

## Serum Adropin Levels in a Preeclampsia Like L-Name Rat Model Treated with Sildenafil Citrate

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### Abstract

**Background:** Preeclampsia, a hypertensive disorder of pregnancy, is detrimental to both mother and fetus. Sildenafil has been proposed as a potential therapy to reduce blood pressure and improve uteroplacental perfusion in preeclamptic patients. Adropin is a novel secreted energy homeostasis protein regulating carbohydrate, lipid and protein metabolism, maternal adropin levels in Preeclampsia were still controversy.

**Aim of the Work:** To evaluate serum levels of adropin in preeclampsia-like rat model and in preeclamptic rats treated with Sildenafil and to investigate their associations with some inflammatory, oxidative stress, and metabolic parameters.

**Material and Methods:** 32 pregnant rats were divided randomly into four groups (n=8): Control group, sildenafil treated group, L-NAME group to induce pre-eclampsia and L-NAME with sildenafil treated group. In all groups, maternal Body Mass Index (BMI), placental weights, fetal weights, maternal mean arterial blood pressure (MAP), total urinary proteins, serum glucose, insulin and calculated Homeostatic Model Assessment of Insulin Resistance index (HOMA-IR), serum urea, creatinine, Triglycerides (TGs), Total Cholesterol (TC), Low Density Lipoproteins-cholesterol (LDL-c), High Density Lipoproteins-cholesterol (HDL-c), C Reactive Protein (CRP), Endothelin-1 (ET-1), and adropin were measured, also placental Malondialdehyde (MDA) levels, and activities of the antioxidant enzymes Superoxide Dismutase (SOD) and glutathione peroxidase (GSH) in placental homogenates were determined plus histopathological examination of placental sections were done.

**Results:** L-NAME induced preeclampsia in rats and they showed significant increase in MAP, total urinary proteins, serum levels of glucose, insulin, calculated HOMA-IR, serum urea, creatinine, CRP, ET-1, TGs, TC, LDL-c, adropin and placental tissue MDA with significant reduction in maternal BMI, placental weights, fetal weights, serum HDL-c and placental tissue SOD and GSH activities as compared to other groups, additionally, L-NAME treated and L-NAME + SILD treated groups revealed positive significant correlations between adropin levels and MAP, total urinary proteins, serum levels of urea, creatinine, CRP, ET-1, TGs, TC, LDL-c, and placental tissue MDA, however they showed negative correlations between adropin and BMI, placental weights, fetal

weights, serum HDL-c and placental tissue SOD and GSH activities.

**Conclusion:** Increased maternal adropin levels in preeclampsia like model in rats might represent a regulatory feedback mechanism against endothelial placental dysfunction, insulin resistance, inflammation and oxidative stress which associated with preeclampsia, hence identify it as a potentially protective agent against preeclampsia development. Sildenafil ameliorates preeclampsia symptoms, inflammation and oxidative stress in a rat model of preeclampsia.

**Key Words:** Adropin – Sildenafil citrate – LNAME – Preeclampsia – Rats.

### Introduction

**PREECLAMPSIA (PE)** is defined by the development of hypertension and proteinuria after 20 weeks of gestation in previously normotensive non-proteinuric pregnant women [1,2]. It has been suggested that PE is a two-stage disorder starting by placental under-perfusion or ischemia (Stage I) followed by secretion of many soluble factors into the maternal circulation with subsequent endothelial dysfunction and multiple organ injuries responsible for its clinical manifestations (Stage II) [3].

As Nitric Oxide (NO) deficiency has been suggested as a contributory factor in pre-eclampsia [4], chronic inhibition of Nitric Oxide Synthase (NOS) in pregnant rats by N (omega)-nitro-L-arginine methyl ester (L-NAME), during mid- to late gestation, results in pre-eclampsia like symptoms such as hypertension, severe renal vasoconstriction, proteinuria, thrombocytopenia and Intra-Uterine Growth Retardation (IUGR) [5].

Sildenafil citrate is a type 5-specific phosphodiesterase inhibitor that inhibits the degradation of nitric oxide leading to vasodilation [6] and increasing fetoplacental blood flow in case of placental vascular insufficiency and preeclampsia [7].

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Adropin is a peptide hormone that is encoded by the energy homeostasis-associated (ENHO) gene. It has been shown to be released in many tissues such as central nervous system, liver, the heart and skeletal muscles [8]. Moreover, it is secreted by vascular endothelium and up regulates Vascular Endothelial Growth Factor 2 (VEGF2) and endothelial Nitric Oxide Synthase (eNOS) which take part in neovascularization [9]. Circulating adropin concentrations are highly regulated by energy intake as well as being involved in cardiovascular function, particularly in endothelial function [10]. In animals, adropin improves insulin sensitivity and protects against obesity associated hyperinsulinemia and hepatosteatosis [11].

Few studies have investigated the role of adropin in some of the high risk pregnancy conditions such as Intrauterine Growth Retardation (IUGR) and gestational diabetes mellitus. However its role in preeclampsia is still unclear. Contra verse studies showed alterations of serum adropin in pregnant women with preeclampsia; Wang et al., [12] revealed for the first time that in preeclampsia serum adropin levels were higher comparing to that of women with normal pregnancy. In contrast, maternal and umbilical cord adropin levels were significantly lower in the preeclamptic women compared to controls [13].

On the previous data, this study was established to investigate serum levels of adropin in L-NAME preeclampsia-like rat model with and without treatment with Sildenafil citrate, furthermore, to assess the associations between adropin with some metabolic, inflammatory and oxidative stress parameters in these models.

### Material and Methods

From 12<sup>th</sup> December 2017 to 30<sup>th</sup> June 2018, this study was carried out on healthy adult albino rats (45 female weighing 180-200g, 15 male weighting 190-220g). They were housed in steel wire cages (6 cages) at the Animal House in Faculty of Medicine of Zagazig University under hygienic conditions. They were fed standard chow, had free access to water, kept at comfortable temperature (20 to 24°C) and were maintained on a 12h light/dark cycle. The rats were accommodated to animal house conditions for one week before the experiments going on. The experimental protocol was approved by physiology department and by Local Medical Ethics Committee in Faculty of Medicine of Zagazig University (Institutional Review Board, IRB). Female rats in estrous cycle were mated with male at a ratio of one male to three females.

Females were inspected daily and the day of appearance of a vaginal plug was regarded as day 1 of pregnancy. Out of 45 females, 32 became pregnant and were randomly divided into four groups (n=8): Control group (pregnant rats were given vehicle (saline) starting from the gestational day 7 to 18, sildenafil treated group (SIL group), L-NAME treated group (L-NAME group) and L-NAME + sildenafil group (L-NAME + SIL group). L-NAME (Sigma-Aldrich, St. Louis, MO, USA) was prepared in saline solution and stored in a freezer at -20°C. L-NAME and L-NAME + SIL groups received L-NAME subcutaneously (50mg/kg/day), from day 7 to day 18 of gestation to induce pre-eclampsia like symptoms. The sildenafil solution was prepared by grinding Viagra tablets (Pfizer Inc., New York, NY, USA) into powder and dissolving in distilled water. SIL and LNAME-SIL groups received sildenafil citrate orally (10mg/kg/day) from day 8 to day 18 of gestation [6,14].

#### *Determination of the first day of pregnancy:*

Vaginal smears taken from the female rats were examined daily by using light microscope to ensure that they were in regular estrus cycle. The estrus phase of the estrus cycle was detected by the presence of cornified epithelial cells which increase in number and eventually predominate as the estrus progresses [15]. The female proved to be in estrus phase was paired with a mature male rat in a separate cage. After mating, females were subsequently isolated until the time of analysis to ensure accurate gestation timing, and in the next morning a vaginal smear taken. Presence of a copulation plug or spermatozoa in the vagina indicated the first day of gestation [16].

*Calculating BMI index:* Each rat was put in closed plastic container and was weighed on gestational day 1 and 19; body length was taken as the distance from the nose tip to the anus. BMI were calculated, BMI equals body weight (gm)/length<sup>2</sup> (cm<sup>2</sup>) [17].

*Urine collection:* On day 18 of pregnancy, rats were housed separately in metabolic cages for 24-hour urine collection. Urine samples were collected, measured for volume and centrifuged 10 minutes at approximately 3000rpm to remove insoluble materials. The supernatant was kept at -20°C. Total proteins: Were assayed using Urinary Protein Assay Kit (Chondrex, Inc. 2607-151 place NE Redmond, WA 98052, USA) as described by Nishi and Elin [18].

*Measurement of blood pressure:* On day 19 of gestation, Systolic and diastolic blood pressures

were measured using a tail-cuff method (NIBP 250, Serial No: 21202-108, BIOPAC system, Inc.; USA). Three measurements with 30 second intervals were recorded and the average of these readings was calculated followed by calculation of the Mean Arterial blood Pressure (MAP) [19].

*Sample collection and morphometric analysis:* Rats were sacrificed at day 19 of gestation by decapitation under light halothane anesthesia. A laparotomy was performed to expose the uterine horns. The number of developed fetuses and their respective placenta in the right uterine horn were counted, removed and weighed on an electronic balance with a least count of 0.001g [6].

*Preparation of placenta homogenates:* The placenta of right uterine horn were sliced, placental tissue were perfused with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16mg mL<sup>-1</sup> to remove any red blood cells and clots. Then, they were homogenized in 5-10mL cold buffer. The homogenate was centrifuged at 10,000 rpm for 15min at 4°C. Supernatant was taken as sample for investigation. The values of parameters are expressed per milligram or gram of tissue protein [20].

*Placental antioxidant system evaluation:* Assay of Superoxide Dismutase (SOD) activity according to the method of Kakkar et al., [21] Glutathione Peroxidase (GPX) activity: According to the method of Reddy et al., [22] and assay of Malondialdehyde (MDA) level: According to the method of Ohkawa et al., [23]. All are measured by using spectrophotometer (spectronic 3000 Array, Germany) at 560, 430 and 535nm respectively.

*Histopathological studies:* The left uterine horn was rapidly removed and the specimens of the placentae were rapidly fixed in 10% formal saline for 48 hours and then washed by tap water. Placental specimens then were processed for paraffin sections. Some sections were cut, mounted on glass slides and stained with hematoxylin and eosin (H & E) for the histological examination [24].

*Blood sampling:* Blood was collected in clean plastic centrifuge tubes and left to clot. Serum was separated by centrifugation of blood at 3000rpm for 15 minutes and stored frozen at -20°C until used.

*Serum analysis for the following:* Urea levels according to Tietz [25] by using rat kits for urea level estimation (Spinreact, S.A.U. ctra. Santa Coloma, 7e-17176 Santesteve de bas (gi), Spain), creatinine levels according to Jaffe [26] using rat

kits for creatinine level estimation (Spinreact, S.A.U. ctra. Santa Coloma, 7e-17176 Santesteve de bas (gi), Spain), insulin levels according to Temple et al., [27] using KAP1251-INS-EASIA (Enzyme Amplified Sensitivity Immunoassay) kits (BioSource Europe S.A., Belgium). Glucose levels according to Tietz [25] using glucose enzymatic (GOD-PAP)-liquizyme rat Kits (Biotechnology, Egypt). Calculation of homeostatic model assessment of insulin resistance index (HOMA-IR) based on serum insulin level ( $\mu\text{U/ml}$ ) and serum glucose level (mg/dl) according to the formula described by Matthews et al., [28] as  $\text{HOMA-IR} = \text{Fasting serum glucose (mg/dl)} \times \text{fasting serum insulin (}\mu\text{U/ml)} / 405$ . Total Cholesterol (TC) levels according to Tietz [25] by using total cholesterol kits estimation (BioSource Europe S.A). High Density Lipoproteins (HDL-c) levels according to Nauk et al., [29] by using kits for HDL-cholesterol (BioSource Europe S.A). Low Density Lipoproteins (LDL-c) calculated according to Friedewald et al. [30],  $\text{LDL} = \text{TC} - \text{HDL} - \text{TG}/5$ . Total triglycerides (TG) levels according to Naito [31] using triglycerides ESPAS SL kits A (Elttech S.A., Lyon, France). C Reactive Proteins (CRP) levels according to Ridker et al., [32] using CRP Kits (Monobind Inc Lake Forest, Ca 92630, USA), endothelin-1 levels using endothelin-1 Enzyme Immunoassay Kit, (EIA kit, Cayman, USA, Cat No., 583151) according to manufacture's instructions, and adropin levels using rat adropin ELISA enzyme-linked immunoassay kits (Sun Red Biotechnology, China catalogue No 201-11-3361) according to manufacture's instructions.

*Statistical analysis:* Results were presented as mean  $\pm$  Standard Deviation (SD). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), Version 20.0 (SPSS Inc., Chicago, IL, United States). Repeated measures of analysis of variance (ANOVA) were applied followed by the Student-Least Significant Deference (LSD), post hoc test to compare means of each two different groups. Pearson's correlation analysis was performed to screen potential relations between serum levels of Adropin and all measured parameters. For all statistical tests done,  $p$ -value  $< 0.05$  was considered to be statistically significant.

## Results

*Regarding (Table 1) & Figs. (1-4):* No statistically significant differences were noted between control and SIL groups in all measured parameters ( $p > 0.05$ ), LNAME preeclampsia rats revealed significant reduction in the mean values of final BMI ( $0.53 \pm 0.06 \text{ gm/Cm}^2$ ), placental weights ( $0.47 \pm$

0.05g) and fetal weights ( $3.81 \pm 0.57$ g) in comparison to those of control group ( $0.65 \pm 0.04$ ,  $0.57 \pm 0.03$  and  $5.36 \pm 0.62$  respectively,  $p < 0.001$ ), SIL group ( $0.64 \pm 0.04$ ,  $0.58 \pm 0.05$ , and  $5.39 \pm 0.53$  respectively,  $p < 0.001$ ), and L NAME + SIL group ( $0.59 \pm 0.02$ ,  $0.52 \pm 0.03$  and  $4.63 \pm 0.6$  respectively,  $p < 0.05$ ,  $p < 0.05$  and  $p < 0.001$  respectively). Moreover, LNAME preeclampsia rats presented significant increase in the mean values of MAP ( $121.65 \pm 11.4$  mmHg) and urinary total proteins ( $50.75 \pm 7.21$  mg) as compared to control ( $80.5 \pm 7.18$  and  $17.62 \pm 2.74$  respectively) ( $p < 0.001$ ), SIL ( $81.75 \pm 6.79$  and  $16.34 \pm 2.62$  respectively,  $p < 0.001$ ) and L NAME + SIL group ( $90.56 \pm 11.37$  and  $22.83 \pm 4.7$  respectively,  $p < 0.01$ ,  $p < 0.001$ ).

Regarding serum biochemical parameters, there was significant increase in the mean values of serum urea ( $40.61 \pm 7.63$ mg/dl), creatinine ( $0.44 \pm 0.07$ mg/dl), CRP ( $1.36 \pm 0.14$ Ug/ml), endothelin-1 ( $2.85 \pm 0.24$ pg/ml), TGs ( $110.72 \pm 10.58$ mg/dl), TC

( $162.7 \pm 12.76$ mg/dl), LDL-c ( $99.26 \pm 9.85$ mg/dl), glucose ( $117.98 \pm 4.78$ mg/dl), insulin ( $17.86 \pm 2.16$  uIU/mL), HOMA-IR ( $5.59 \pm 0.45$ ) and serum adropin levels ( $60.22 \pm 6.28$ ng/ml) in LNAME preeclampsia rats in contrast to those in control group ( $21.21 \pm 2.70$ ,  $0.21 \pm 0.048$ ,  $0.77 \pm 0.09$ ,  $1.54 \pm 0.17$ ,  $69.37 \pm 6.03$ ,  $110.22 \pm 8.37$ ,  $55.98 \pm 3.37$ ,  $91.98 \pm 7.87$ ,  $8.06 \pm 2.11$ ,  $1.80 \pm 0.17$  and  $32.47 \pm 4.03$  respectively,  $p < 0.001$ ), SIL group ( $18.64 \pm 1.39$ ,  $0.20 \pm 0.03$ ,  $0.75 \pm 0.07$ ,  $1.45 \pm 0.15$ ,  $68.38 \pm 7.79$ ,  $106.76 \pm 11.48$ ,  $55.31 \pm 7.28$ ,  $92.25 \pm 7.45$ ,  $7.92 \pm 1.89$ ,  $1.79 \pm 0.19$  and  $31.46 \pm 3.76$  respectively,  $p < 0.001$ ) and L NAME + SIL group ( $27 \pm 6.3$ ,  $0.26 \pm 0.05$ ,  $0.92 \pm 0.09$ ,  $1.96 \pm 0.12$ ,  $79 \pm 7.81$ ,  $123.31 \pm 9.55$ ,  $65.98 \pm 12.3$ ,  $100.19 \pm 5.26$ ,  $11.43 \pm 1.13$ ,  $2.59 \pm 0.64$  and  $38.18 \pm 4.44$ , respectively;  $p < 0.001$ ). However, LNAME preeclampsia rats showed significant decrease in the mean values of serum HDL-c levels ( $39.5 \pm 6.06$ mg/dl) versus the corresponding levels in control ( $58.36 \pm 8.57$ ,  $p < 0.001$ ), SIL group ( $60.47 \pm 3.64$ ,  $p < 0.001$ ) and L NAME + SIL group ( $50.42 \pm 6.07$ ,  $p < 0.01$ ).

Table (1): Statistical analysis of maternal BMI (gm/Cm<sup>2</sup>), placental weight (g), fetal weight (g), MAP (mmHg), urinary proteins (mg/24h) and all serum parameters of the studied groups.

N=8	Control	SIL	LNAME	L NAME + SIL
BMI (gestational 1)	0.52±0.03	0.53±0.01	0.52±0.02	0.52±0.05
final BMI (gm/Cm <sup>2</sup> )	0.65±0.04	0.64±0.04NS <sup>a</sup>	0.53±0.06, $p < 0.001$ <sup>a,b</sup>	0.59±0.02, $p < 0.05$ <sup>a,b,c</sup>
Placental weight (g)	0.57±0.03	0.58±0.05NS <sup>a</sup>	0.47±0.05, $p < 0.001$ <sup>a,b</sup>	0.52±0.03, $p < 0.05$ <sup>a,b,c</sup>
Fetal weight(g)	5.36±0.62	5.39±0.53NS <sup>a</sup>	3.81±0.57, $p < 0.001$ <sup>a,b</sup>	4.63±0.6, $p < 0.05$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
MAP (mmHg)	80.5±7.18	81.75±6.79NS <sup>a</sup>	121.65±11.4, $p < 0.001$ <sup>a,b</sup>	90.56±11.37, $p < 0.05$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
Urea (mg/dl)	21.21±2.70	18.64±1.39NS <sup>a</sup>	40.61±7.63, $p < 0.001$ <sup>a,b</sup>	27±6.3, $p < 0.05$ <sup>a</sup> , $p < 0.01$ <sup>b</sup> , $p < 0.001$ <sup>c</sup>
Creatinine (mg/dl)	0.21±.048	0.20±0.03NS <sup>a</sup>	0.44±0.07, $p < 0.001$ <sup>a,b</sup>	0.26±0.05, $p < 0.05$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
Total urinary proteins (mg/24h)	17.62±2.74	16.34±2.62NS <sup>a</sup>	50.75±7.21, $p < 0.001$ <sup>a,b</sup>	22.83±4.7, $p < 0.05$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
TG (mg/dl)	69.37±6.03	68.38±7.79NS <sup>a</sup>	110.72±10.58, $p < 0.001$ <sup>a,b</sup>	79±7.81, $p < 0.05$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
TC (mg/dl)	110.22±8.37	106.76±11.48NS <sup>a</sup>	162.7±12.76, $p < 0.001$ <sup>a,b</sup>	123.31±9.55, $p < 0.05$ <sup>a</sup> , $p < 0.01$ <sup>b</sup> , $p < 0.001$ <sup>c</sup>
LDL-c (mg/dl)	55.98±3.37	55.31±7.28NS <sup>a</sup>	99.26±9.85, $p < 0.001$ <sup>a,b</sup>	65.98±12.3, $p < 0.05$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
HDL-c (mg/dl)	58.36±8.57	60.47±3.64NS <sup>a</sup>	39.5±6.06, $p < 0.001$ <sup>a,b</sup>	50.42±6.07, $p < 0.05$ <sup>a</sup> , $p < 0.01$ <sup>b,c</sup>
Glucose (mg/dL)	91.98±7.87	92.25±7.45NS <sup>a</sup>	117.98±4.78, $p < 0.001$ <sup>a,b</sup>	100.19±5.26, $p < 0.05$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
Insulin (uIU/mL)	8.06±2.11	7.92±1.89NS <sup>a</sup>	17.86±2.16, $p < 0.001$ <sup>a,b</sup>	11.43±1.13, $p < 0.01$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
HOMA-IR	1.80±0.17	1.79±0.19NS <sup>a</sup>	5.59±0.45, $p < 0.001$ <sup>a,b</sup>	2.59±0.64, $p < 0.01$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
CRP (Ug/ml)	0.77±0.09	0.75±0.07NS <sup>a</sup>	1.36±0.14, $p < 0.01$ <sup>a,b</sup>	0.92±0.09, $p < 0.01$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
Placental MDA (nmol/gm tissue)	31.9±2.5	31.2±2.5NS <sup>a</sup>	55.65±6.35, $p < 0.001$ <sup>a,b</sup>	37.02±4.5, $p < 0.05$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
Placental SOD (U/mg protein)	10.16±1.6	10.46±0.69NS <sup>a</sup>	6.26±1.87, $p < 0.001$ <sup>a,b</sup>	8.64±1.45, $p < 0.05$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
Placental GSH-Px levels (U/mg protein)	13.73±1.78	13.95±1.41NS <sup>a</sup>	9.68±2.08, $p < 0.001$ <sup>a,b</sup>	11.92±1.27, $p < 0.05$ <sup>a,b,c</sup>
Endothelin-1 (pg/ml)	1.54±0.17	1.45±0.15NS <sup>a</sup>	2.85±0.24, $p < 0.001$ <sup>a,b</sup>	1.96±0.12, $p < 0.01$ <sup>a,b</sup> , $p < 0.001$ <sup>c</sup>
Adropin (ng/ml)	32.47±4.03	31.46±3.76NS <sup>a</sup>	60.22±6.28, $p < 0.001$ <sup>a,b</sup>	38.18±4.44, $p < 0.05$ <sup>a</sup> , $p < 0.01$ <sup>b</sup> , $p < 0.001$ <sup>c</sup>

NS: No Significant ( $p > 0.05$ ).

<sup>a</sup> :  $p$ -value of significance versus control group.

<sup>b</sup>:  $p$ -value of significance versus SIL group.

<sup>c</sup>:  $p$ -value of significance versus LNAME group.

Concerning placental tissue oxidative activities, there was significant increase in the mean values of placental MDA levels ( $55.65 \pm 6.35$ nmol/gm tissue) in LNAME preeclampsia rats in contrast to those in control group ( $31.9 \pm 2.5$ ,  $p < 0.001$ ), SIL group ( $31.2 \pm 2.5$ ,  $p < 0.001$ ) and L NAME + SIL group ( $37.02 \pm 4.5$ ,  $p < 0.001$ ), while there was significant decrease in the mean values of placental SOD ( $6.26 \pm 1.87$ U/mg protein) and GSH-Px levels ( $9.68 \pm 2.08$ U/mg protein) as compared to those in control ( $10.16 \pm 1.6$ ,  $13.73 \pm 1.78$  respectively,

$p < 0.001$ ), SIL group ( $10.46 \pm 0.69$ ,  $13.95 \pm 1.41$  respectively,  $p < 0.001$ ) and L NAME + SIL group ( $8.64 \pm 1.45$ ,  $11.92 \pm 1.27$  respectively). Concerning (Table 2); serum adropin levels in LNAME preeclampsia rats and in L NAME + SIL group showed significant positive correlations with each of following; MAP ( $r = 0.838$ ,  $p < 0.01$ ;  $r = 0.849$ ,  $p < 0.01$  respectively), total urinary proteins levels ( $r = 0.842$ ,  $p < 0.01$ ;  $r = 0.789$ ,  $p < 0.05$  respectively), serum levels of; urea ( $r = 0.791$ ,  $p < 0.05$ ;  $r = 0.808$ ,  $p < 0.05$  respectively), creatinine ( $r = 0.830$ ,  $p < 0.05$ ;  $r = 0.860$ ,

$p < 0.05$  respectively), CRP ( $r = 0.765, p < 0.05$ ;  $r = 0.795, p < 0.05$  respectively), endothelin-1 ( $r = 0.773, p < 0.05$ ;  $r = 0.939, p < 0.01$  respectively), TGs ( $r = 0.814, p < 0.05$ ;  $r = 0.828, p < 0.05$  respectively), TC ( $r = 0.898, p < 0.01$ ;  $r = 0.934, p < 0.01$  respectively), LDL-c ( $r = 0.900, p < 0.01$ ;  $r = 0.891, p < 0.01$  respectively), glucose ( $r = 0.906, p < 0.01$ ;  $r = 0.961, p < 0.001$  respectively), insulin ( $r = 0.917, p < 0.01$ ;  $r = 0.950, p < 0.001$  respectively), HOMA-IR ( $r = 0.943, p < 0.001$ ;  $r = 0.816, p < 0.05$  respectively) and Placental tissue MDA levels ( $r = 0.993, p > 0.001$ ;  $r = 0.912, p < 0.01$  respectively), however, they revealed significant negative correlation with maternal BMI ( $r = -0.848, p < 0.01$ ;  $r = -0.889, p < 0.01$  respectively), placental weights ( $r = -0.767, p < 0.05$ ;  $r = -0.881, p < 0.01$  respectively), fetal weights ( $r =$

$0.874, p < 0.01$ ;  $r = 0.765, p < 0.05$  respectively), serum HDL-c levels ( $r = -0.763, p < 0.05$ ;  $r = -0.920, p < 0.01$  respectively), placental SOD tissue levels ( $r = -0.854, p < 0.01$ ;  $r = -0.780, p < 0.05$  respectively) and placental GSH tissue levels ( $r = -0.890, p < 0.01$ ;  $r = -0.839, p < 0.01$  respectively).

Additionally, in controls and SIL groups, significant correlations were found between adropin and MAP ( $r = 0.742, p < 0.05$ ;  $r = 0.818, p < 0.05$  respectively), endothelin-1 ( $r = 0.768, p < 0.05$ ;  $r = 0.862, p < 0.01$  respectively), while negative correlations with TGs levels ( $r = -0.730, p < 0.05$ ;  $r = -0.842, p < 0.01$  respectively). Control group showed positive significant correlations between adropin and HDL-c ( $r = 0.756, p < 0.05$ ).

Table (2): Pearson's correlation analysis between serum adropin and all other parameters in studied groups.

	Control		SIL		LNAME		L NAME + SIL	
	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value
Maternal BMI	-0.695	0.056	-0.260	0.535	-0.848	0.008 **	-0.889	0.003**
Placental weight (g)	-0.595	0.120	-0.588	0.126	-0.767	0.026*	-0.881	0.004**
Fetal weight (g)	-0.408	0.316	-0.103	0.808	-0.874	0.005 **	-0.765	0.027*
MAP (mmHg)	0.742	0.035*	0.818	0.013*	0.838	0.009**	0.849	0.008**
Urea (mg/dl)	0.263	0.529	0.391	0.388	0.791	.019*	0.808	0.015*
Creatinine (mg/dl)	0.204	0.628	0.253	0.545	0.830	0.011*	0.860	0.006**
urinary proteins (mg/24h)	0.433	0.284	0.448	0.266	0.842	0.009**	0.789	0.020*
CRP (Ug/ml)	0.666	0.071	0.256	0.451	0.765	0.027*	0.795	0.018*
Endothelin-1 (pg/ml)	0.768	0.026*	0.862	0.006**	0.773	0.024*	0.939	0.001**
Placental MDA (nmol/gm tissue)	0.252	0.547	0.109	0.798	0.993	>0.001***	0.912	0.002**
Placental SOD (U/mg protein)	0.527	0.179	-0.354	0.390	-0.854	0.007**	-0.780	0.022*
Placental GSH-Px (U/mg protein)	-0.139	0.743	0.105	0.804	-0.890	0.003**	-0.839	0.009**
Glucose (mg/dl)	0.123	0.702	0.230	0.461	0.906	0.002**	0.961	>0.001***
Insulin (uIU/mL)	0.656	0.077	0.621	0.082	0.917	0.001**	0.950	>0.001***
HOMA-IR	0.467	0.302	0.104	0.802	0.943	>0.001***	0.816	0.014*
TG (mg/dl)	-0.730	0.040*	-0.842	0.009**	0.814	0.014*	0.828	0.011*
TC (mg/dl)	-0.419	0.302	-0.576	0.135	0.898	0.002**	0.934	0.001**
LDL-c (mg/dl)	-0.064	0.881	-0.015	0.973	0.900	0.002**	0.891	0.003**
HDL-c (mg/dl)	0.756	0.030*	0.299	0.472	-0.763	0.028 *	-0.920	0.001**

\*: Significant ( $p < 0.05$ ).

\*\*: Highly significant ( $p < 0.001$ ).

*r*: Correlation coefficient.

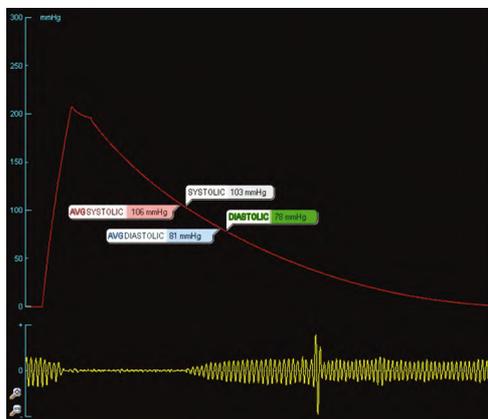


Fig. (1): Recorded blood pressure from control group.

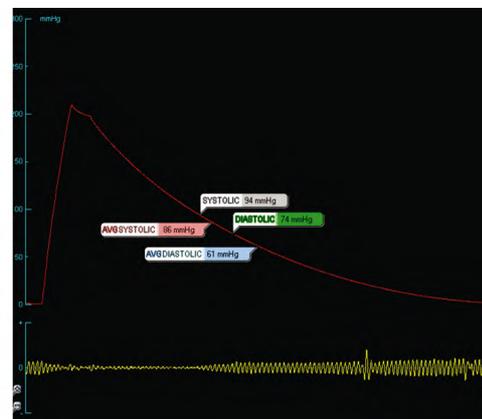


Fig. (2): Recorded blood pressure from SIL group.

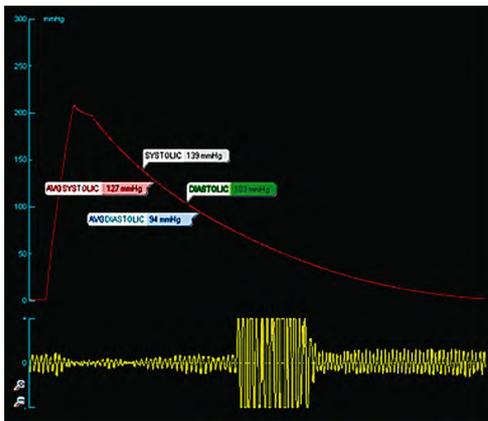


Fig. (3): Recorded blood pressure from L-NAME group.

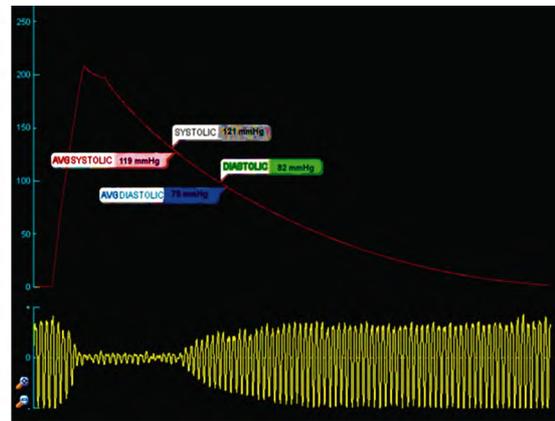


Fig. (4): Recorded blood pressure from L-NAME + SIL group.

*Histopathological results:*

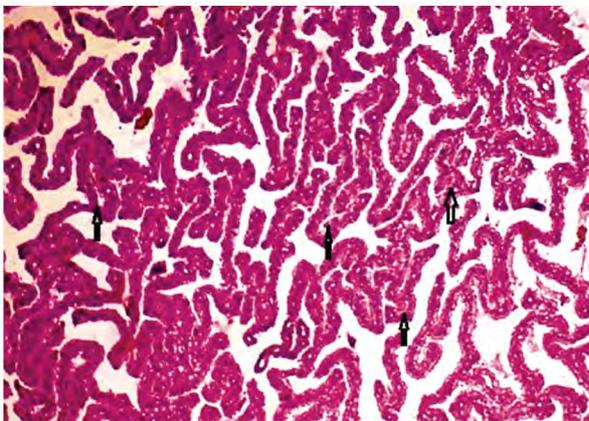


Photo (1): Photomicrograph of placental section from control rats showing normal placental tissue of normal-sized chorionic villi lined by cytotrophoblastic cells (O) (H & E X400).

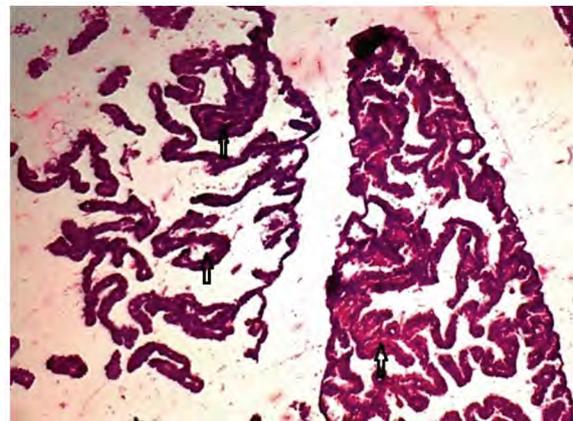


Photo (2): Photomicrograph of placental section from SIL treated rats showing normal placental tissue of normal-sized chorionic villi lined by cytotrophoblastic cells (O) (H & E X400).

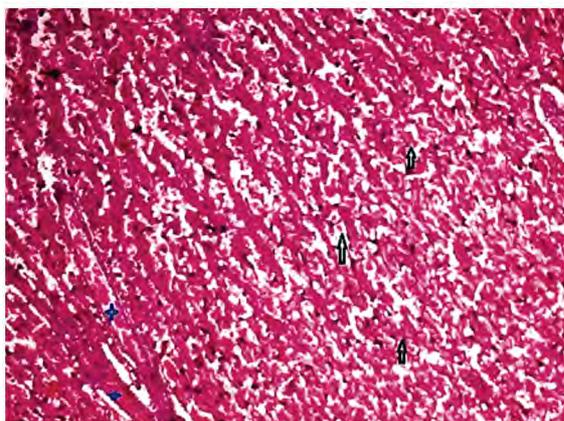


Photo (3): Photomicrograph of placental tissue from LNAME preeclampsia group showing; large areas of necrosis (O) infiltrated by aggregates of inflammatory cells. The blood vessels are dilated and thrombosed (TM) (H & E X400).

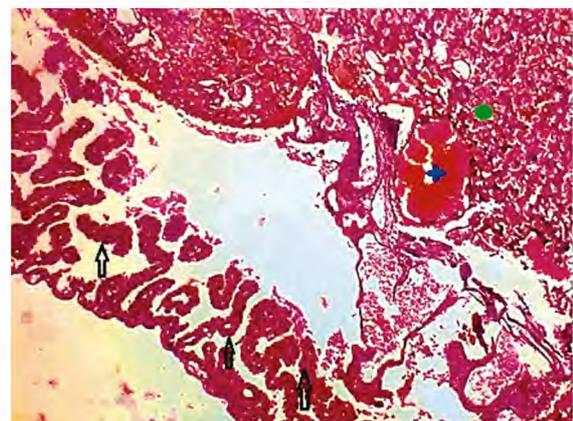


Photo (4): Photomicrograph of placental section from LNAME + SIL group Showing areas of normal-sized chorionic villi lined by cytotrophoblastic cells (O) with dilated vascular spaces (TM) surrounded by small areas of necrosis and inflammation (O) (H & E X400).

## Discussion

In this study, experimental preeclampsia model induced by L-NAME were successfully established and proved by maternal hypertension, proteinuria, increased serum levels of urea and creatinine, besides decreased placental and fetal weights, these findings were consistent with other reports of Zhou et al., [33] and Amaral et al., [34]. Moreover, the histopathological findings were agreed with Powe et al., [35].

L-NAME was found to be a potent competitive NOS inhibitor with subsequently decreasing NO synthesis [36]. This leads to increasing the adhesion molecules expression with subsequent acceleration of the inflammation in systemic vasculature and placenta (which indicated in the current work by increased CRP) and causing endothelial dysfunction with utero-placental perfusion failure [37]. In the kidney, vascular endothelial dysfunction leads to altered renal hemodynamics and reduced renal excretory function causing hypertension and proteinuria [38]. Moreover, increase in ET-1 may be attributed to the reduction in NO synthesis as NO was known to be a potent inhibitor of ET-1 production [39]. Additionally, Sandrim et al., [40] revealed that NO synthesis was inversely related to the serum levels of anti-angiogenic factors including soluble endoglin (sEng), soluble fms-like tyrosine kinase-1 (sFlt-1). So, inhibition of NO synthesis will be associated with an increase in these anti-angiogenic factors that inhibit the angiogenic factors; Vascular Endothelial Growth Factor (VEGF), Placental Growth Factor (PlGF), so these factors were strongly incriminated in the pathophysiology of PE and promotes the development of hypertension [41,42]. In addition to its NO inhibitory effect, L-NAME was found to decrease prostacyclin and increase the plasma endothelin and thromboxane A2 with more vasoconstriction, oxidative stress and hypertension [43].

In current study, rats with preeclampsia showed significant decrease in their BMI on gestational day 18, placental and fetal weights, these results are similar with Ma et al., [44] and Huai et al., [14], however, Ramesar et al., [45] and Motta et al., [6] found decrease in placental weights but not fetal weights in the same model, L-NAME induces a reduction in NO in fetal trophoblast vascular area and consequently decreases placental blood flow and fetal oxygen supply, which could be causing IUGR [6]. NOS inhibitors reduce food intake and body weight gain via affecting serotonin metabolism in the cortex, diencephalon causing anorexic effect [46,47].

Increased serum glucose, insulin levels and HOMA-IR in rats with preeclampsia in this work suggested development of insulin resistance. Insulin resistance is well established to be associated with preeclampsia in animal [48,49] and human studies [50,51], blockade of NOS causes peripheral insulin resistance secondary to blockade of the hepatic parasympathetic reflex release of hepatic insulin-sensitizing substance in response to insulin [52]. Also, Oxidative stress and inflammation has been suggested to contribute to insulin resistance

[53].

It is clearly seen that L-NAME administration to pregnant rats exhibited placental tissue oxidative damage (increased MDA and reduced activities of the antioxidant enzymes SOD and GSH), inflammation (increased CRP), dyslipidemia (increased TC, LDL-cholesterol and TGs but significant decreased in HDL-cholesterol) and these findings were supported by Ma et al., [44] and Huai et al., [14] who found increased in TC, TGs and free fatty acid levels in LNAME preeclampsia-like mice. In the L-NAME model, the free fatty acid oxidation disorders occur, with decreased levels of mRNA and protein expression of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), FFA levels were negatively correlated with LCHAD levels [54]. Oxidative stress has been implicated in the pathophysiology of preeclampsia because it damages the maternal vascular endothelium as reported by human [34,55,56] and animal studies [57,58].

In rats received sildenafil only, no alterations in maternal BMI, fetal and placental weights, and MAP and biochemical parameters versus control. These data are similar to studies recorded by Sasser and Baylis [59] and Motta et al., [6] who revealed that use of sildenafil in rats with normal gestation did not induce any change in fetal number or growth. Sildenafil in women with IUGR produced no adverse maternal side effects [60]. It may mediated endothelial relaxation of uterine vessels and improves uterine blood flow via NO-cGMP pathway [61]. Whereas, in LNAME plus Sildenafil treated rats, sildenafil counteracted the effect of L-NAME and improved placental weights and fetal growth and these results were agreed with Dastjerdi et al., [62] who showed that sildenafil used as a therapeutic agent may improve myometrial perfusion in foetal growth restriction pregnancies, Ramesar et al., [63] proved that sildenafil citrate improves uterine artery blood flow and endometrial development in women undergoing in vitro fertilization.

Moreover, in the current work, Sildenafil had beneficial effects in decreasing the severity of PE by ameliorating the MAP, renal functions, oxidative stress and insulin resistance. And these results were supported by Gillis et al., [64] who showed that Sildenafil improved renal function, reduced MAP, and increased fetal growth in preeclamptic Dahl salt sensitive rats, Sildenafil also enhances preeclampsia symptoms in LNAME and in reduced uterine perfusion pressure models [45,65]. Additionally, sildenafil improves fetal growth and maternal blood pressure in human subjects with preeclampsia [35]. Interestingly, chronic treatment with sildenafil improved insulin action and diminished obesity in high-fat-fed mice [66].

Regarding serum adropin levels, rats with preeclampsia showed significantly increase in adropin levels when compared to both control and SIL groups and these levels were positively correlated with MAP, proteinuria, serum levels of urea, creatinine, endothelin-1, CRP, TGs, TC, LDL-cholesterol, glucose, insulin, HOMA-IR and placenta tissue of MDA, however were negatively correlated with maternal BMI, placental tissue of SOD, GSH levels and serum HDL-cholesterol levels while in LNAME+ sildenafil treated group adropin levels were significantly decreased compared to LNAME group, and still correlated with all previous parameters.

These results were in line with Wang et al., [12] who showed that serum adropin levels were significantly increased in women with preeclampsia versus with normal pregnancy, however in contrast with those of Cakmak et al., [13] who found that maternal and umbilical cord adropin levels were significantly lower in the preeclamptic women compared to normal subjects and that maternal levels were much lower in the severe preeclampsia than mild preeclampsia, and maternal adropin levels were negatively correlated with systolic and diastolic blood pressures.

Adropin has been shown to be released in many tissues in rats and is also released by vascular endothelial cells and plays a role in the neovascularization [12]. It was reported that adropin had a protective effect on endothelium by up-regulating endothelial nitric oxide synthase in the endothelium via activation of vascular Endothelial Growth Factor Receptor (EGFR) 2 phosphatidylinositol 3-kinase Akt and EGFR 2 extracellular signal regulated kinase 1/2 pathways and promoting endothelial function [9]. Also, Topuz et al., [67] showed low levels of adropin in subjects with the endothelial dysfunction. Gözal et al., [10] demonstrated

that adropin concentration is reduced in children with obstructive sleep apnea and endothelial dysfunction.

Adropin plays a role in hypertension via endothelial dysfunction [68]. Gu et al., [69] found that patients with essential hypertension had lower levels of serum adropin, and serum adropin was inversely correlated with blood endothelin-1, systolic and diastolic blood pressures. Moreover, Gulen et al., [70] found significantly higher levels of adropin in normotensive patients compared to hypertensive ones with and without target organ damage and they reported that adropin was not correlated with systolic and diastolic blood pressure. However, Bolayır et al., [71] showed decreased adropin levels in nocturnal hypertensive and non-dipper hypertensive patients and a strong correlation was found between nighttime blood pressure levels with adropin and hsCRP levels. Animal studies indicated that adropin regulates angiogenesis, increases blood flow and capillary density in an ischemia model [9].

It was suggested that increased maternal serum adropin in this study might serve as a protective feedback mechanism against the pathogenesis of preeclampsia: Endothelial dysfunction, insulin resistance, oxidative stress, and inflammation.

It is possibly proper to assume that adropin may be involved in the regulation of fetal growth. In current study, maternal adropin levels were negatively correlated with placental and fetal weights in preeclampsia group and in preeclampsia treated with sildenafil group and these results were agreed by Celik et al., [72] who reported a negative correlation between umbilical cord adropin levels, gestational age at delivery and birth weight in pregnancies complicated with GDM, also Baka et al., [73] showed that maternal serum adropin levels in severe IUGR cases were increased and they explained this increased adropin as a compensatory feedback mechanism to counteract endothelial placental dysfunction in IUGR.

Contrary to these findings, Cakmak et al., [13] found a positive correlation between umbilical cord adropin levels and both birth weight and gestational age at delivery and they assumed that an increased adropin expression may be a representative of normally functioning placenta and consequently healthy fetal maturation, Qui et al., [74] observed a positive correlation between cord blood adropin concentrations and placental weight and gestational age at birth in preterm deliveries. Moreover, Aydin et al., [75] reported decreased

levels of adropin in both umbilical cord and maternal blood in IUGR cases and they identified positive correlations between endothelin and adropin in healthy pregnancies but not in pregnancies with IUGR.

Respect to adropin correlations with glucose metabolism parameters in other studies; serum adropin positively correlated with elevated HbA1c in gestational diabetes mellitus [76], in contrast, Aydın et al., [75] reported that there was a negative correlation between adropin concentrations and HbA1c (%), also serum adropin level is inversely associated with glucose and insulin resistance in T2DM patients [77] and in polycystic ovary [78]. Kumar et al., [79] showed that increased adropin decrease insulin resistance and glucose intolerance that occur in response to metabolic stress, Gao et al., [80] concluded that adropin improves glucose clearance, ameliorates insulin resistance and reverses dyslipidemia in obese mice, intraperitoneal administration of adropin significantly decreased the levels of fasting blood glucose, HbA1c (%), HOMA-IR and insulin levels [81] however, no correlations with glucose parameters in End Stage Renal Disease (ESRD) [82], or in essential hypertension [69]. Accordingly, it can be speculated that higher adropin in experimental preeclampsia in rats is one of the multiple adaptive responses on adverse glucose metabolism.

Some researchers have noted negative relationships between serum adropin and cholesterol, very low-density lipoprotein cholesterol, and triglyceride in women with polycystic ovary syndrome [78]. Moreover, serum adropin levels negatively correlated with triglycerides and BMI in patients with myocardial infarction and those with metabolic syndrome after gastric bypass [83,84]. Akcılar et al., [81] found that administration of adropin significantly decreased inflammatory cytokines, levels of LDL-C and increased the levels of HDL-C in rat model with hyperlipidemia and they suggested that adropin could have a strong anti-hyperlipidemia activity and also ameliorate other lipid metabolism disorder-related complications. In contrast, no associations between adropin and lipid profile in patients with ESRD [82] or amongst young adolescents [85]. Interestingly, control rats in this study revealed negative correlations of adropin with triglycerides and positive with HDL-cholesterol and these observations were recorded by Kalu et al., [82], similarly, Nowak et al., [86] detected a positive correlation between adropin level and HDL cholesterol but no correlation with BMI in healthy women. Recently, Yang et al., [87] indicated that adropin levels negatively correlate with mark-

ers of oxidative stress injury and endothelial dysfunction in Rat Brain of old age.

#### Conclusion:

Increased maternal adropin levels in preeclampsia like model in rats might represent a regulatory feedback mechanism against insulin resistance endothelial placental dysfunction; inflammation and oxidative stress which associated with origin of preeclampsia, hence identify it as a potentially protective agent against preeclampsia development. Sildenafil citrate can alleviate the gestational hypertension and proteinuria and improve the pregnancy outcomes by reducing inflammation insulin resistance and oxidative stress in experimental preeclampsia. Further studies are required in order to have a better understanding of the relation between preeclampsia and adropin.

#### Acknowledgment:

To Kamal El-Kashishi, Professor of Pathology, Faculty of Medicine, Zagazig University for performing the histological study.

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## مستويات هرمون الأدرابين في مصل نموذج الجرذان المصابة بتسمم الحمل بعقار ال-ل-نيم والمعالجة بعقار السلدنافيل سيترات

خلفية البحث: تسمم الحمل هو اضطراب إرتفاع ضغط الدم من الحمل، وهو ضار على كل من الأم والجنين. وقد أقترح السيلدينافيل كعلاج محتمل لخفض ضغط الدم وتحسين التروية في الرحم في مرضى تسمم الحمل. الأدرابين هو بروتين جديد يتحكم في توازن الطاقة وتنظيم التمثيل الغذائي للكربوهيدرات والدهون والبروتين، وقد وجد أن مستويات الأدرابين في دم الأمهات المصابة بتسمم الحمل لا تزال قيد الجدول.

الهدف من العمل: لتقييم مستويات الأدرابين في مصل الجرذان المصابة بتسمم الحمل بعقار ال-ل-نيم وأيضا الجرذان المصابة بتسمم الحمل والمعالجة بعقار السلدنافيل سيترات وللتحقق من إرتباطها ببعض المعلمات الأيضية ودلائل الإلتهابات والأكسدة.

المواد والأساليب: تم تقسيم الجرذان الحوامل (٣٢ جرذا) عشوائيا إلى أربع مجموعات (ن=٨): مجموعة ضابطة، ومجموعة معالجة بالسيلدينافيل، ومجموعة معالجة بال-ل-نيم) للتحقق على حدوث تسمم الحمل ومجموعة معالجة بال-ل-نيم مع السلدنافيل. في جميع المجموعات، تم قياس كلا من مؤشر كتلة الجسم للجرذان الأم، وأوزان المشيمة، وأوزان الجنين، وضغط الدم الشرياني، وإجمالي البروتينات البولية، والجلوكوز والأنسولين ومقاومة الأنسولين، والدهون الثلاثية، والكوليسترول الكلي، والكوليسترول العالى الكثافة، والكوليسترول المنخفض الكثافة، وبروتين سى التفاعلى واليوريا في المصل، والكرياتينين، والأندوثيلين والأدرابين، أيضا مستويات المألونديالديهيد في المشيمة، والإنزيمات المضادة للأكسدة سوبرأكسيد ديسموتاز وجليوتاثين بيروكسيداز في أنسجة المشيمة بالإضافة إلى فحص أنسجة المشيمة هيستولوجيا.

النتائج: تسبب لل-نيم في إصابة الجرذان بتسمم الحمل وأظهرت هذه الجرذان زيادة ذات دلالة إحصائية في كلا من ضغط الدم الشرياني، والبروتينات البولية الكلية ومستويات المصل من اليوريا، والكرياتينين، والجلوكوز، والأنسولين ومقاومة الأنسولين، والدهون الثلاثية، والكوليسترول الكلي، والكوليسترول العالى الكثافة وبروتين سى التفاعلى والأندوثيلين والأدرابين وكذلك مستويات المألونديالديهيد في المشيمة في حين إنخفاض ذات دلالة إحصائية في كلا من مؤشر كتلة الجسم للجرذان الأم، وأوزان المشيمة، وأوزان الجنين والكوليسترول المنخفض الكثافة والإنزيمات المضادة للأكسدة سوبرأكسيد ديسموتاز وجليوتاثين بيروكسيداز في أنسجة المشيمة بالمقارنة مع المجموعات الأخرى، بالإضافة إلى ذلك، كشفت مجموعات الجرذان المعالجة بال-ل-نيم وال-نيم مع السلدنافيل عن إرتباطات موجبة ذات دلالة إحصائية بين مستويات الأدرابين وضغط الدم الشرياني، والبروتينات البولية الكلية، ومستويات المصل من اليوريا، الكرياتينين، والجلوكوز، والأنسولين ومقاومة الأنسولين، والدهون الثلاثية، والكوليسترول الكلي، والكوليسترول العالى الكثافة، وبروتين سى التفاعلى والأندوثيلين ومستويات المألونديالديهيد في المشيمة، على الرغم من أنها أظهرت وجود إرتباطات سلبية ذات دلالة إحصائية بين الأدرابين من مؤشر كتلة الجسم للجرذان الأم، وأوزان المشيمة، وأوزان الجنين والكوليسترول المنخفض الكثافة والإنزيمات المضادة للأكسدة سوبرأكسيد ديسموتاز وجليوتاثين بيروكسيداز في أنسجة المشيمة.

الخلاصة: زيادة مستويات الأدرابين في مصل نموذج الجرذان المصابة بتسمم الحمل بعقار ال-ل-نيم) يمثل آلية لتعويض ما يسببه ضعف المشيمة البطانى، ومقاومة الأنسولين، والإلتهاب والأكسدة التي ترتبط مع تسمم الحمل، وبالتالي التعرف على أنه وكيل الحماية المحتملة ضد تطور تسمم الحمل بينما وجد أن السلدنافيل يحسن أعراض تسمم الحمل والإلتهاب والإجهاد التأكسدى في نموذج الجرذان المصابة بتسمم الحمل.