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L-arginine supplementation lowers blood pressure, protein excretion and plasma lipid profile in experimental salt-induced hypertension in pregnancy: Relevance to preeclampsia

A.P. Arikawe[a,*], I.C. Udenze[b], A.W. Olusanya[c], O.A. Akinnibosun[a], I. Dike[a], B.N. Duru[c]

[a] Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria
[b] Department of Clinical Pathology, Faculty of Clinical Sciences, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria
[c] Department of Pharmacology, Therapeutics & Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria

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ABSTRACT

This study aimed to investigate the effects of L-arginine supplementation on blood pressure, protein excretion, lipid profile in salt-induced hypertensive pregnant rats.

Female Sprague-Dawley rats were divided into 4 groups. Control Preg (normal rat chow). Control Preg + L-ARG (normal rat chow and daily oral L-Arginine from 16th – 20th week). Salt Preg (high salt diet, 8%). Salt Preg + L-ARG (high salt diet, 8% and daily oral L-Arginine from 16th – 20th week. Non-invasive BP was recorded using a tail-cuff machine at 1 st and 2nd trimesters. On day 19 of pregnancy, invasive BP was obtained by carotid artery cannulation connected to LabChart-7 pro software. This was followed by blood samples collection for lipid profile analysis.

L-arginine significantly reduced ($P < 0.05$) systolic, diastolic, MAP at 1 st, 2nd trimesters, day 19 of pregnancy, LDL, plasma and urinary creatinine and protein levels in Control Preg + L-ARG and Salt Preg + L-ARG groups compared to other groups. Urinary Na + and K + were significantly higher ($P < 0.05$) in Salt Preg + L-ARG group compared to other groups. Total cholesterol level was significantly higher ($P < 0.05$) in salt groups compared to control groups. Triglyceride level and urine volume were significantly higher ($P < 0.05$) in Salt Preg group compared to other groups. It also significantly increased ($P < 0.05$) HDL in Control Preg + L-ARG and Salt Preg + L-ARG groups compared to other groups. L-arginine supplementation ameliorates some deleterious effects in salt- induced hypertensive pregnant rats possibly through its known NO vasodilatory effect and might also mediate a diuretic like action.

1. Introduction

Normal pregnancy is associated with significant haemodynamic and cardiovascular changes to meet the metabolic needs of the mother and fetus. For example, maternal cardiac output and plasma volume increase during pregnancy, whereas the total vascular resistance and arterial pressure tend to decrease [1]. This is due to a reduction in the pressor response and vascular reactivity to vasoconstrictor agonists [2,3] and a reduction in angiotensin II in normal pregnant rats [4]. The changes during normal pregnancy have been attributed, in part, to increased synthesis/release of nitric oxide (NO) and perhaps other vasodilator substances such as prostacyclin (PGI2) and hyperpolarizing factor by various maternal cells including vascular endothelial cells [5–7].

Hypertension is one of the most common medical complications in pregnancy and it affects about 10% of pregnancies [8]. Hypertensive disorders during pregnancy are classified into 4 categories i.e. chronic hypertension, preeclampsia-eclampsia, preeclampsia superimposed on chronic hypertension and gestational hypertension [9]. Preeclampsia is a pregnancy-specific disorder characterized by hypertension and proteinuria [10,11]. It is defined by blood pressure of 140/90 mm of Hg or a rise in the systolic blood pressure of more than 30 mm of Hg or diastolic blood pressure of more than 15 mm of Hg after 20 weeks of gestation accompanied by proteinuria ≥ 300 mg/24 h [12,13]. It is a major cause of maternal morbidity worldwide increasing the mother's risk of developing renal failure, pulmonary oedema, and stroke,

* Corresponding author at: Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi Araba, Lagos, Nigeria.
E-mail address: aarikawe@unilag.edu.ng (A.P. Arikawe).

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and it is also associated with grave risks for the foetus including intrauterine growth restriction (IUGR), death and prematurity with attendant complications [14,15].

The aetiology of preeclampsia is not well understood; however, some pathways have been suggested. These include endothelial dysfunction, defective placentation, inflammatory responses, oxidative stress, activation of thrombosis and the renin-angiotensin system (RAS) [16–18].

Other implicated aetiology entail defective nitric oxide (NO) synthesis and abnormal lipid synthesis. For example, studies in pregnant rats administered nitro-L-arginine methyl ester (L-NAME) have shown decreased NO production during late pregnancy (days 14–20) [19,20]. This reduction in NO synthesis is associated with a significant increase in maternal blood pressure, loss of vascular refractoriness to pressor stimuli, and a decrease in the weight of the placenta as well as that of the offspring [19,20]. It is also characterized by generalized vasoconstriction and increased pressor response to vasoconstrictor agonists such as angiotensin II (ANG II) [21]. The increased vascular reactivity to vasoconstrictors could be due to decreased endothelium-dependent mechanisms of vascular relaxation and/or enhanced mechanisms of vascular smooth muscle contraction. This implies that the inhibition of NO synthesis in pregnant rats could provide a useful model of human preeclampsia [22].

High salt diet has been implicated in the pathogenesis of human hypertension particularly in salt-sensitive individuals [23]. For example, the level of salt in a diet has been correlated with blood pressure levels in humans [24,25] and in experimental animals [26,27].

Several mechanisms have been demonstrated to mediate high salt-induced hypertension and impairment of vascular function in several vascular beds of laboratory animals [28–30]. Increased reactive oxygen species (ROS) generation and increased endothelial NO synthase (eNOS) uncoupling have been implicated in this phenomenon [31,32] which is characterized by attenuated vasodilators and enhanced vasoconstrictors responses to vasoactive agents [26]. There seems to be an overlap in the mechanisms through which a high salt diet leads to hypertension and eventually to preeclampsia. Thus, salt-induced hypertension in pregnant rats could be another model of human preeclampsia.

L-arginine, a precursor for the synthesis of nitric oxide (NO) is an amino acid that plays multiple roles in the cardiovascular system largely through NO production [33–35]. Several studies have established the antihypertensive and antioxidant properties of L-arginine, showing the benefits in hypertension and hypercholesterolemia [33,36–42]. L-arginine supplementation has been reported to reduce blood pressure and related cardiovascular risk factors in humans and animals.

Nitric oxide is an important protective molecule in the vasculature and eNOS is responsible for most of the vascular NO produced [43]. Nitric oxide maintains vascular integrity by inhibiting platelet aggregation, leukocyte-endothelium adhesion and vascular smooth muscle cell proliferation [43,44]. The NO produced in the cardiac muscle also helps regulate cardiac contractility. Several diseases such as atherosclerosis, hypertension, diabetes mellitus, congestive heart failure, thrombosis, stroke as well as preeclampsia have been associated with abnormalities in NO signalling or diminished bioavailability [43–47]. This property leads to the hypothesis that L-arginine supplementation may have potential blood pressure lowering effect by increasing the production of nitric oxide in salt-induced hypertension in the pregnant rat model of preeclampsia. Thus, this study aimed to investigate the possible protective effects of oral L-arginine supplementation on blood pressure, protein excretion and lipid profile in salt-induced hypertensive pregnant rats.

2. Methodology

2.1. Animals

Forty (40) female Sprague-Dawley nulliparous rats weighing between 70–80 g and aged 5 weeks, in the beginning, were used for this study. The rats were obtained from the animal laboratory centre, College of Medicine, University of Lagos. All the animals were housed in groups of 5 rats per clear polypropylene cage lined with wood shavings and acclimatized for one week. Rats were kept under normal light conditions (12 h light/dark cycle) and normal room temperature ($23 \pm 1\,^{\circ}C$). Pelletized normal rat chow and water was made available ad libitum, and all rats were weighed weekly.

All experimental procedures were carried out in compliance with the international principles for laboratory animals as obtained in the Helsinki's declaration (NIH1985) guide for the care and use of laboratory animals. The research protocol was also in line with the guidelines of the College of Medicine, University of Lagos, Health Research Ethics Committee. The whole study lasted for 20 weeks (6 weeks before salt diet; 10 weeks of salt diet; and 4 weeks oral L-Arginine administration).

2.2. Experimental design and animal groupings

The rats were divided into four groups as described below:

Group I (Control Preg) had access to normal rat chow and water ad libitum and no drug was administered to them throughout the length of study.

Group II (Control Preg + L-ARG) had access to normal rat chow and water ad libitum. Rats in this group also received daily L-Arginine administration (100 mg/kg) in distilled water and administered orally via oral gavage from the 16th week till 20th week (28 consecutive days).

Group III (Salt Preg) fed high salt diet (8% salt) [26,48] for 10 weeks and had access to water ad libitum and no drug was administered to them throughout the length of study.

Group IV (Salt Preg + L-ARG) fed high salt diet (8% salt) [26,48] for 10 weeks and had access to water ad libitum. Rats in this group also received daily L-Arginine administration (100 mg/kg) in distilled water and administered orally via oral gavage from the 16th week till 20th week (28 consecutive days).

2.3. Assessment of oestrous cycle and induction of pregnancy

From the 15th week, vaginal smears were carried out daily in each rat during early hours of the morning to determine the specific phase of the oestrous cycle by viewing the predominant cell types in the vaginal smears under a microscope using the Marcondes technique [49]. The smears were done for two consecutive weeks to confirm the animals were cyclic. At the 17th week, rats on proestrous phase were allowed to mate with male rats on the evening of proestrous. The presence of sperm cells in the smears of the rats on the next day confirmed mating and was assumed as day 1 of pregnancy.

2.4. Non-invasive blood pressure measurement

Non-invasive blood pressure (BP) parameters i.e. systolic blood pressure (SBP); diastolic blood pressure (DBP), and mean arterial blood pressure (MAP) were recorded using a tail-cuff machine at 1 st trimester (day 7 of pregnancy) and 2nd trimester (day 14 of pregnancy). The caudal BP of animals was measured with a tail-cuff BP apparatus (Kent Scientific CODA system).

2.5. Sacrifice, invasive blood pressure and foetal weight measurements

On day 19 of pregnancy, the rats were anaesthetized with a solution of 25% (w/v) urethane and 1% (w/v) α-chloralose injected intraperitoneally at a dose of 5 ml/kg body weight. The rat that has totally lost its righting reflex was placed in supine position on the dissecting board, limbs were fastened and the trachea was exposed and cannulated. BP measurements were obtained by cannulation of one carotid artery. A polyethylene cannula filled with 1% heparinized saline was inserted into the artery, tied in place.

The cannula inserted into the artery was connected to the transducer to obtain BP values. The abdominal cavity of each rat was cut open, foetuses were counted, isolated and weighed. Invasive BP measurement was carried out via arterial cannulation as described above using a pressure transducer (model SP 844, Physiological Pressure Transducer, AD Instruments) which was attached through MLAC11 Grass adapter cable to a computerized data acquisition system with LabChart-7 pro software (Power Lab-4/24T, model MLT844/P; AD Instruments Pty Ltd., Castle Hill, Australia) [18].

2.6. Collection of blood and urine samples

Blood samples were collected from each rat immediately after invasive recording of BP parameters according to animal groupings and markings. The blood samples were collected from the left carotid artery using a capillary tube, collected into EDTA coated test tubes and centrifuged at 3000 (rpm) for 10 min. to extract the plasma. The plasma was stored at −20 °C for measurement of the fasting lipid profile, fasting electrolyte, urea and creatinine levels.

Twelve-hour urine samples were collected on day 17 or 18 of pregnancy using a locally constructed metabolic cage. The urine collected was preserved with toluene and used for determining urinary protein, electrolyte, urea and creatinine levels and urine volume. Total protein in the urine was assayed using Randox Biuret kits [50]. Urinary creatinine content was determined using the principle of a colourimetric reaction of creatinine with alkaline picrate measured kinetically at 490 nm.

2.7. Lipid profile assessment

High-density lipoprotein (HDL) and Low-density lipoprotein (LDL) were measured as previously described [51]. Total cholesterol and triglycerides were quantified using the Callegari S.P.A (Parma, Italy) Autoanalyzer system. This system employs the wet chemistry and photometric technology to perform absorbance readings which measure the colour of the sample. The absorbance is converted automatically into concentration based on the standard calibration curves stored in the equipment's microprocessor.

2.8. Plasma and urinary electrolytes

Sodium and potassium levels in the plasma and urine were measured using the flame photometry method (410 flame photometer, Chiron Diagnostics) following the manufacturer's guidelines. Urea and Creatinine were determined using the standard assay kit following diacetyl monoxime, and alkaline picrate methods, respectively.

2.9. Statistical analysis

The data were analyzed with Graph Pad Prism 8 software (Graph pad software San Diego, CA, USA). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by student-Newman-Keuls post hoc test. All data are presented as mean ± S.E.M. Level of statistical significance was taken at $P < 0.05$.

Table 1
Effect of L-Arginine on Systolic Blood Pressure (mmHg) in hypertensive pregnant rats.

Systolic blood pressure (mmHg)	1st trimester	2nd Trimester	19th day Gestation
Control Preg	120.0 ± 2.89	100.0 ± 2.76	111.1 ± 3.42
Control Preg + L-ARG	118.3 ± 2.72	98.2 ± 2.82	105.2 ± 3.13
Salt Preg	145.1 ± 2.80*	152.6 ± 2.46*μ	123.3 ± 3.67*
Salt Preg + L-ARG	127.2 ± 2.64μα	121.4 ± 2.41*μα	107.9 ± 3.48α

* significant vs Control Preg., μ significant vs Control Preg + L-ARG., α significant vs Salt Preg.

Table 2
L-Arginine effects on Diastolic Blood Pressure (mmHg) in hypertensive pregnant rats.

Diastolic blood pressure (mmHg)	1st trimester	2nd Trimester	19th day Gestation
Control Preg	81.4 ± 2.70	58.0 ± 2.56	85.8 ± 3.08
Control Preg + L-ARG	89.8 ± 2.62	59.6 ± 2.78	86.4 ± 3.24
Salt Preg	118.7 ± 2.84*μ	111.7 ± 2.62*μ	94.4 ± 3.90
Salt Preg + L-ARG	107.9 ± 2.90*μα	93.4 ± 2.67*μα	82.7 ± 3.68α

* significant vs Control Preg., μ significant vs Control Preg + L-ARG., α significant vs Salt Preg.

Table 3
Mean Arterial Pressure (mm Hg) in hypertensive pregnant rats following L-Arginine supplementation.

Mean arterial pressure (mmHg)	1st trimester	2nd Trimester	19th day Gestation
Control Preg	94.1 ± 2.83	71.2 ± 2.12	94.2 ± 3.01
Control Preg + L-ARG	99.3 ± 2.69	73.4 ± 2.34	96.0 ± 3.87
Salt Preg	127.2 ± 2.87*	116.8 ± 2.56*μ	104.1 ± 3.78*
Salt Preg + L-ARG	114.0 ± 2.56*α	102.6 ± 2.46*μα	91.1 ± 3.64α

* significant vs Control Preg., μ significant vs Control Preg + L-ARG., α significant vs Salt Preg.

3. Result

3.1. Blood pressure

SBP, DBP, and MAP measurements were recorded using the tail-cuff non-invasive method during the 1 st and 2nd trimesters of pregnancy. All three blood pressure parameters were significantly higher ($P < 0.05$) in the Salt Preg and Salt Preg + L-ARG groups compared to the Control Preg and Control Preg + L-ARG groups during the 1 st and 2nd trimesters of pregnancy (Tables 1–3). On the 19th day of gestation SBP, DBP, and MAP measurements were recorded using the invasive method, which is the gold standard for BP measurements [52] in lower mammals. All three blood pressure parameters were significantly higher ($P < 0.05$) in the Salt Preg group only compared to the other groups (Tables 1–3). This could imply L-Arginine is more effective at the 3rd trimester probably due to a longer duration of supplementation.

3.2. Lipid profile

L-Arginine supplementation significantly increased ($P < 0.05$) HDL levels in Control Preg + L-ARG and Salt Preg + L-ARG groups compared to Control Preg and Salt Preg groups. On the other hand, it significantly decreased ($P < 0.05$) LDL levels in these same groups (Fig. 1). Total cholesterol level was significantly higher ($P < 0.05$) in Salt Preg and Salt Preg + L-ARG groups compared to the Control Preg and Control Preg + L-ARG groups. Triglyceride level was sig-

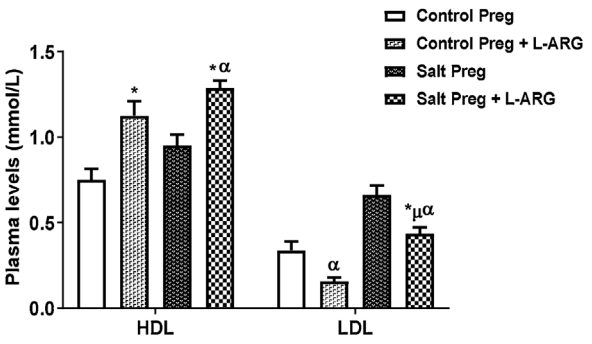

Fig. 1. Plasma levels of HDL and LDL in hypertensive pregnant rats following L-Arginine supplementation *significant vs Control Preg., $^{\mu}$significant vs Control Preg + L-ARG., $^{\alpha}$significant vs Salt Preg.

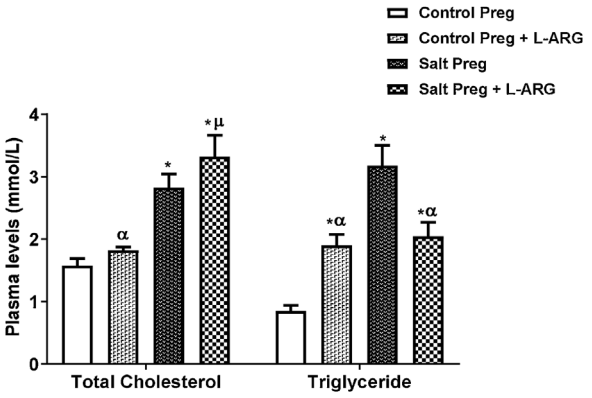

Fig. 2. Total cholesterol and triglyceride levels in hypertensive pregnant rats following L-Arginine *significant vs Control Preg., $^{\mu}$significant vs Control Preg + L-ARG., $^{\alpha}$significant vs Salt Preg.

nificantly highest ($P < 0.05$) in the Salt Preg group compared to the other three groups. It was also significantly higher ($P < 0.05$) in the Control Preg + L-ARG and Salt Preg + L-ARG groups compared to the Control Preg group (Fig. 2).

3.3. Electrolytes

Plasma sodium and potassium were not significantly different amongst the four groups. On the other hand, urinary sodium was significantly higher ($P < 0.05$) in Salt Preg and Salt Preg + L-ARG groups compared to the Control Preg and Control Preg + L-ARG groups. Likewise, urinary potassium was significantly highest ($P < 0.05$) in Salt Preg + L-ARG group compared to the three other groups (Figs. 3 and 4).

Plasma urea and urinary urea were not significantly different amongst the four groups. On the other hand, plasma creatinine and urinary creatinine levels were significantly lower ($P < 0.05$) in the Control Preg + L-ARG and Salt Preg + L-ARG groups compared to Control Preg and Salt Preg groups (Figs. 5–7).

Urinary protein level was significantly highest ($P < 0.05$) in Salt Preg group compared to the three other groups (Fig. 8).

3.4. Foetal weight (gm)

Foetal weight was significantly lower ($P < 0.05$) in the Salt Preg and Salt Preg + L-ARG groups compared to the Control Preg and Control Preg + L-ARG groups (Fig. 9).

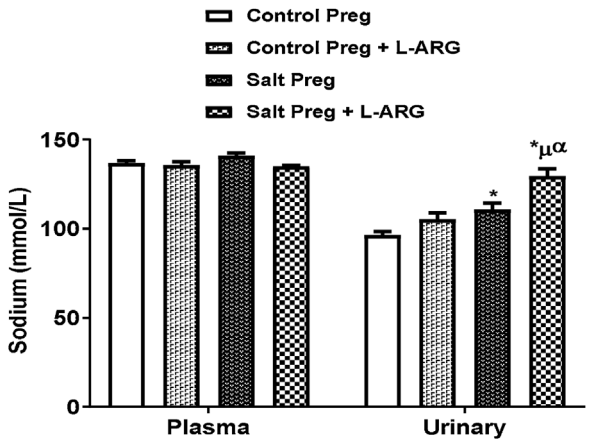

Fig. 3. Plasma and urinary sodium in hypertensive pregnant rats following L-Arginine supplementation *significant vs Control Preg., $^{\mu}$significant vs Control Preg + L-ARG., $^{\alpha}$significant vs Salt Preg.

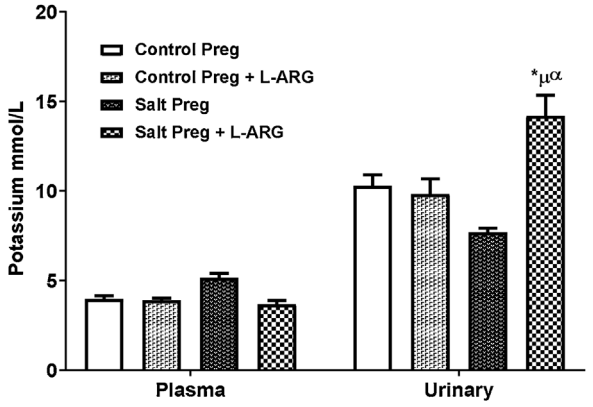

Fig. 4. Plasma and urinary potassium in hypertensive pregnant rats following L-Arginine supplementation *significant vs Control Preg., $^{\mu}$significant vs Control Preg + L-ARG., $^{\alpha}$significant vs Salt Preg.

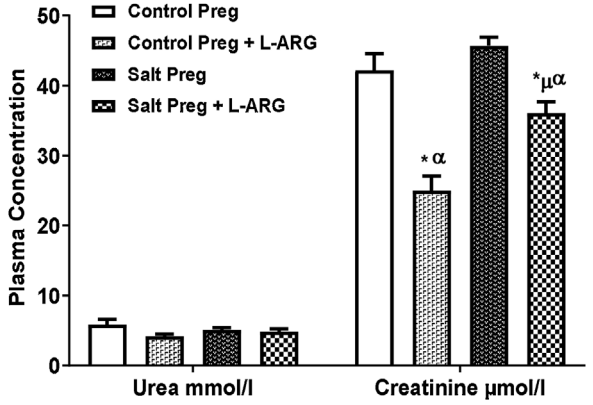

Fig. 5. Plasma urea and creatinine in hypertensive pregnant rats following L-Arginine supplementation *significant vs Control Preg., $^{\mu}$significant vs Control Preg + L-ARG., $^{\alpha}$significant vs Salt Preg.

3.5. Twenty-four hour urine volume (mls)

Twenty-four hours urine volume was significantly highest ($P < 0.05$) in the salt Preg group compared to the Control Preg and Control Preg + L-ARG groups (Fig. 10).

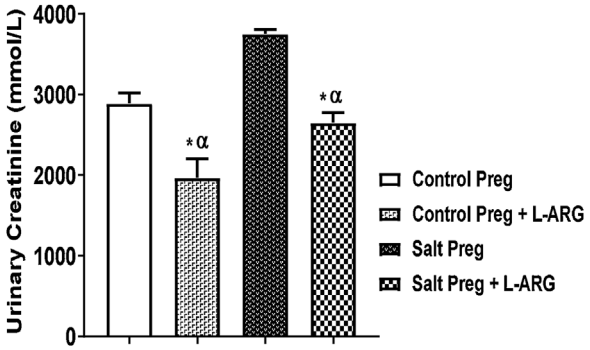

Fig. 6. Urinary creatinine in hypertensive pregnant rats following L-Arginine supplementation.
*significant vs Control Preg., and $^{\alpha}$significant vs Salt Preg.

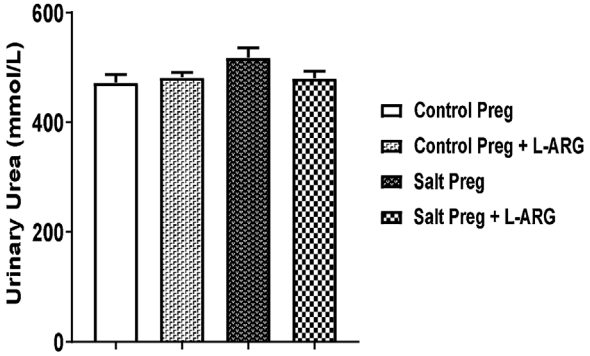

Fig. 7. Urinary urea in hypertensive pregnant rats following L-Arginine supplementation.

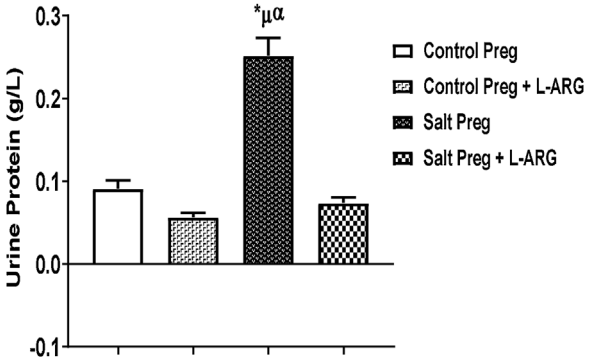

Fig. 8. Urine protein level in hypertensive pregnant rats following L-Arginine supplementation.
*significant vs Control Preg., $^{\mu}$significant vs Control Preg + L-ARG., $^{\alpha}$significant vs Salt Preg.

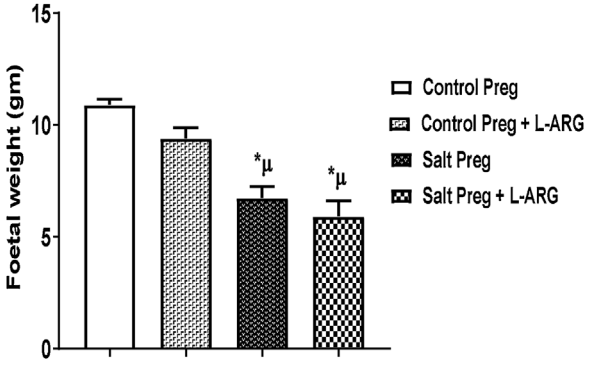

Fig. 9. Foetal weight in hypertensive pregnant rats following L-Arginine supplementation.
*significant vs Control Preg., $^{\mu}$significant vs Control Preg + L-ARG.

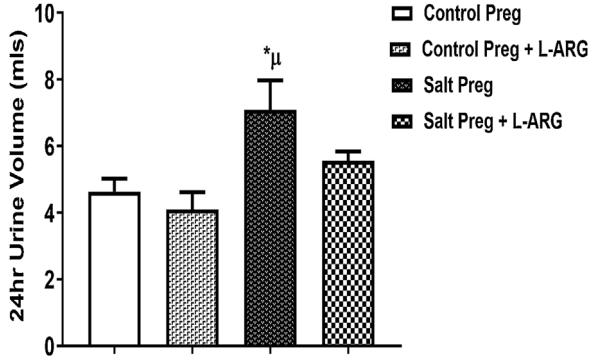

Fig. 10. Twenty-Four Hour Urine Volume in hypertensive pregnant rats following L-Arginine supplementation. *significant vs Control Preg., $^{\mu}$significant vs Control Preg + L-ARG.

4. Discussion

The relationship between low plasma L-arginine concentration and higher blood pressure in Pregnancy-induced hypertension (PIH) has been previously documented and it has been shown that decreased L-arginine plasma levels might be a useful tool to predict the development of PIH [53–56]. These suggest that L-arginine supplementation may reduce the risk of developing PIH. Our findings showed that hypertensive pregnant rats on oral L-arginine supplementation had lower blood pressures which were comparable to non-hypertensive pregnant control rats (Tables 1–3).

These results are consistent with a report showing that L-arginine reduced the blood pressure in pregnant rats with uterine hypoperfusion induced hypertension [57], likewise in humans [58]. Altun et al. [59], also observed a decrease in blood pressure in stress-induced preeclamptic rats with SBP lower in the L-arginine supplemented group than in the preeclamptic group without treatment. However, some studies have reported that oral or intravenous L-arginine supplementation did not reduce blood pressure during preeclampsia [60,61].

One of the suggested pathophysiologic basis of PIH is impaired NO synthesis leading to endothelial dysfunction [53,62–64]. The beneficial effect of L-arginine is linked to the release of NO which causes an increase in 3′,5′-cyclic-guanosine monophosphate (cGMP) production. This leads to the activation of cGMP-dependent protein kinase (PKG) that mediates the relaxation of the vascular smooth muscle cells (VSMCs), with a subsequent reduction in blood pressure as a result of vasodilatation [65,66].

Increased salt intake is a known risk factor for developing hypertension [67]. Our model used chronic salt intake to induce hypertension in rats. This causes a corresponding increase in renal salt excretion until the excretory capacities of renal tubules are overwhelmed. Thus, there is a reset of the total amount of sodium content in the extracellular fluid with a concomitant increase in the total vascular volume thus resulting in hypertension. Therefore, reducing the total body store of sodium is another mechanism of managing hypertension. Diuretics produce their anti-hypertensive effects by increasing the excretion of sodium through inhibiting transporters and co-transporters that reabsorb electrolytes [68,69].

Similarly, calcium channel blockers also possess natriuretic property in addition to their vasodilatory effects [68]. Increased natriuresis causes a reduction of total extracellular fluid volume as a result of increased diuresis and this is responsible for its anti-hypertensive effects. Nitric oxide has also been shown to inhibit the Na+–K+–Cl– cotransporter (NKCC2), which is responsible for reabsorption of sodium, potassium and chloride in the loop of Henle [69]. The inhibition of this NKCC2 transporter results in increased excretion of sodium and potassium in the urine.

Our findings showed that L-arginine supplementation significantly increased urinary excretion of sodium and potassium without affecting the serum levels of the electrolytes (Figs. 3–7). This has been reported in a previous study [70]. Our results also showed a significantly higher ($P < 0.05$) urine volume in the hypertensive pregnant rats compared to the control pregnant rats (Fig. 10).

The lipid-lowering benefits of L-arginine have also been documented. Pahlavani et al. [71] reported that L-arginine supplementation significantly decreased triglycerides, LDL and cholesterol levels while it significantly increased HDL levels compared to the control group. Nascimento et al. [72], also reported similar findings although, these authors stated no significant effect on triglycerides and total cholesterol levels. Our results are similar to these findings that L-arginine supplementation significantly decreased LDL, triglyceride levels while it significantly increased HDL levels in hypertensive pregnant rats (Figs. 1 and 2).

Maternal dyslipidemia is associated with increased maternal morbidity and mortality and poor fetal outcomes. Increased triglycerides level has been linked to increased pregnancy complications. High levels of triglyceride during early pregnancy has been reported to be associated with increased incidence of PIH, preeclampsia, large for gestational age babies, and a higher rate of preterm deliveries [73]. It has been reported that hypertriglyceridemia is associated with a higher risk of maternal obesity and preeclampsia [74]. A recent study also reported that both high total cholesterol and high triglyceride levels were associated with increased rates of preterm deliveries [75]. Similarly, increased LDL fraction and reduced HDL levels have also been reported to be associated with PIH, gestational DM and preeclampsia [76].

Preeclampsia complicates about 3–5% of all pregnancies and is defined as hypertension occurring from 20 weeks of gestation in association with proteinuria of >300 mg/24 h [77]. The results from this study showed that L-arginine supplementation significantly reduced ($P < 0.05$) urinary protein levels in the hypertensive pregnant rats compared to control pregnant rats (Fig. 8). This is different from a previous study that showed no effect on proteinuria in preeclamptic women administered L-arginine [78].

Our findings suggest that oral L-arginine may be beneficial in preventing preeclampsia superimposed on chronic hypertension. Though the mechanisms through which L-arginine sub-serves this anti-proteinuric effect is unknown, we hypothesized that it may be related to the vasodilatory effect of NO which in turn reduces glomerular filtration pressure [79] across glomerular capillaries.

Foetal weight was significantly lower ($p < 0.05$) in the hypertensive pregnant rats compared to the control pregnant rats and oral L-arginine supplementation did not reverse this trend (Fig. 9). This is in line with a study that showed that L-arginine supplementation does not enhance weight gain in pregnant spontaneously hypertensive rats [80].

5. Conclusion

The results of our study indicate that L-arginine supplementation attenuates several deleterious effects of salt-induced hypertension in pregnant rats possibly through the described role of L-arginine in NO synthesis which may support beneficial vasodilatory effects; L-arginine may also have a diuretic-like action, however, this concept is still speculative and will require further study.

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