Niger. J. Physiol. Sci. 32(December 2017) 129-136
www.njps.com.ng

Niger. J. Physiol. Sci.

Effects of Lipopolysaccharide and High Saline Intake on Blood Pressure, Angiogenic Factors and Liver Enzymes of Pregnant Rats

***Oludare G.O., Ilo O.J. and Lamidi B.A.**

Department of Physiology, College of Medicine of the University of Lagos, Lagos, Nigeria.

Summary: This study investigated the effect of high salt water intake and lipopolysaccharide injection as probable rat models of preeclampsia during pregnancy. Thirty-three female Sprague-Dawley rats weighing between 150-170g were divided into 4 groups of the control (normal saline), high salt water intake (1.8% NaCl from days 13-18 of pregnancy), lipopolysaccharide group (40 µg/kg b.w. ip injection from day 16-18 of pregnancy) and high salt water (1.8% NaCl) and lipopolysaccharide injection (40 µg/kg b.w i.p. from days 16-18 of pregnancy). Urine samples were collected on day 18 of pregnancy and the animals were sacrificed on day 19 of pregnancy for blood pressure parameters, angiogenic and liver enzyme assays. The systolic, diastolic and mean arterial blood pressure of pregnant rats on high saline intake increased significantly when compared with control. Fetal weight was decreased while VEGF levels were increased in this group and no difference was found in the protein and liver enzyme levels. Decreased fetal weight was also observed in HS + lip group accompanied with an increase in PIGF, and VEGF levels with no change in blood pressure, protein and liver enzymes. Similar result was found in the Lipopolysaccharide alone group but with additional changes such as increase in VEGFR-1 levels and protein levels. Both high salt and lipopolysaccharide presents preeclampsia symptoms but the absence of protein in the urine in high salt water intake group as well as the inability of lipopolysaccharide to increase blood pressure suggest that both substances might not be ideal for preeclampsia research in rats.

Keywords*:* Preeclampsia, Lipopolysaccharide, Placental growth factors, High salt, Vascular endothelial growth factor

**Address for correspondence*: gooludare@cmul.edu.ng; +2347035363115

Manuscript Accepted: December, 2017

INTRODUCTION

Preeclampsia is the most common and serious antenatal complication of pregnancy characterized by hypertension, proteinuria and sometimes abnormal fluid retention. It remains a major cause of maternal and perinatal morbidity and mortality worldwide and affect about 2–8% of all pregnancies (Steegers *et al*., 2010). Although the ultimate cause of preeclampsia remains elusive, several pathophysiological mechanisms have been proposed for preeclampsia, such as defective placentation (Zhou *et al*., 1993), alteration of immunological responses (Sacks *et al*., 1998), or genetic defects (Ward *et al*., 1993). In animal studies, different models have been suggested to mimic preeclampsia however; no suitable substance or model has satisfactorily mimicked the complete symptoms in pregnant women with preeclampsia. This study set out to ascertain the roles of high saline intake and lipopolysaccharide injection as models of preeclampsia.

Epidemiological studies do demonstrate a small, yet significant correlation between salt intake and hypertension in humans (Kitiyakara *et al*., 2003, Beausejour *et al*., 2003). High sodium intake was reported to prevent pregnancy-induced decrease of blood pressure in rats and that these rats showed some physiological responses that resemble manifestations observed in preeclampsia (Beausejour *et al*., 2003). The mechanism by which high salt intake increases blood pressure include the disruption of the endothelial cells leading to decreased nitric oxide production (Dharmashankar and Widlansky, 2010; Dickson *et al*., 2011, Mohamed, 2016). Endothelial dysfunction is also one of the mechanisms proposed to cause preeclampsia (Ferris, 1991). The disruption of the endothelium affects the activities of growth factors important in the formation of vascular networks required for oxygen and nutrient delivery to the developing embryo (Chappell *et al*., 2013).

Lipopolysaccharide (LPS), a toxic component from the cell walls in gram-negative bacteria has been well established to trigger inflammation and has been used to study the pathophysiology of various disease states including pathological pregnancies (Faas *et al*., 1994). With ultra-low doses, its effect has been strongly associated with histopathological clinical events known to mimic human preeclampsia (Xue *et al*., 2015). Numerous animal studies have revealed that

administration of LPS to pregnant rats induced preeclampsia-like symptoms (Cotechini *et al*., 2014; VanderGraaf *et al*., 2013; Faas *et al.,* 2004). Dai *et al.*, (2011) also demonstrated that LPS inhibits the invasion of human trophoblast cells thereby inhibiting spiral artery remodeling contributing to poor placentation.

LPS is associated with adverse developmental outcomes, including preterm delivery, fetal death, teratogenicity, and intrauterine growth restriction (IUGR). Humans are constantly exposed to low levels of LPS through infection (Chen *et al*., 2012). Gastrointestinal inflammatory diseases and excess alcohol intake are known to increase the permeability of LPS from the gastrointestinal tract into blood (Zhou *et al*., 2003). High levels of LPS have been detected in women with bacterial vaginosis (Platz-Christensen, 1993). In humans, Gram-negative bacterial infections are a recognized cause of fetal loss and preterm labor (Romero, 1998). Mimicking maternal infection by exposing pregnant rodents to LPS at early gestational stages resulted in embryonic resorption and fetal death (Gendron *et al*., 1990, Ogando *et al*., 2003). Maternal LPS exposure at the middle gestational stages caused fetal death and abortion (Leazer *et al*., 2002). Maternal LPS exposure at late gestational stages led to fetal death, intrauterine growth restriction (IUGR), skeletal development retardation, and preterm delivery (Chen *et al*., 2012). In another study, maternal LPS exposure during pregnancy lead to offspring vascular dysfunction at 12 weeks of age, which appear to be genetically predisposed to cardiovascular disease later in life (Zhao *et al*., 2014)

Other recent advances in understanding preeclampsia and fetal growth restriction have also elucidated important biological roles for placental-derived angiogenic factors (Maynard and Karumanchi, 2011). In normal pregnancy, placental growth factor (PlGF), a member of the vascular endothelial growth factors, synthesized by syncytiotrophoblast (Shore *et al.,* 1997), increases with gestation in maternal circulation, with low levels in early gestational stage and dramatically increasing concentrations afterwards, peaking at 26 to 30 weeks (Knudsen *et al*., 2011) and declining towards term. PlGF is abnormally low in women with preeclampsia in comparison with gestational age-matched controls (Levine *et al*., 2004) and is reduced further in severe preeclampsia (Robinson *et al*., 2006). Studies have also shown that plasma concentration of Vascular Endothelial Growth Factor (VEGF) is significantly reduced in severe pre-eclampsia (Jeffrey *et al*., 2000). The tendency to derive a spectacular model for preeclampsia in animal models will prove to be a potential way forward in the identification of the causes and management of preeclampsia. This study therefore, investigated the roles of high saline intake and lipopolysaccharide on blood pressure, liver enzymes, urinary protein and growth factors.

MATERIALS AND METHODS

Animal

Thirty-three (33) female Sprague-Dawley rats obtained from the Animal House of the College of Medicine University of Lagos were used for this study. The rats were kept under standard conditions of 12-hours light and dark cycles. They were acclimatized for 3 weeks, kept at room temperature and were allowed to feed and drink water *ad libitum* prior to the experimental stage. 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 care and use of laboratory animals. The rats were divided into four groups of rats as described below:

- Group I (control group**)**: 6 female rats received 0.2 ml normal saline from day 13-18 of pregnancy
- Group II (High salt water only): nine female rats received 1.8% NaCl solution in drinking water from day 13-18 of pregnancy
- Group III (Lipopolysaccharide only): nine female rats' female Sprague-Dawley rats received intraperitoneal injection of 40µg/kg lipopolysaccharide from day 16-18 of pregnancy (Cotchini *et al*., 2008).
- Group IV (high salt water + lipopolysaccharide): nine female rats received 1.8% NaCl solution in drinking water from day 13-18 of pregnancy and intraperitoneal injection of 40µg/kg lipopolysaccharide from day 16-18 of pregnancy.

Determination estrous cycle, mating and pregnancy

Vaginal smears were collected from each rat and the cell patterns were observed under a light microscope and the phase of each rat's cycle was determined by the dominant type of cell present in the vaginal fluids. Rats on the proestrous phase received a male rat on the evening of proestrous for mating. The following day, the presence of sperm plugs in the vagina of the rats or sperm cells in their smears confirmed mating and was taking as day 1 of pregnancy.

Collection of urine samples and determination of urinary protein, albumin and creatinine

Twelve hours urine samples were collected on day 17 or 18 of pregnancy using a metabolic cage. The urine collected was preserved with toluene and used for determining urine protein, albumin and creatinine. Urinary total protein was assayed using Randox Biuret kits as described by (Tietz, 1995). Urinary creatinine content was determined using the principle of a colorimetric reaction of creatinine with alkaline picrate measured kinetically at 490 nm while total Albumin content in the urine was determined using Randox Bromocresol green (BCG) concentrate (comprised 75 mM/L Succinate buffer at pH 4.2 0.15 mM/L BCG, brij 35 and preservative and standard (comprised 4.5 g/dL human plasma albumin and 100 mM/L tris buffer at pH 7.3) were used to assay urine albumin concentration as described by (Grant *et al*., 1987).

Blood Pressure Measurement

Invasive blood pressure measurement was carried out via arterial cannulation. 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 anaesthetized rat was placed on its back on the operating table, the limbs were fastened to the table, and the trachea was exposed and cannulated. The blood pressure measurements were obtained by cannulation of one carotid artery. A polyethylene cannula filled with 1% heparinised saline was inserted into the artery, tied in place, and connected via a pressure transducer (model SP 844, Physiological Pressure Transducer. AD Instruments) that 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). The LabChart-7 Pro software computes the HR by applying the cyclic measurement function, which is a channel calculation that analyzes periodic blood pressure waveforms in real time. Data of the detected cycles are displayed as a continuous data trace for HR in another channel of the data acquisition system. Recordings were taken at a sampling frequency of 5/seconds.

Determination of PIGF, VEGF and VEGFR1 (sFLT-1) concentration in serum

Blood samples were collected via the carotid artery from the rats during sacrifice for the estimation of the levels of placental growth factor (PIGF), vascular endothelial growth factor (VEGF) and Vascular endothelial growth factor receptor 1 using Enzyme-linked-immunosorbent serologic assay (ELISA) techniques. The samples were centrifuged for 15min at 3000rpm using a bench top centrifuge and the serum was stored at -20^{0}C. The assay was carried out using the protocol booklet of the manufacturers of the ELISA kits for PIGF Elabscience Rat PGF ELISA kit catalog No: E-EL-R0742), VEGF (Elabscience Rat VEGF ELISA kit catalog No: E-EL-R0020) and VEGFR1 (Elabscience Rat sFLT-1/sVEGFR1 ELISA kit catalog No: E-EL-R0911).

Determination of liver enzymes ALT, AST and ALP

Alanine aminotransferase (ALT) was measured using Randox kit by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylthydrazine (DPNH) as described by Schmidt and Schmidt, (1963). Aspartate aminotransferase (AST) was measured using Randox kit by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4- DPNH as described by Schmidt and Schmidt, (1963), while alkaline phosphatase (ALP) was estimated using the method of King and Armstrong (1988).

Statistical Analysis

All the values are expressed as mean ± standard error of mean (SEM). The values were analysed by one-way ANOVA followed by Student's Newman-Keuls post-hoc test using the Graph Pad software. Differences were considered significant when $p < 0.05$.

RESULTS

Fetal parameters

Fetal weight (Day 19) was significantly reduced in HS, LPS and HS+ LPS groups when compared with control ($p< 0.05$). There was a significant increase in fetal weight in the LPS and HS+ LPS groups when compared with HS. Fetal weight was also found to be increased in HS + LPS group compared to the LPS group. The percentage of resorption sites in the HS (45.23%), LPS (48.78%) and HS +LPS (34.88%) were significantly increased compared to the control (13.3%) (Table 1).

Blood pressure parameters

As shown in table 2, systolic blood pressure, diastolic blood pressure and mean arterial blood pressure were significantly increased in HS administered pregnant rat than control ($p < 0.05$), while no increase was found with LPS and HS + LPS administered pregnant rats when compared with control ($p > 0.05$). However, the LPS administered pregnant rats showed a decrease in SBP, DBP and MABP when compared with the HS rats ($p< 0.05$).

Table 1: Effect of high salt water intake and lipopolysaccharide injection feotal parameters in pregnant rats

	Control	HS	LPS	HS + LPS
Fetuses weight (Day 19) (g)	4.05 + 0.08	2.51 ± 0.03 *	3.93 ± 0.14*#	3.03 ± 0.05 *#$
Total number of fetus and resorptions sites	45	42	41	43
Number of resorptions	6	19	20	15
Percentage of resorptions (%)	13.33	45.23	48.78	34.88

*signifies significant difference from control, # signifies significant difference from HS and **$** signifies significant difference from LPS ($p< 0.05$). HS = High salt water intake, LPS = Lipopolysaccharide.

Table 2: Blood pressure parameters of pregnant rats on high salt water intake and lipopolysaccharide injection

	Control	HS	LPS	HS + LPS
SBP (mm Hg)	124.53 ± 9.37	151.45± 3.32*	114.54 ± 7.26#	135.56 ± 7.81
DBP (mm Hg)	95.28 ± 9.06	122.85± 5.81*	84.11 ±7.54#	102.15 ± 7.29
HR (Beats/min)	366.00±22.07	420.00± 12.77	406.00 ± 19.20	428.00 ± 3.99
MABP (mm Hg)	105.03 ± 8.96	132.38 ± 4.98*	94.26 ± 7.36#	113.28 ± 7.40
PP (mm Hg)	29.23 ± 4.09	28.59 ± 2.56	30.43 ± 2.44	33.40 ± 2.13

*signifies significant difference from control, # signifies significant difference from HS ($p< 0.05$). HS = High salt water intake, LPS = Lipopolysaccharide, SBP = systolic blood pressure, DBP= diastolic blood pressure, HR = heart rate, MABP = Mean arterial blood pressure, PP= pulse pressure.

Table 3: Effect of high salt water intake and lipopolysaccharide injection on urine protein, albumin and creatinine levels in pregnant rats

	Control	HS	LPS	HS + LPS
Protein (g/L)	0.09 ± 0.03	0.15 ± 0.02	1.29 ± 0.35*#	0.13 ± 0.02$
Albumin (g/L)	0.09 ± 0.05	0.09 ± 0.02	0.78 ± 0.17*#	0.08 ± 0.01$
Creatinine (umol/L)	742.18 ± 34.53	849.06 ± 49.95	821.02 ± 53.48	817.48 ± 56.97

*signifies significant difference from control, # signifies significant difference from HS and **$** signifies significant difference from LPS ($p < 0.05$). HS = High salt water intake, LPS = Lipopolysaccharide.

Table 4: Effect of high salt water intake and lipopolysaccharide injection on liver enzymes (ALP, AST and ALT) levels in pregnant rats

	Control	HS	LPS	HS + LPS
ALP (iu/L)	22.55 ± 1.57	21.53 ± 1.75	24.16 ± 0.67	22.9 ± 1.28
AST (iu/L)	14.33 ± 1.28	18.33 ± 1.94	20.33 ± 2.96	23.66 ± 2.53*
ALT (iu/L)	4.33 ± 0.80	4.83 ± 0.74	5.66 ± 0.95	6.50 ± 0.72

*signifies significant difference from control ($p < 0.05$). HS = High salt water intake, LPS = Lipopolysaccharide. ALP = alkaline phosphatase, AST= aspartate aminotransferase ALT= alanine aminotransferase

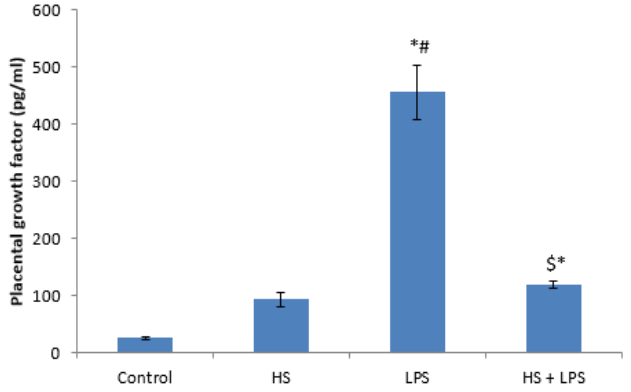

Figure 1: Effect of high salt water intake and lipopolysaccharide injection on placental growth factor levels in pregnant rats. *signifies significant difference from control ($p < 0.05$). # signifies significant difference from high salt water intake ($p < 0.05$). $ signifies significant difference from lipopolysaccharide ($p < 0.05$). HS = High salt water intake LPS = Lipopolysaccharide PIGF= placenta growth factor.

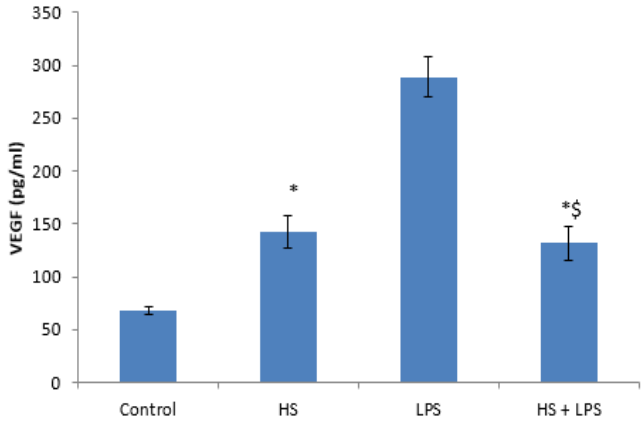

Figure 2: Effect of high salt water intake and lipopolysaccharide injection on vascular endothelial growth factor levels in pregnant rats. *signifies significant difference from control ($p < 0.05$). # signifies significant difference from high salt water intake ($p < 0.05$). $ signifies significant difference from lipopolysaccharide ($p < 0.05$). HS = High salt water intake LPS = Lipopolysaccharide, VEGF = Vascular endothelial growth factor

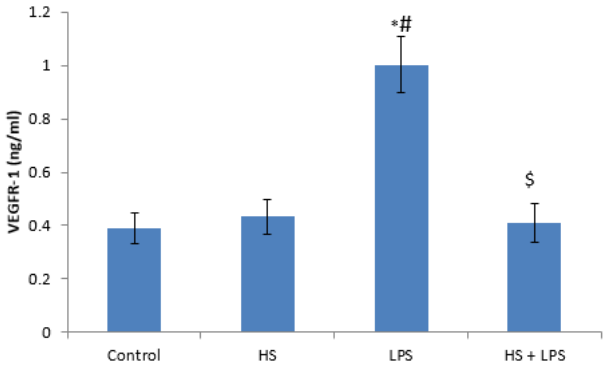

Figure 3: Effect of high salt water intake and lipopolysaccharide injection on vascular endothelial growth factor receptor 1 levels in pregnant rats. *signifies significant difference from control ($p < 0.05$). # signifies significant difference from high salt water intake ($p < 0.05$). $ signifies significant difference from lipopolysaccharide ($p < 0.05$). HS = High salt water intake LPS = Lipopolysaccharide, VEGFR-1 = Vascular endothelial growth factor receptor 1

Liver enzymes

There was no significant difference in the levels of ALP and ALT in the HS, LPS and HS + LPS groups when compared with control ($p > 0.05$). However, in the HS + LPS administered pregnant rats, AST levels was significantly increased when compared to control values ($p < 0.05$).

Growth factors PIGF, VEGF and VEGFR1

A significant increase in the PIGF levels was found in the LPS and HS+LPS rats when compared with control ($p < 0.05$). In addition, the LPS group showed a significant increase in PIGF levels when compared with both the HS and HS + LPS groups (figure 1). The VEGF levels was significantly increased in the HS, LPS and HS+ LPS groups when compared with the control ($p < 0.05$). The increase in VEGF levels in LPS administered pregnant rats was also found to be significant when compared to both HS and HS + LPS groups (figure 2). The levels of the expression of VEGFR1 (FLT-1) was increased in the LPS group when compared with control, HS and HS+LPS groups (figure 3).

DISCUSSION

This study demonstrated that 1.8% saline intake in mid gestation for 7 days in rats is associated with increased blood pressure, decreased fetal weight and increased VEGF levels. Lipopolysacharide administration on the other hand is associated with increases in VEGF, PIGF and VEGFR-1 as well as protein levels. Fetal weight was also reduced and 48% resorption of foetus occurred. It is known that preeclampsia is characterized by the new onset of hypertension, and proteinuria during the last trimester of pregnancy and severe preeclampsia may also lead to small for gestational age babies (Maynard *et al.,* 2005). Intrauterine growth retardation is one of the consequences of preeclampsia. Previous study on lipopolysaccharide and fetal growth and outcome showed a decrease fetal weight. This was hypothesized to be as a result of the maternal blood vessels response to LPS induced inflammation which could lead to hypoperfusion of nutrients to the fetus (Stephen *et al.,* 2011, Leazar *et al.,* 2002). Beaseajour *et al.,* (2003) has also reported reduced fetal weight in the dams of rats on high saline intake which was attributed to decrease feed intake and decreased blood volume.

Increased systolic and diastolic blood pressure was found in the high saline intake pregnant rats but not in the LPS administered rats. LPS administration in pregnant rats on days 16-18 appears to have a blood pressure lowering effect with values in the LPS alone group comparable with the control values. The use of LPS in inducing blood pressure is bi-phasic. While some studies have reported increase in blood pressure using ultra-low dose infusion of LPS (Cotechini *et al.,* 2014; VanderGraaf *et al*., 2013, Fass *et al.,* 1994,), others have reported that LPS administration causes a decrease in peripheral resistance leading to vascular dilation and thus a low blood pressure (Ehrentraut *et al.,* 2007, Shindo *et al.,* 2000, Thiemermann and Vane 1990). The large LPS dose used in this study over a period of 3 days might have been responsible for the decreased systolic and diastolic blood pressure since inflammation is one of the causes of low blood pressure. Also, an increase in total protein and albumin excretion in the urine of these pregnant rats might indicate a dysfunction of the kidneys caused by LPS induced inflammation (Pingping *et al.,* 2015). While the increased blood pressure in the high saline intake pregnant rats is consistent with the work of Beaseajor *et al.,* (2003) using 1.8% saline.

Vascular endothelial growth factor receptor-1 also known as soluble fm-like tyrosine 1 (sflt-1) receptor is produced by alternate splicing of the flt-1 gene and is known to bind VEGF and PIGF. It also has antagonistic effects on the biological effects of PIGF and VEGF (Torry *et al*., 2003, Maynard *et al.,* 2005). The flt-1 gene has been associated with angiogenesis and vasculogenesis. In this present study, there was a significant increase in the VEGFR-1 serum concentration in lipopolysaccharide only treated animals. This is in line with various other preeclamptic studies (Ana and Karumanchi, 2012; McKeeman *et al.,* 2004; Maynard *et al*., 2003). Many other factors have also been shown to increase placental production of sFlt-1 such as angiotensin II and autoantibodies against angiotensin receptor-1 (Zhou *et al.,* 2007). Furthermore, several animal models of preeclampsia showed an increase in sFlt-1, suggesting that this molecule may be a central culprit in the pathogenesis of preeclampsia which originates with different insults to the placenta converging on a final common pathway mediated by excess release of sFlt-1 into the maternal circulation.

The PIGF is basically involved in angiogenesis as well as vasculogenesis. An imbalance between angiogenic and antiangiogenic factors plays a fundamental role in the pathogenesis of preeclampsia. Serum levels of placental growth factor (PLGF), a factor promoting angiogenesis, in patients with preeclampsia are significantly lower than in non-preeclamptic pregnancies. The increased PIGF levels reported in this study in LPS administered rats and LPS + HS administered rats suggest that the rats did not develop preeclampsia. This is in line with the result on blood pressure which is a significant factor in preenclampsia. However, the presence of protein in the urine in the LPS administered rats as well as increased VEGFR-1 suggest contrarily. Accumulating evidences have documented that angiogenesis is closely linked to inflammation and regulators of angiogenesis play key roles in various inflammatory conditions. PlGF is an angiogenic protein belonging to the VEGF family and is upregulated mainly in pathologic conditions. Recently, PlGF was discovered to have a proinflammatory role in inflammatory arthritis and its serum level drew attention not only as a useful surrogate biomarker but also a potential therapeutic target in atherosclerosis and various cancers (Kim *et al.,* 2012). The link of LPS with inflammation as well PIGF with inflammation might also help understand the outcome of this study in the LPS pregnant rats. Thus, since this dosage of LPS did not produce hypertension but produced proteinuria and increased VEGFR-1 one might suggest that higher doses of LPS and co-administration of LPS with salt is not a likely model for preenclampsia. However, lesser single dosage of LPS might prove effective as reported by some studies (VanderGraaf *et al*., 2013; Faas *et al.,* 2004).

In conclusion, this study has shown in line with previous studies the effects of LPS in generating an animal model of preeclampsia in relation to proteinuria and fetal growth restriction. However, questions its angiogenesis enhancing effect which contradicts the given set-point of use of relevant biomarkers like PIGF as a determinant of preeclampsia by various studies,

though this could be related to the link of PIGF expression during inflammation. In addition to this, is the decreased blood pressure caused by LPS making this dosage unsuitable as a marker for preeclampsia. This study has also showed that high salt water intake only during the late gestational age in rats cannot serve as a model of preeclampsia since proteinuria is a vital factor to determine preeclampsia. The combination of high saline intake and LPS also did not produce preenclampsia like effects as LPS had a hypotensive effect on HS and HS appears to eliminate the chances of proteinuria. Therefore, both high salt and lipopolysaccharide presents preeclampsia symptoms but the absence of protein in the urine in high salt water intake group as well as the inability of lipopolysaccharide to increase blood pressure suggest that both substances might not be ideal for preeclampsia research in rats.

REFERENCES

Ana, S.C. and Karumanchi, S.A. (2012) Angiogenic factors in preeclampsia and related disorders. Cold Spring Harb Perspect medicine; doi: 10.1101/cshperspect.a006585.

Beausejour, A., Auger, K., St Louis, J. and Brochu, M. (2003) High-sodium intake prevents pregnancy-induced decrease of blood pressure in the rat. Am. J. Physiol, Heart Circulation and Physiology 285: H375–H383.

Chappell, J.C., Mouillesseaux, K.P. and Bautch, V.L. (2013) Flt-1 (Vascular Endothelial Growth Factor Receptor-1) is essential for the vascular endothelial growth factor–notch feedback loop during angiogenesis Arterioscler Thromb Vasc Biol. 33:1952-1959

Chen, Y., Zhao, M., Chen, X., Zhang, Y., Wang, H., Huang, Y. *et al.*, (2012) Zinc supplementation during pregnancy protects against lipopolysaccharide-induced fetal growth restriction and demise through its anti-inflammatory effect. The Journal of Immunology 189: 454–463.

Chen, Y., Zhao, M., Chen, X., Zhang, Y., Wang, H., Huang, Y., *et al.*,(1994) Effects of Escherichia coli and Porphyromonas gingivalis lipopolysaccharide on pregnancy outcome in the golden hamster. Infect. Immun 62: 4652–4655.

Cotechini, T., Komisarenko, M., Sperou, A., Macdonald-Goodfellow, S., Adams, M.A. *et al.*, (2014) Inflammation in rat pregnancy inhibits spiral artery remodeling leading to fetal growth restriction and features of preeclampsia. J Exp Med. 211:165–179.

Dai, Y., Diao, Z., Sun, H., Li, R., Qiu, Z. and Hu, Y. (2011) Micro RNA-155 is involved in the remodeling of human trophoblast derived HTR-8/SVneo cells induced by lipopolysaccharides. Hum Reprod. 26:1882–1891.

Dharmashankar, K., and Widlansky, M.E. (2010) Vascular Endothelial Function and Hypertension: Insights and Directions Curr Hypertens Rep 12(6): 448–455

Dickinson, K.M., Clifton, P.M. and Keogh, J.B. (2011) Endothelial function is impaired after a high-salt meal in healthy subjects *Am J Clin Nutr* 93(3): 500-505

Ehrentraut, S., Frede, S., Stapel, H., Mengden, T., Grohe, C., Fandrey, J. *et al.*, (2007) Antagonism of lipopolysaccharide-induced blood pressure attenuation and vascular contractility Arterioscler Thromb Vasc Biol. 27:2170-2176

Faas, M.M., Broekema, M., Moes, H., vanderSchaaf, G., Heineman, M.J. and deVos P. (2004) Altered monocyte function in experimental preeclampsia in the rat. Am J Obstet Gynecol. 191:1192–1198.

Faas, M.M., Schuiling, G.A., Baller, J.F., Visscher, C.A. and Bakker, W.W. (1994) A new animal model for human preeclampsia: ultra-low-dose endotoxin infusion in pregnant rats. Am. J. Obstet. Gynecol. 171:158–164.

Ferris, T.F. (1991) Pregnancy, preeclampsia, and the endothelial cell. N Engl J Med 1991; 325:1439–1440

Gendron, R.L., Nestel, F.P., Lapp, W.S. and Baines, M.G. (1990) Lipopolysaccharide-induced fetal resorption in mice is associated with the intrauterine production of tumour necrosis factor-alpha. J. Reprod. Fertil. 90: 395–402.

Grant, G.H., Silverman, L.M., Christenson, R.H. (1987) 3th ed. WB Saunders Company; Philadelphia: 1987. Aminoacids and proteins. (Fundamental of Clinical Chemistry).

Hoareau, L., Bencharif, K., Rondeau, P., Murumalla, R., Ravanan, P., Tallet, F. *et al.*, (2010) Signaling pathways involved in LPS induced TNFalpha production in human adipocytes Journal of Inflammation 7:1

Jeffrey, C.L., Robert, C., Bassam, H., Elizabeth, T.M., Robert, C. *et al.*, (2090) Reductions of vascular endothelial growth factor and placental growth factor concentrations in severe preeclampsia. American Journal of Obstetrics and Gynecology 183 (6): 1554-1557.

Kim, K., Cho, C. and Kim, W. (2012) Role of placenta growth factor in cancer and inflammation. Exp Mol Med 44(1): 10–19.

Kitiyakara, C., Chabrashvili, T., Chen, Y., Blau, J., Karber, A., Aslam, S. *et al.*, (2003) Salt intake, oxidative stress, and renal expression of NADPH Oxidase and superoxide dismutase Journal of American Society of Nephrology 14: 2775–2782.

Knudsen, U.B., Kronborg, C.S., vonDadelszen, P., Kupfer, K., Lee, S.W., Vittinghus, E. *et al.*, (2011). A single rapid point-of-care placental growth factor determination as an aid in the

diagnosis of preeclampsia. Pregnancy Hypertens 2:8–15.

Lanning, J.C., Hilbelink, D.R. and Chen, L.T. (1983) Teratogenic effects of endotoxin on the golden hamster. Teratog. Carcinog. Mutagen 3: 145–149.

Leazer, T.M., Barbee, B., Ebron-McCoy, M., Henry-Sam, G.A., Rogers, J.M. (2002) Role of the maternal acute phase response and tumor necrosis factor alpha in the developmental toxicity of lipopolysaccharide in the CD-1 mouse. Reprod.Toxicol 16: 173–179.

Levine, R.J., Maynard, S.E., Qian, C., Lim, K.H., En gland, L.J., Yu, K.F. (2004). Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 350:672–683.

Maynard, S.E., Min, J.Y., Merchan, J., Lim, K.H., Li, J., Mondal, S. (2003) Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 111: 649–658

Maynard, S.E., Venkatesha, S., Thadhani, R., Karumanchi, S.A. (2005) Soluble Fms-like tyrosine kinase 1 and endothelial dysfunction in the pathogenesis of preeclampsia. Pediatric research 57: 1R-7R.

Maynard, S.E. and Thadhani, R. (2009). Pregnancy and the Kidney J Am Soc Nephrol 20: 14–22.

Maynard, S.E. and Karumanchi, S.A. (2011). Angiogenic factors and preeclampsia. Semin Nephrol 31:33–46.

McKeeman, G.C., Joy, E.S., Carolyn, M.C., Alyson, J.H., Neil, M. (2004) Soluble vascular endothelial growth factor receptor-1 (sFlt-1) is increased throughout gestation in patients who have preeclampsia develop. American journal of Obstetrics and gynecology 191(4): 1240–1246

Mohamed, A.W. (2016) Vascular effects of a single high salt meal The Egyptian Heart Journal 68(3): 159-164

Ogando, D.G., Paz, D., Cella, M., Franchi, A.M. (2003) The fundamental role of increased production of nitric oxide in lipopolysaccharide-induced embryonic resorption in mice. Reproduction 125: 95–110.

Ornoy, A. and Altshuler, G. (1976) Maternal endotoxemia, fetal anomalies, and central nervous system damage: a rat model of a human problem. Am. J. Obstet. Gynecol 124: 196–204.

Pingping, X., Mingming, Z., Ping, G., Caimei, L., Jianjun, Z., Yujing, L., *et al.*, (2015) Single Administration of Ultra-Low-Dose Lipopolysaccharide in Rat Early Pregnancy Induces TLR4 Activation in the Placenta Contributing to Preeclampsia. PLOS one 10(4):e0124001.

Platz-Christensen, J.J., Mattsby-Baltzer, I., Thomsen, P., Wiqvist, N. (19930 Endotoxin and interleukin-1 alpha in the cervical mucus and vaginal fluid of pregnant women with bacterial vaginosis. Am. J. Obstet. Gynecol 169: 1161–1166.

Robinson, C.J., Johnson, D.D., Chang, E.Y., Armstro ng, D.M., Wang, W. (2006) Evaluation of placenta growth factor and soluble Fms-like tyrosine kinase 1 receptor levels in mild and severe preeclampsia. Am J Obstet Gynecol 195:255–259.

Romero, R., Roslansky, P., Oyarzun, E., Wan, M., Emamian, M., Novitsky, T.J., *et al.,* (1988) Labor and infection. II. Bacterial endotoxin in amniotic fluid and its relationship to the onset of preterm labor. Am. J.Obstet. Gynecol 158: 1044–1049.

Sacks, G.P., Studena, K., Sargent, I.L., Redman, C.W.G. (1998) Normal pregnancy andpreeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. Am. J. Obstet. Gynecol 179, 80–86.

Shore, V.H., Wang, T.H., Wang, C.L., Torry, R.J., Ca udle, M.R., Torry, D.S. (1997). Vascular endothel-ial growth factor, placenta growth factor and their receptors in isolated human trophoblast. Placenta 18:657–665.

Steegers, E.A., vonDadelszen, P., Duvekot, J.J., Pijne nborg, R. (2010) Pre-eclampsia. Lancet 376:631–644.

Torry, D.S., Mukherjea, D., Arroyo, J., Torry, R.J. (2003) Expression and function of placental growth factor: implications for abnormal placentation. J Soc Gynecolog Investig 10: 178-188

Thiemermann, C. and Vane, J. (1990) Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat in vivo European Journal of Pharmacology 182 (3): 591-595.

VanderGraaf, A.M., Wiegman, M.J., Plösch, T., Zeeman, G.G., vanBuiten, A. *et al.,* (2013) Endothelium dependent relaxation and angiotensin II sensitivity in experimental preeclampsia. PLoSOne 2013; 8:e79884.

Ward, K., Hata, A., Jeunemaitre, X., Helin, C., Nelson, L., Namikawa C, *et al.*, (1993) A molecular variant of angiotensinogen associated with preeclampsia. Nat Genet 4:59–61

Xu, D. (2012) Zinc supplementation during pregnancy protects against lipopolysaccharide-induced fetal growth restriction and demise through its anti-inflammatory effect. The J. of Immunology 189: 454–463.

Xue, P., Zheng, M., Gong, P., Lin, C., Zhou, J., Li, Y., *et al.*, (2015) Single administration of ultra-low-dose lipopolysaccharide in rat early pregnancy induces TLR4 activation in the placenta contributing to preeclampsia. PLoS ONE 10(4): e0124001.

Zhao, S., Zhang, H., Cao, D., Liu, Y., Li, X. (2014) Lipopolysaccharide exposure during pregnancy

leads to aortic dysfunction in offspring rats. PLoS ONE 9(7): e102273

Zhou, C.C., Zhang, Y., Irani, R.A., Zhang, H., Mi, T., Popek, E.J. *et al.,* (2008) Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in pregnant mice. Nat Med 14: 855–862

Zhou, Z., Wang, L., Song, Z., Lambert, J.C., McClain, C.J. and Kang, Y.J. (2003) A critical involvement of oxidative stress in acute alcohol-induced hepatic TNF alpha production. Am. J. Pathol 163: 1137–1146.

Zhou, Y., Damsky, C.H., Chiu, K., Roberts, J.M. and Fisher, S.J. (1993) Preeclampsia is associated with abnormal expression of adhesion molecules by invasive cytotrophoblasts. J Clin Invest 91:950–960