properties of heart: Electrocardiography

In the present study, we recorded the derangement in electrical impulses in the heart (Figure 3). PE group had elevated P wave dispersion (measured by the difference between maximum and minimum values of P wave segment) in comparison to the pregnant and non-pregnant control rats (8.9 ± 0.1 vs. 2.12 ± 0.03 and 1.05 ± 0.02). We have also observed a significantly elevated ST segment in PE rats (8.13 ± 0.06) as compared to control and pregnant control rats (1.02 ± 0.01and,1.45 ± 0.03).

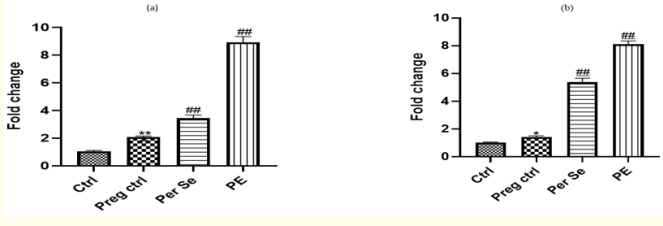

Figure 3: P wave dispersion (a) and ST elevation (b) have been depicted respectively. The above figure represents changes in ECG properties in PE groups as compared to all the groups. Values are expressed as Mean ± SEM for 8 animals in each group. The results are significant with * p value < 0.05, **p value < 0.01 as compared to control and #p value < 0.05, # #p value < 0.01 as compared to pregnant control. Control group were administered normal saline, Preg ctrl is normal pregnant, per se is L-NAME administered non pregnant control and Preeclamptic were pregnant rats administered with L-NAME.

Biochemical parameters

In the present study, we have observed that the cardiac biomarker Creatine Kinase-Muscle Brain (CK-MB) was increased in the PE group (142.01 ± 3.51) when compared with healthy pregnant and nonpregnant controls (32.54 ± 1.7 and 10.44 ± 0.1 respectively). In PE, changes in liver enzymes occur that are indicative of placental and fetal stress. Therefore, it is very important to check for these parameters during pregnancy. During normal pregnancy, these enzymes increase around the second trimester and then get back to normal or remain slightly elevated during the third trimester. In our study, AST levels were assessed in the plasma collected from all the groups. A significant increase was observed in PE samples (80.83 ± 2.07) as compared to normal control (36.91 ± 1.7) and pregnant controls (38.57 ± 2.3). ALT levels were also found to be elevated in PE samples (173.91 ± 1.13) as compared to normal controls (96.37 ± 1.7) as well as pregnant controls (103.74 ± 2.54). Elevated alkaline phosphatase (ALP) shows a positive correlation with fetal distress and IUGR occurring in PE and could be an important tool for monitoring placental damage [25]. The level of ALP observed in PE groups (362.78 ± 8.72) was increased when compared to pregnant and normal controls (158.39 ± 3.64 and 98.49 ± 0.83). ANOVA revealed statistical significance in all the results ($p < 0.05$) among the groups (Figure 4).

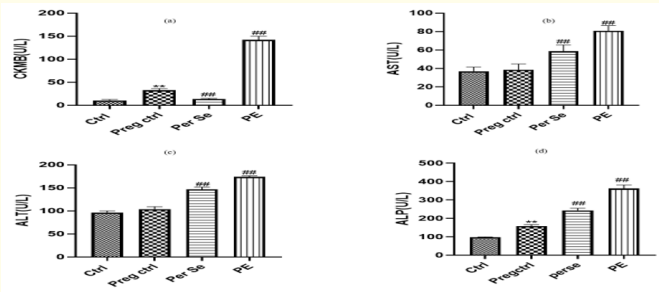

Figure 4: Represents the Creatine kinase-MB(a), AST(b), ALT(c) and ALP (d) levels in control, pregnant control, L-NAME treated control and pre-eclamptic animal model. Values are expressed as Mean ± SEM for 8 animals in each group. The result is significant with **p value < 0.01 as compared to control and #p value <0.05, # #p value <0.01 as compared to pregnant control.

Nitric oxide and oxidative stress

Nitric oxide is an important determinant of PE. We have observed that the level of nitric oxide was lower in people with PE than in normal pregnant and nonpregnant controls (0.23 ± 0.03 vs. 1.94 ± 0.04 and 1.12 ± 0.04, respectively). We have analyzed the oxidative stress by measuring prooxidants such as lipid expression, and the percentage of inhibition of superoxide dismutase and glutathione in all the groups The level of lipid peroxidation in the PE group is elevated when compared with normal pregnant and nonpregnant controls (794.97 ± 2.96 vs. 523.76 ± 4.9 and 377.95 ± 3.4 respectively). SOD levels were found to be maximally inhibited in PE as compared to their normal and nonpregnant counterparts (82.58 ± 1.21 vs. 38.66 ± 0.8 and 55. 46 ± 1.84 respectively). Reduced glutathione (GSH) levels were found to be greatly reduced when compared with pregnant and non-pregnant groups (21.45 ± 0.8 vs.75.7 ± 1.22 and 54.28 ± 1.83 respectively). Within the group, all of the results are significant with a p-value of< 0.05 (Figure 5).

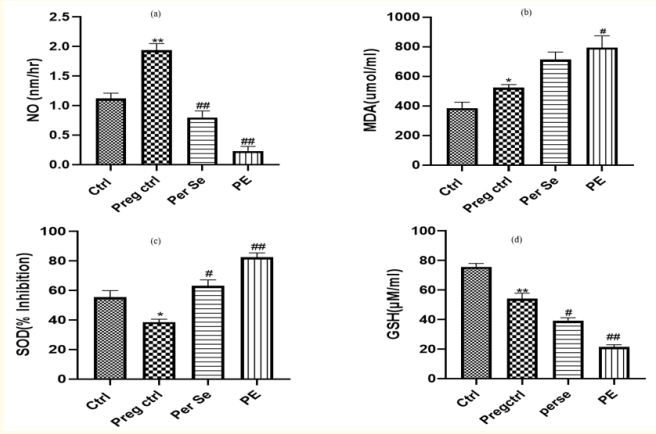

Figure 5: Represent nitric oxide(a), MDA(b), SOD(c) and GSH (d) levels in control, pregnant control, L-NAME treated control(perse) and pre-eclamptic (PE) animal model. Values are expressed as Mean ± SEM for 8 animals in each group. The result is significant with*p value<0.05, **p value < 0.01 as compared to control and #p value <0.05, # #p value <0.01 as compared to pregnant control. NO = Nitric Oxide, MDA = Malondialdehyde, SOD = Superoxide Dismutase and GSH = Reduced Glutathione.

Discussion

L-NAME-induced Wistar rat model of preeclampsia may be a potential model for exploring the pathophysiology of preeclampsia and evaluating treatments for this syndrome. L-NAME treatment resulted in raised blood pressure, proteinuria, oxidative stress, unfavorable pregnancy outcomes, deranged liver enzymes, and a cardiac abnormality in rats.

Since ancient times, the PE disease has been known. PE is a significant pregnancy complication that increases maternal and perinatal morbidity and death, affecting approximately 10% of all pregnancies. Despite considerable scientific efforts, very little is known about its complex and multifaceted origin and pathogenesis. For many years, it was debatable whether an animal model might be useful in PE research because the development of PE spontaneously is unique to human pregnancies. So, in animal models, the symptoms can only be approximated rather than replicated. Chronic NO inhibition raises blood pressure in a volume-dependent manner, and the related physiological and pathological features are comparable to those of primary hypertension [26]. Furthermore, acute suppression of NO production by L-NAME injection resulted in arterial hypertension and vasoconstriction [27]. In this study, we developed a novel PE model by injecting the NOS inhibitor L-NAME intraperitoneally. We propose that L-NAME administration in pregnant rats suppresses NOS and mimics preeclampsia. In our study, we have observed that our PE model replicates most of the clinical symptoms such as the increase in systolic blood pressure, proteinuria, IUGR, deranged liver enzymes, cardiac anomalies (electrical: ECG and structural: cardiac hypertrophy and CKMB) reduced nitric oxide and increased oxidative stress. These changes were not observed in L-NAME administered virgin female rats. This increase in blood pressure and proteinuria could result from endothelial dysfunction as it contributes to increased systemic vascular resistance, leading to an impaired balance between vasoconstrictors and vasodilators [28,29]. We have observed that the phenotypic characteristics in the preeclamptic model mimic the phenotypic characteristics found in preeclamptic patients, such as low birth weight, the small size of the fetus, reduced litter size, and reduced placental weight (indicative of IUGR). We have also observed certain developmental anomalies, mortality, and preterm birth in our study**.** In the current study, we observed that the weight of the placenta in preeclamptic model rats was less than the weight of the placenta of normal pregnant rats. Pre-eclampsia can also cause cardiovascular problems in the mother and cardio-metabolic disorders in the offspring later in life. It could lead to a greater risk of eventual heart failure and an increased risk of coronary heart disease, stroke, and death from cardiovascular disease. In 2011, the American Heart Association guidelines acknowledged PE as an independent gender-specific cardiovascular risk factor [30]. It has been estimated that compared with women without a history of pregnancy-related complications, the calculated ten-year CVD risk based on the Framingham score is 31% greater with PE history and 27% greater with gestational hypertension [31]. In ECG, ST-segment or T-wave abnormalities, or both ('ST-T abnormalities'), are the most common ischemic ECG findings. These anomalies have been linked to an elevated risk of cardiovascular disease in prospective studies (CVD). S-T elevation is the first signal on an ECG which leads to myocardial infarction. The duration and height of the ECG waves changed dramatically in our study. Hence, S-T elevation observed in PE rats in our study could be indicative of a potential risk of myocardial infarction. Various cardiovascular risk factors have been shown to impact P-wave dispersion (Pd) (Dawood Darbar, Arshad Jahangir, Stephen C. Hammill And Gersh 2002). Increased pulse wave velocity (PWV), which indicates increased arterial stiffness due to endothelial dysfunction, is a crucial component of PE. It is thought that permanent vascular damage caused due to increased oxidative stress initiates endothelial dysfunction, leading to PE, which further contributes to the pathogenesis of CVD. When normal and pregnant controls were compared, we observed that P-wave dispersion was considerably increased in the case of preeclamptic rats. Furthermore, greater Pd values could distinguish severe forms of PE from moderate PE patients, which might be employed as a clinical diagnostic tool in the differential diagnosis of preeclamptic patients [32]. Such alterations in ECG recordings point to the cardiac damage, which was further validated by the cardiac biomarker CK-MB. We observed that CK-MB levels were significantly higher in PE groups as compared to the control and pregnant control. When comparing the biochemical reports of the PE rats to those of all other groups, we have discovered that the levels of liver enzymes ALT, AST, and ALP are highly increased in PE rats. In addition to hypertension and proteinuria, PE is associated with hepatic dysfunction [33]. It has been hypothesized that in PE, various mediators are released from the liver and blood vessel endothelium, causing vasoconstriction and hepatic hypoxia, which lead to other systemic illnesses such as neural edema and cardiovascular disease.

Normal pregnancy is a prooxidant stage characterized by ROS generation and oxidative stress, characterized by decreased plasma levels of free antioxidants. This prooxidant feature is considerably more pronounced in several pregnancy-related diseases, including PE [34]. Although the cause of PE is uncertain, a placental origin is hypothesized and supported by the quick remission of symptoms after delivery. Implantation, trophoblast invasion, and spiral artery remodelling all take place until the 20-22nd week of pregnancy in a normal pregnancy [35]. Due to poor trophoblastic invasion and abnormal placentation, the placenta develops oxidative stress. In rats, this corresponds to the commencement of the final week of pregnancy [36]. Various reports show that increased superoxide production causes placental oxidative stress, which is instrumental in the development of PE [37]. In this case, superoxides and free radicals produced by maternal oxidative stress may target lipids, proteins, and nucleic acids, causing damage to placental cells, tissues, and organs. In this study, we have reported increased levels of MDA and nitric oxide and decreased levels of the antioxidants GSH and SOD. The deficiency of NO may directly or indirectly commence a cascade of physiological mechanisms in PE, such as hypertension, increased glomerular filtration rate, proteinuria, and platelet dysfunction [38]. Nitric oxide (NO) is one of the key players in the regulation of placental blood flow. It is actively engaged in cytotrophoblast endovascular invasion and development of the placenta, through its unique angiogenic and vasculogenic properties [39]. Therefore, oxidative stress plays as an important part in the pathophysiology of PE by altering the oxidative stress of the placenta.

Conclusion

Animal-based research in PE is crucial in understanding its pathophysiological mechanisms. Despite the fact that this unfortunate pregnancy complication has been reported for thousands of years, no effective therapy or cure has been devised, and delivery of the placenta is the only cure available till date. In our study, we have developed and validated the L-NAME based model of PE. We have observed that PE model was characterized by hypertension and proteinuria along with the involvement of multi organ dysfunctions such as liver and heart. This model mimics the clinical symptoms of PE. Therefore, it is plausible to say that L-NAME-induced animal models may allow us to explore surgical, pharmacological, and genetic modifications, facilitating substantial advances in this area to understand the processes that govern PE and beyond.

Acknowledgement

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Declaration of Interest

We hereby declare that the manuscript has not been previously published in any language anywhere and that it is not under simultaneous consideration by another journal. None of the authors have any conflict of interest or any financial ties to disclose.

Ethical Approval

Animals were procured from the animal house, Jamia Hamdard, New Delhi after taking permission from the Institutional Animal Ethical Committee (IAEC; Protocol No.1689) of Jamia Hamdard, New Delhi, India. We follow the standards of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Consent to Participate

Not Applicable.

Consent to Publish

All authors read the manuscript and approved the same for publication.

Authors Contributions

The research was conceived and planned by HQ, GK and MIA. MIA, GK and HQ helped in designing the animal experiments. HQ, SN and ND performed the experiments. HQ and SB performed molecular work. HQ, SN and ND performed animal work. HQ and MIA wrote the manuscript. All authors read and approved the final manuscript and have no conflict of interest. All authors read and approved the manuscript and all data were generated in-house and no paper mill was used.

Funding

Not applicable.

Competing Interests

No competing interests are present among the authors.

Availability of Data and Materials

All the data were generated and analyzed by the author and all the resources were available in the laboratory of the author.

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