Delta University Scientific Journal
Journal home page: https://digitalcommons.aaru.edu.jo/dusj/

Protective Effect of Artemisinin on the Placenta of L-NAME Induced rat model of Preeclampsia

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Abstract

Preeclampsia (PE) is a pregnancy induced hypertensive disorder and an important cause of maternal and fetal morbidity and mortality. It affects about 3 - 8% of all pregnancies worldwide. Placental factors that arise from placental hypoxia/ischemia set off molecular and cellular cascades, resulting in vascular endothelial dysfunction. The aim of study was to clarify the possible protective effect of Artemisinin (ART) on the placenta of L-NAME induced rat model of PE and to explore possible involved mechanisms. Thirty pregnant female Sparge Dawley rats were randomly divided into 3 groups (10 rats/each): The control group, PE-group, and ART treated group. L-Nitro arginine methyl ester (L-NAME; 75 mg/kg body weight/day) was injected subcutaneous from day 10 to day 19 of gestation to induce hypertension during pregnancy. The mean arterial blood pressure (MABP) and 24-hour protein in urine were determined before scarification. Rats were sacrificed on day 20 of gestation, Placental tissues homogenate was prepared for evaluation of oxidative stress markers. Additionally, Slices from placenta were studied by light and electron microscopes. The MABP, 24-hour protein in urine and oxidative stress markers in the PE-group were significantly higher when compared to control group. ART treated group had a non-significant decrease in MABP although had a significant decrease in proteinuria and oxidative markers when compared to PE-group. The histopathological examination of ART group showed partial improvement in both light and electron microscopic findings as regard placental vascular changes and cellular parameters when compared to PE group. The present study concluded that ART exerted protective effect in preeclampsia model through oxidative stress correction and reduction of proteinuria along with improvement of placental vascular and cellular changes in both light and electron microscopies.

Keywords: Artemisinin, Preclampsia, LNAME.

Introduction

Preeclampsia [PE] is defined as a de novo hypertensive progressive disorder with significant proteinuria appearing during the second or third trimester of gestation (Augusto et al., 2020). Hypertensive disorders of pregnancy complicate 10% of all pregnancies worldwide. Pregnancies complicated by pre-eclampsia show an increase in maternal and perinatal morbidity and mortality in the developing world (Hurrell et al., 2020).

The main pathophysiology of PE includes poor placental perfusion, endothelial cell dysfunction, and disturbed balance of angiogenic factors. Poor placental perfusion is a stimulus for reactive oxygen species (ROS) production, increased maternal inflammation, immunologic dysfunction, suppression of endothelial nitric oxide (eNOS), enhanced inducible nitric oxide (iNOS), endothelin-1production (ET-1) and increased circulating levels of both the anti-angiogenic protein the plasma soluble factor-like tyrosine kinase-1 (sFlt-1), caspase-3 expression, decrease placental growth factor (PLGF), and decrease vascular endothelial growth factor; VEGF (Goulopoulou and Davidge, 2015).

In pre-eclampsia, oxidative stress and maternal inflammatory response seem to be exaggerated due to the release of inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10), and C-reactive protein; CRP (Gilbert et al., 2008). Poor placental perfusion is a trigger for ROS formation and production like nitric oxide (·NO), superoxide ($O^{\cdot -}2$), hydrogen peroxide (H2O2), hydroxyl radical (·OH), and peroxyl nitrite; $ONOO^-$ (Aouache et al., 2018b).

There are multiple animal models of PE, and the ideal animal model should help to understand the pathophysiological mechanisms of the PE disease and the therapeutic strategies involved in its management (Jim and Karumanchi, 2017). The most popular one is induced by the administration of Nitro L-arginine methyl ester (L-NAME) which inhibit nitric oxide synthase (NOS) during mid to late period of gestation (Soobryan et al., 2017).

Artemisinin (ART) is isolated from the plant Artemisia annua, sweet wormwood, an herb employed in Chinese traditional medicine (Shu et al., 2018). It is a well-known antimalarial drug showing antibacterial, antifungal, antileishmanial, antioxidant, antitumor, anti-inflammatory activity, and immunomodulatory effects (Shakir et al., 2011). It had been proven to be protective in models of post-infarct myocardial remodeling, lupus nephritis, experimental autoimmune encephalomyelitis, and Alzheimer disease (Zhang et al., 2016). ART is a clinically safe medication with few side effects, so it is suitable for long-term clinical applications (Yan et al., 2017). ART was reported to exert an inhibitory effect on inducible nitric oxide synthase (iNOS) synthesis (Wang et al., 2017). Also, several studies had demonstrated that ART can decrease inflammation by down-regulation of TNF-α ,interleukin1B (IL-1β), nuclear factor kappa B (NF-kB) and transforming growth factor-β1;TGF- β1 (Zhang et al., 2016). ART may be a promising drug in prophylaxis of PE due to anti-inflammatory and antioxidant effect.

Materials and Methods

Chemicals and drugs used

Artemisinin (ART) is raw material powder was purchased from Nanjing Yuan Sen Thai Biological Technology Co., China. Carboxy methyl cellulose (CMC) (0.5%) is raw white powder material from (Sigma - Aldrich, USA). N(gamma)-nitro-L-arginine methyl ester (L-NAME) is white powder obtained from (Sigma - Aldrich, USA).

Experimental animals

Thirty adult female Sprague-Dawley rats weighing 200-250 grams and thirty adult male Sprague- Dawley rats were used throughout this study. They were obtained from the medical experimental research center (MERC), Mansoura faculty of medicine. Seven days prior to the experiment, the animals were acclimatized to standard laboratory conditions, and they were put in similar optimum housing condition with free access to food and water. The animals were housed in plastic cages lined with sawdust that was renewed daily and were observed for food intake. They were kept in cages at a room with controlled temperature (23 ± 2°C), humidity (50 ± 5%) and 12-h light–dark cycle. The care and use of animals were done according to the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Research and published by the National Research Council, USA, 2011.The study design and protocol was revised and approved by Mansoura Faculty of Medicine, Institutional Research Board (IRB) code number: MS.19.10.852.

Grouping of animals in experimental design

- Thirty adult female pregnant Sprague-Dawley rats were divided into three groups (10 rats in each group) as the following:
- Control group: Pregnant rats were received carboxy methyl cellulose (0.5 % CMC) by oral gavage (Gu et al., 2012a).

- Preeclampsia (PE) group: Pregnant rats were administered L-NAME (75 mg/kg / body weight/ once daily) starting from gestational day (GD 10) till end of pregnancy via subcutaneous injection (Shu et al., 2018).
- Artemisinin-treated group: Pregnant rats were received ART powder dissolved in 0.5% CMC as vehicle at dose of (75 mg/kg / body weight/ once daily) by oral gavage from gestational day (GD7) till end of gestation period and were administered L-NAME (75 mg/kg / body weight/ once daily) starting from gestational day (GD 10) till end of pregnancy via subcutaneous injection (Gu et al., 2012b).

Methods of Diagnosis of Pregnancy in current study

A. Vaginal Smear

1.Day Zero of pregnancy: Female rats in the estrus period were mated with male rats at a ratio of one female to one male. They were confined in wire-bottomed cages overnight.

2.On the next day: Using a standard saline solution and a tiny pipette, the females' vaginal lavages were tested for the presence of vaginal plug and/or sperms (Marcondes et al., 2002).

3. The first day of pregnancy: vaginal fluid was inspected under a microscope. Females with sperm positive were thought to be pregnant

B. Ultrasonography (US):

Female rats were anaesthetized using a nose cone and 3.0% isoflurane in oxygen. A 15 mega -hertz (MHz) transducer was used for transabdominal ultrasound. The presence of gestational sacs confirm pregnancy (Stasinopoulou et al., 2014). On day 20 of pregnancy, rats were weighed and fasted overnight before being scarified by decapitation under light halothane anesthesia at dose of 30 mg/kg/body weight (Kissin et al., 1983). The gravid uterine horns were exposed by lower midline abdominal incision, the sacs were opened, the fetus and the umbilical cord were exposed. The placentae were detached, and the specimens of the placentae were rapidly fixed in 10% neutral buffer formalin for 48 hours and then washed by tap water.

Biochemical assay oxidative stress marker using placental homogenates:

Placental tissues were rinsed in phosphate buffer saline (PBS) to remove excess blood, homogenized in 5 ml of PBS per gram tissue and stored at < 20 °C overnight. The homogenate was centrifuged at 2000xg for 5 minutes super mutant was removed and delivered into Eppendorf's and frozen at 80 °C until assay (Simpson, 2010).

Assay of reduced glutathione (GSH) level in placental tissue homogenates:

GSH was measured using reduced glutathione colorimetric determination kits (Bio diagnostic, Giza, Egypt). (Rahman et al., 2006).

Assay of lipid peroxidase (malondialdehyde); MDA level in placental tissue homogenates:

Malondialdehyde was measured using lipid peroxidase (MDA) colorimetric determination kits (Bio diagnostic, Giza, Egypt). (Ohkawa et al., 1979).

Assay of inducible nitric oxide; iNO level in placental tissue homogenates:

Nitric oxide was measured using NO colorimetric assay kits (Bio diagnostic, Giza, Egypt). (Bryan and Grisham, 2007).

Measurement of 24-hour protein in urine

The rats were placed separately in metabolic cages for 24-hour urine collection on day 19 of gestation. Urine protein concentrations were measured by the principle of turbidimetry by adding 5% trichloroacetic acid according to (Polkinghorne, 2006).

Determination Mean arterial blood pressure (MABP)

Using Doppler ultrasonography to analyses maternal and fetal hemodynamic changes. The peak systolic velocity (PSV) of the umbilical cord at the insertion site was measured (Cnossen et al., 2008, Elmetwally et al., 2016). The mean spiral artery (SA) resistance index (RI) of the implantation site was calculated using the equation (RI = [PSV – EDV]/PSV) by measuring the end diastolic flow velocity (EDV) of each SA. In healthy pregnancies, (pulsatility index (PI), resistance index (RI) decreased. Higher PI was found in SA of complicated pregnancies compared to healthy pregnancies (Cnossen et al., 2008, Lloyd-Davies et al., 2021). Doppler parameters and data collection were automatically measured.

Histopathological assessment by L.M. (Hematoxylin and Eosin stain; H&E):

The placenta was preserved in 10% neutral buffer formalin for 24 hours, embedded in paraffin, sectioned at 5 µm, and stained with H&E. The histological examination was performed by an investigator blinded to the interventions.

Electron microscopy (E.M):

The placenta was cut into very small pieces (1 mm3) and fixed immediately in 0.1 mol/L phosphate buffer containing 2.5% glutaraldehyde and 2% paraformaldehyde at 4 ºC for 2 – 4 hours in the refrigerator, then post-fixed in 1% osmium tetraoxide for two hours at 4 ºC. Specimens were then dehydrated, embedded in epon, then ultra-thin silvery golden sections were cut by ultra-microtome and picked up on uncoated grids, then stained with uranyl acetate and lead citrate and examined by transmission electron microscope (TEM- JEOL JEM- 100 SX) in electron microscopy unit (Faculty of Agriculture, Mansoura University). (Suvarna et al., 2018).

Statistical analysis

Data were analyzed using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 21). Continuous variables were presented as mean ± SD (standard deviation) for normally distributed data. The three groups were compared with ANOVA test and in-between group's comparison was tested by post hoc Tukey test. The results was considered significant when $p \leq 0.05$.

Results

Mean arterial blood pressure (MABP)

In Table (1) &Figure (1), administration of L -NAME in PE group showed significant increase in the MABP (156.02±1.04) in comparison to control group (96.01±0.89). Treatment with ART showed non-significant decrease in the MABP (152.30±5.21) in comparison to PE group.

Table (1)

Effects of ART on MABP (mmHG) in L-NAME induced PE in rats

Groups	MABP (mmHg)			Post hoc Tukey test	
	Mean ± SD	Min-Max	ANOVA (p value)	P1	P2
Control group	96.01± 0.89	95.12-97.54	F=1011 P≤0.001*	-	-
Preeclampsia group	156.02± 1.04	154.33-157.26		≤0.001*	-
ART group	152.30± 5.21	139.0-156.0		≤0.001*	0.023*

Data are presented as means (of 10 rats) ± SD. The three groups were compared with ANOVA test and in-between groups comparison was tested by post hoc Tukey test. P1: Comparison between Preeclampsia, ART groups and control groups. P2: Comparison between ART and Preeclampsia groups.

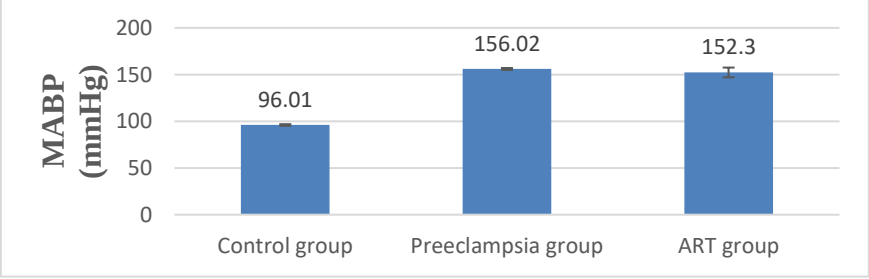

Figure (1): Effects of ART on MABP (mmHG) in L-NAME induced PE in rats

Urinary Protein value during / 24h:

In table (2) &Figure (2), administration of L -NAME in PE group showed significant increase in the 24h urine protein in (81.18±3.84) in comparison to control normal group (34.13±2.51). Treatment with ART showed significant decrease in the 24h urine protein (65.06±5.42) when compared to PE group.

Table (2)

Effects of ART on 24-hour urine protein value in L-NAME in rats

Groups	Urine protein / 24h			Post hoc Tukey test	
	Mean ± SD	Min-Max	ANOVA (p value)	P1	P2
Control group	34.13±2.51	29.50-37.90	F=251.3 P≤0.001*	-	-
Preeclampsia group	81.18±3.84	74.30-87.80		≤0.001*	-
ART group	65.06±5.42	55.80-73.00		≤0.001*	≤0.001*

Data are presented as means (of 10 rats) ± SD. The three groups were compared with ANOVA test and in-between groups comparison was tested by post hoc Tukey test. P1: Comparison between Preeclampsia, ART groups and control groups. P2: Comparison between ART and Preeclampsia groups

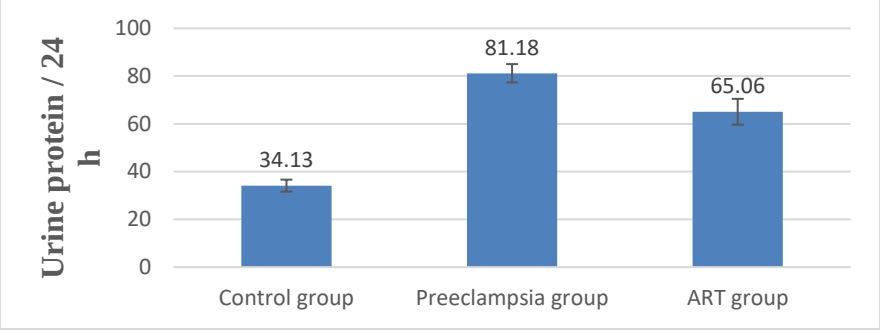

Figure (2): Effects of ART on 24-hour urine protein value in L-NAME in rats

Assessment of oxidative stress markers in placental tissue

1) Placental tissue levels of reduced Glutathione (GSH):

In table (3) &Figure (3), administration of L -NAME in PE group showed significant decrease in the placental tissue GSH levels (2.69±0.28) in comparison to control group (5.45±0.30). Treatment with ART showed significant increase in the placental tissue GSH levels (3.40±0.29) when compared to PE group.

Table (3)

Effects of ART on GSH (mmol/g tissue) in L-NAME induced PE in rats

Groups	GSH (mmol/g tissue)			Post hoc Tukey test	
	Mean ± SD	Min-Max	ANOVA (p value)	P1	P2
Control group	5.45±0.30	4.90-5.90	F=152.8 P≤0.001*	-	-
Preeclampsia group	2.69±0.28	2.10-3.10		≤0.001*	-
ART group	3.40±0.29	2.90-4.00		≤0.001*	≤0.001*

Data are presented as means (of 10 rats) ± SD. The three groups were compared with ANOVA test and in-between groups comparison was tested by post hoc Tukey test. P1: Comparison between Preeclampsia, ART groups and control groups. P2: Comparison between ART and Preeclampsia groups

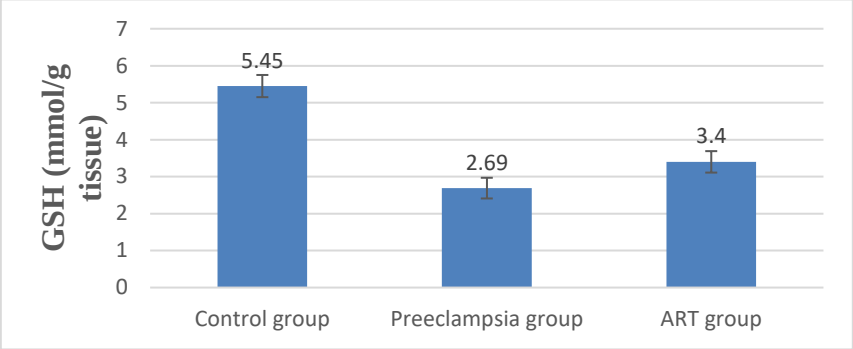

Figure (3): Effects of ART on GSH (mmol/g tissue) in L-NAME induced PE in rats

2) Placental tissue levels of malondialdehyde (MDA):

In table (4) &Figure (4), administration of L -NAME in PE group showed significant increase in the placental tissue MDA levels (5.27±0.22) in comparison to control group (2.41±0.28). Treatment with ART showed significant decrease in the placental MDA levels (4.81±0.19) when compared to PE group.

Table (4)

Effects of ART on MDA (nmol/g tissue) in L-NAME induced PE in rats

Groups	MDA (nmol/g tissue)		**Post hoc Tukey test**		
	Mean ± SD	**Min-Max**	**ANOVA (p value)**	**P1**	**P2**
Control group	2.41±0.28	1.80-2.90	F=262.7 P≤0.001*	-	-
Preeclampsia group	5.27±0.22	4.90-5.50		≤0.001*	-
ART group	4.81±0.19	4.40-5.10		≤0.001*	≤0.001*

Data are presented as means (of 10 rats) ± SD. The three groups were compared with ANOVA test and in-between groups comparison was tested by post hoc Tukey test. P1: Comparison between Preeclampsia, ART groups and control groups. P2: Comparison between ART and Preeclampsia groups

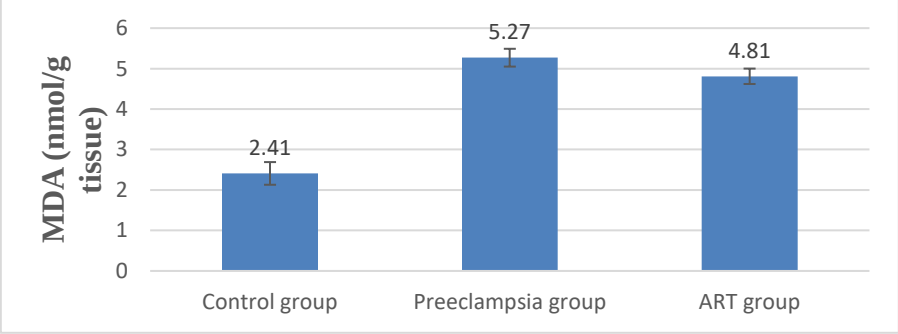

Figure (4): Effects of ART on MDA (nmol/g tissue) in L-NAME induced PE in rats

3) Placental tissue levels of inducible nitric oxide (iNO):

In table (5) &Figure (5), administration of L -NAME in PE group showed significant increase in the iNO (11.29±1.12) in comparison to control group (6.28±0.42). Treatment with ART showed significant decrease in the placental tissue iNO levels (9.35±0.85) when compared to PE group.

Table (5)

Effects of ART on iNO (µmol/g tissue) in L-NAME induced PE in rats

Groups	iNO (µmol/ g tissue)			Post hoc Tukey test	
	Mean ± SD	**Min-Max**	**ANOVA (p value)**	**P1**	**P2**
Control group	6.28±0.42	5.50-6.80	F=82.5 P≤0.001*	-	-
Preeclampsia group	11.29±1.12	9.80-13.10		≤0.001*	-
ART group	9.35±0.85	8.00-10.80		≤0.001*	≤0.001*

Data are presented as means (of 10 rats) ± SD. The three groups were compared with ANOVA test and in-between groups comparison was tested by post hoc Tukey test. P1: Comparison between Preeclampsia, ART groups and control groups. P2: Comparison between ART and Preeclampsia groups

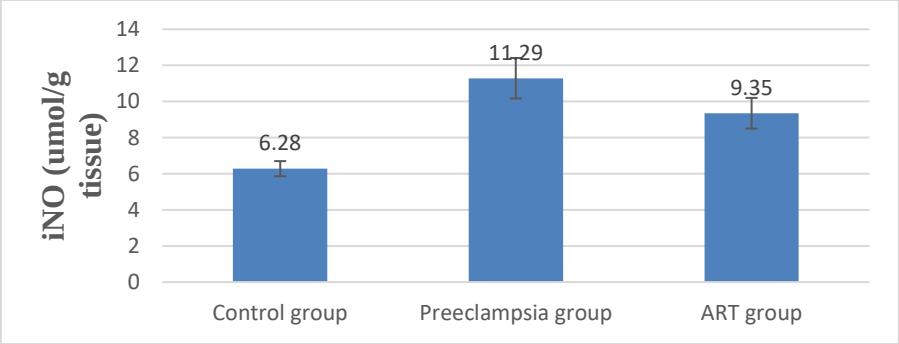

Figure (5): Effects of ART on iNO (nmol/g tissue) in L-NAME induced PE in rats

Light microscopic examination:

Examination of H&E stained sections of rat placenta of the control group showed that it is formed of tropospongium and labyrinth. Tropospongium is composed of 3 types of cells including glycogen cells with cytoplasm, spongiotrophoblasts with basophilic cytoplasm and giant trophoblast cells with basophilic cytoplasm in addition to maternal venous spaces (Figure 6A). The labyrinth is formed of interdigitating maternal blood sinusoids and fetal capillaries that are separated from each other by interhaemal membrane. This membrane is formed of cytotrophoblast cells and syncytiotrophoblast cells (Figure 6B).

In the PE group, tropospongium appeared thinner than in control group with areas of degenerating glycogen cells (Figure 7A). Labyrinth showed narrowing of maternal blood sinusoids with deposition of homogenous acidophilic material in their lumen (Figure 7B).

In placenta of the ART group, tropospongium showed regenerating glycogen cells. However, some are still degenerated (Figure 8A). Labyrinth showed apparently wider maternal blood sinusoids compared with PE group, however, some are still narrow and contained hyaline acidophilic material in their lumen (Figure 8B).

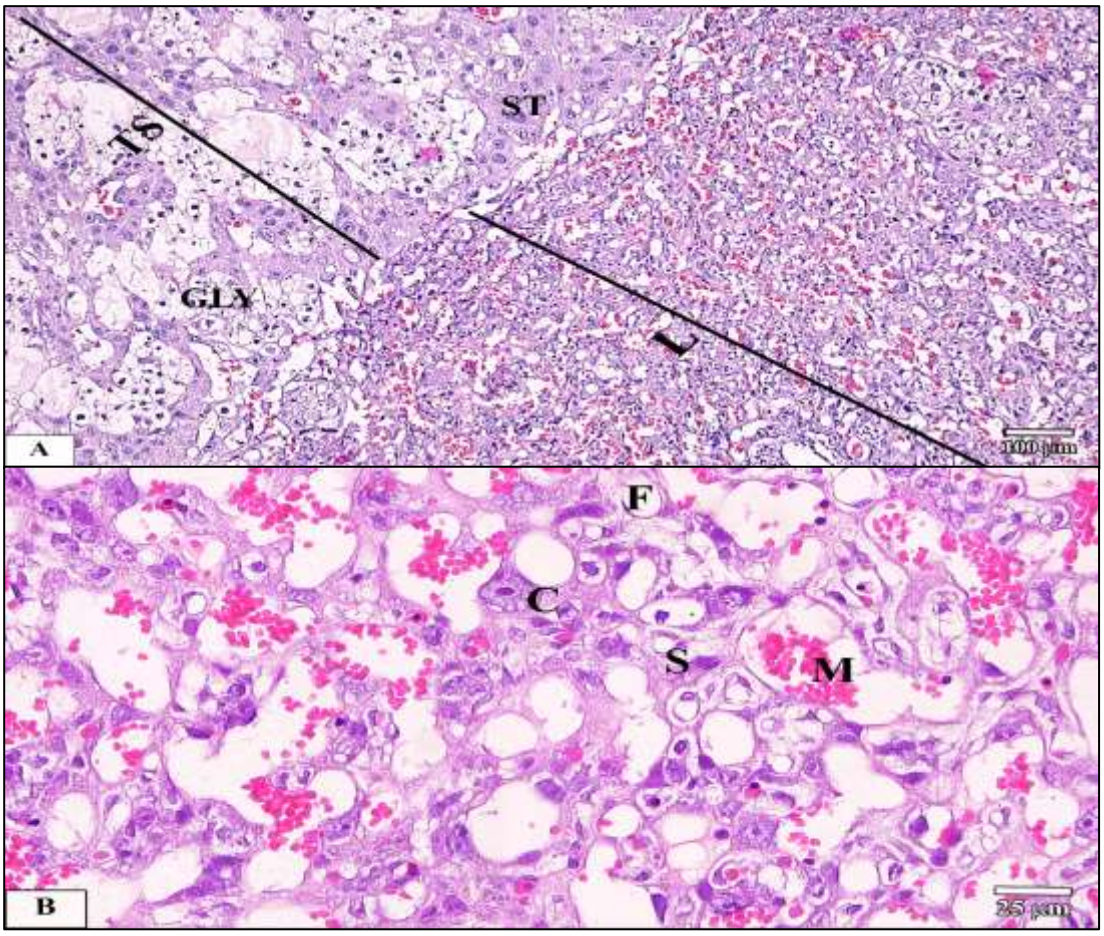

Fig. (6) The histological structure of the control rat placenta. (A) TS: tropospongium, GLY: glycogen cells, ST: spongiotrophoblasts, L: labyrinth. (B) M: maternal blood sinusoids, F: fetal capillaries, S:

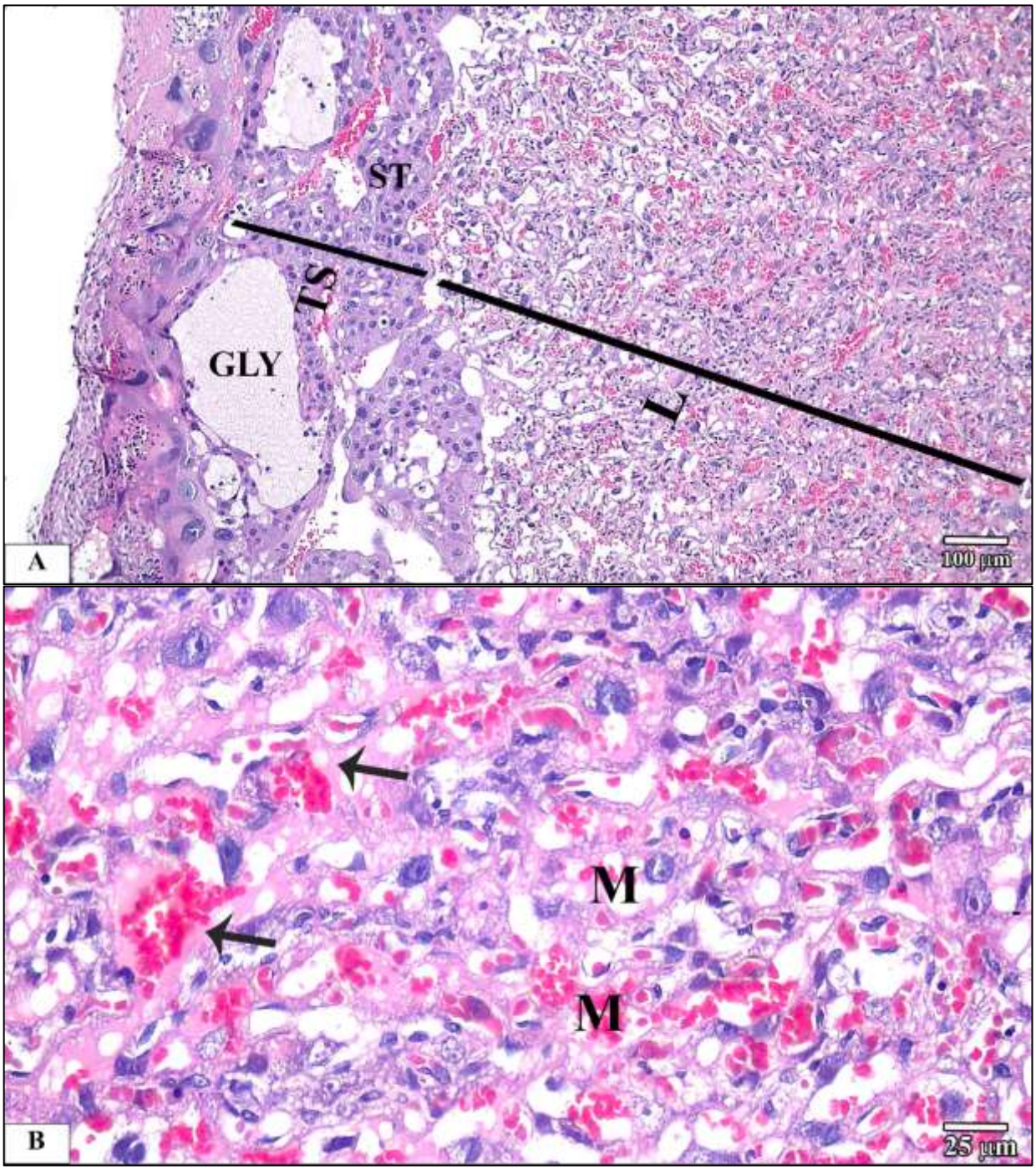

Fig. (7) The histological structure of the placenta of the PE group. (A) TS: tropospongium, GLY: glycogen cells, ST: spongiotrophoblasts, L: labyrinth. (B) M: maternal blood sinusoids, arrow: hyaline acidophilic material. H&E staining.

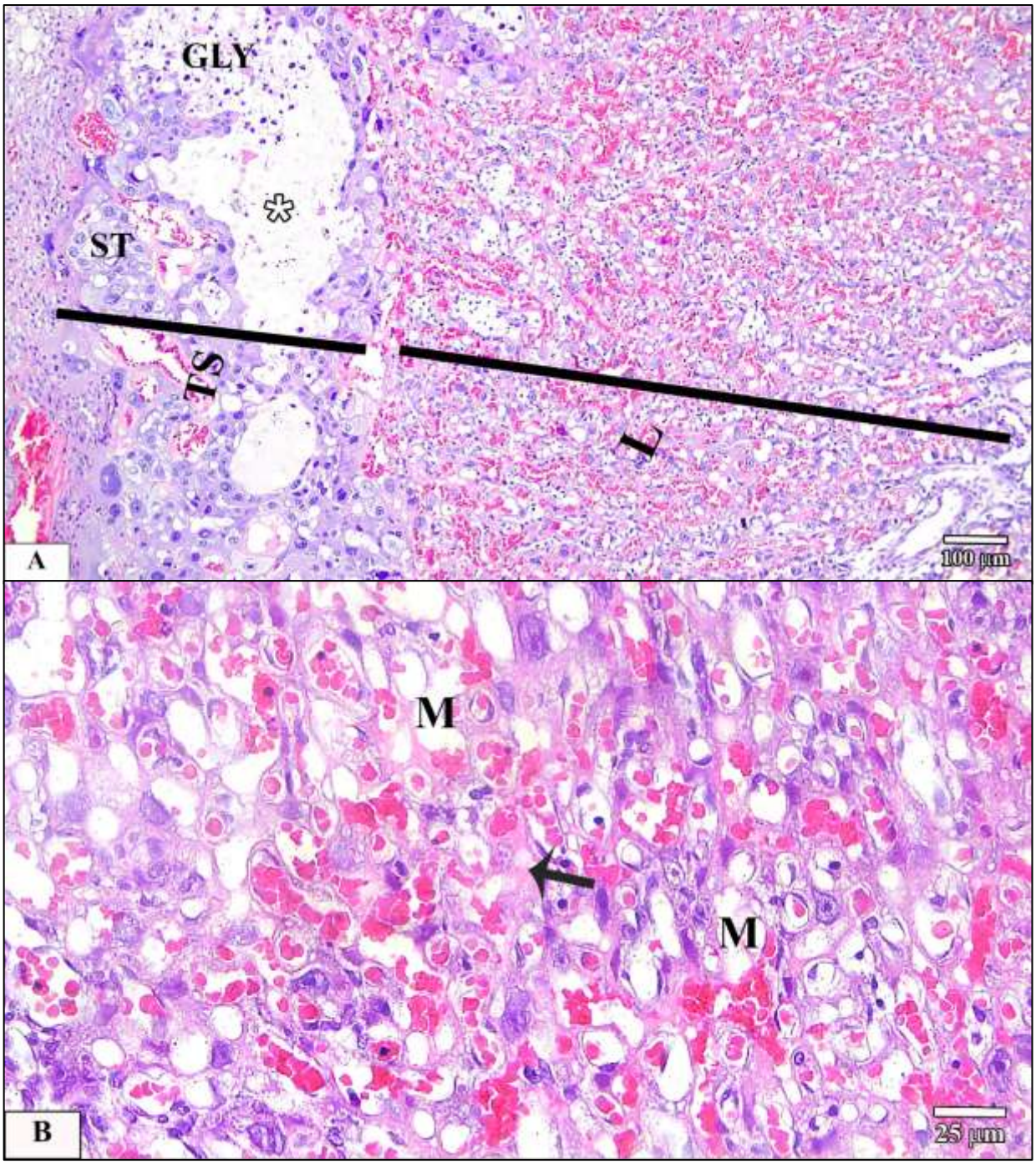

Fig. (8) The histological structure of the placenta of the ART group. (A) TS: tropospongium, GLY: glycogen cells, ST: spongiotrophoblasts, L: labyrinth. Asterix: degenerating glycogen cells. (B) M: maternal blood sinusoids, arrow: hyaline acidophilic material. H&E staining.

Electron microscopic examination:

Examination of ultrathin sections of placenta of control rats showed that maternal blood sinusoids were lined by syncytiotrophoblast with regular euchromatic nucleus. Cytoplasm contained rounded mitochondria and profiles of rER. Numerous microvilli were projecting from its surface into maternal blood sinusoids **(Fig. 9)**.

In the PE group, syncytiotrophoblast had irregular dark nucleus. Cytoplasm contained numerous vacuoles, dilated rER and dense small mitochondria. Cell membrane was disrupted with lost microvilli **(Fig. 10)**

Compared with PE placenta, placenta of ART treated group showed that syncytiotrophoblast had regular euchromatic nucleus. Cytoplasm contains rounded mitochondria, and rER similar to control placenta however, some dilated rER and few vacuoles were still seen. Cell membrane appeared intact with some projecting microvilli **(Fig. 11)**.

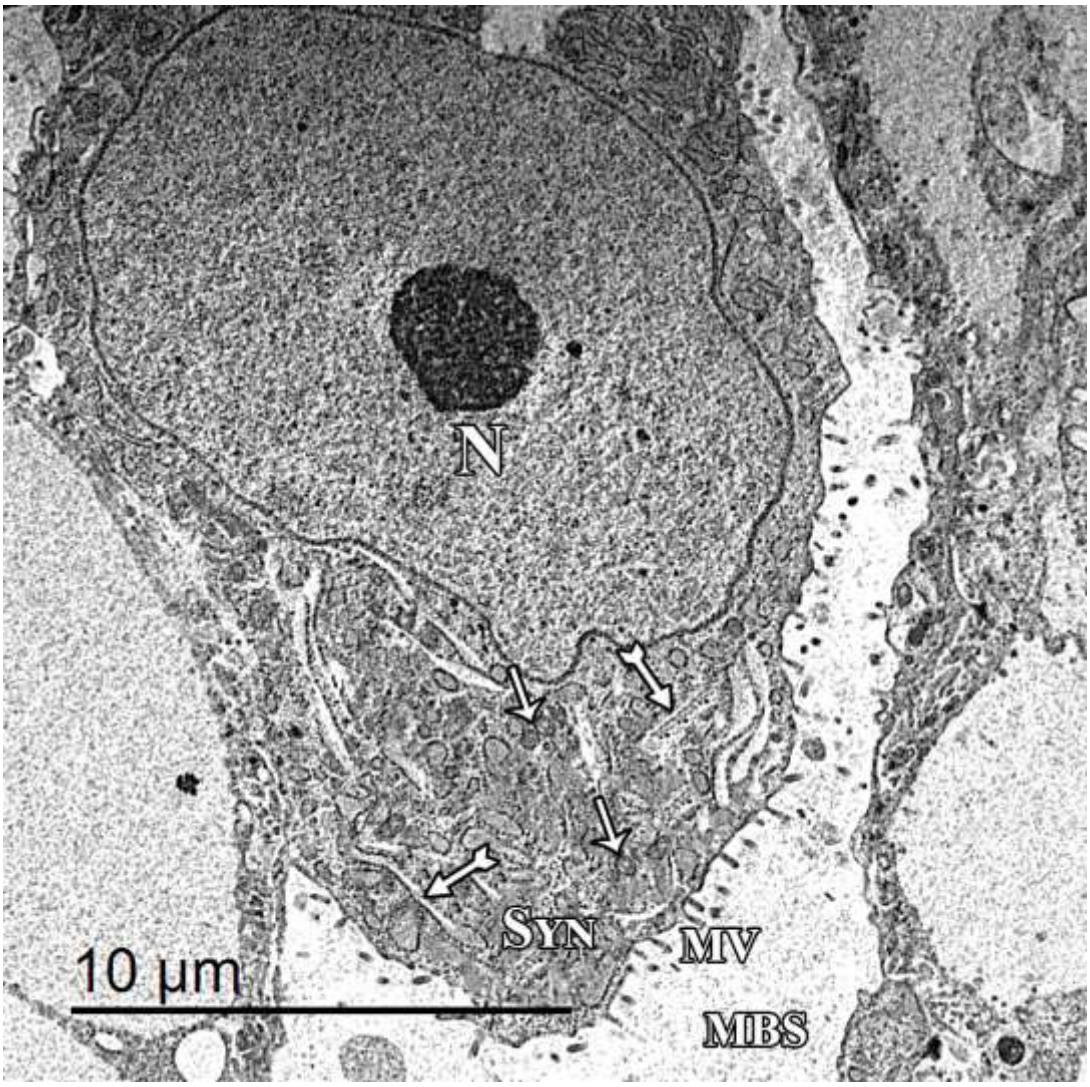

Fig. (9): A transmission electron photomicrograph of the placenta of the control group. SYN: syncytiotrophoblast, N: nucleus, MV: microvilli, MBS: maternal blood sinusoid, arrow: mitochondria, forked arrow: rough endoplasmic reticulum (rER).

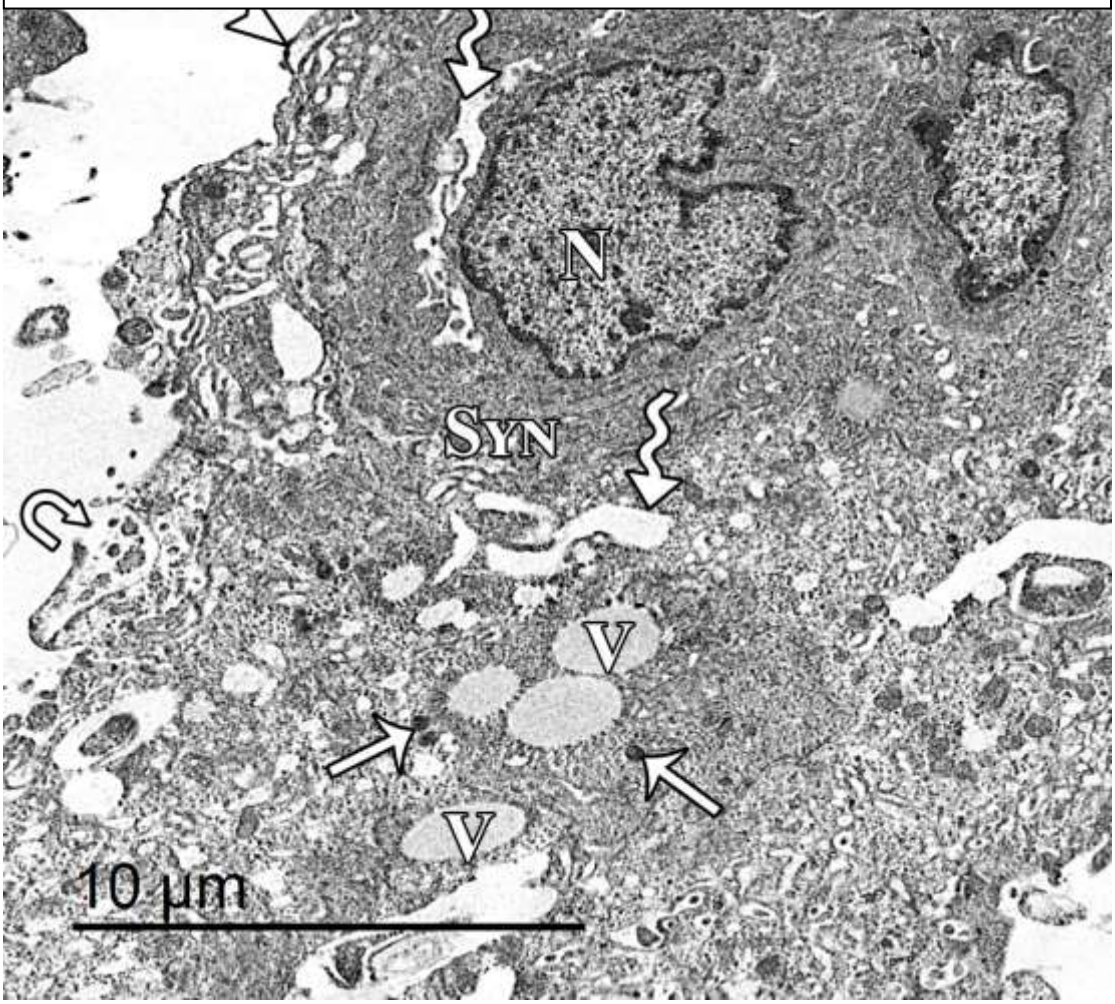

Fig. (10): A transmission electron photomicrograph of placenta of PE group. SYN: syncytiotrophoblast, N: nucleus, V: vacuole, arrowhead: absent microvilli, arrow: mitochondria, wavy arrow: dilated rER, curved arrow: disrupted cell membrane.

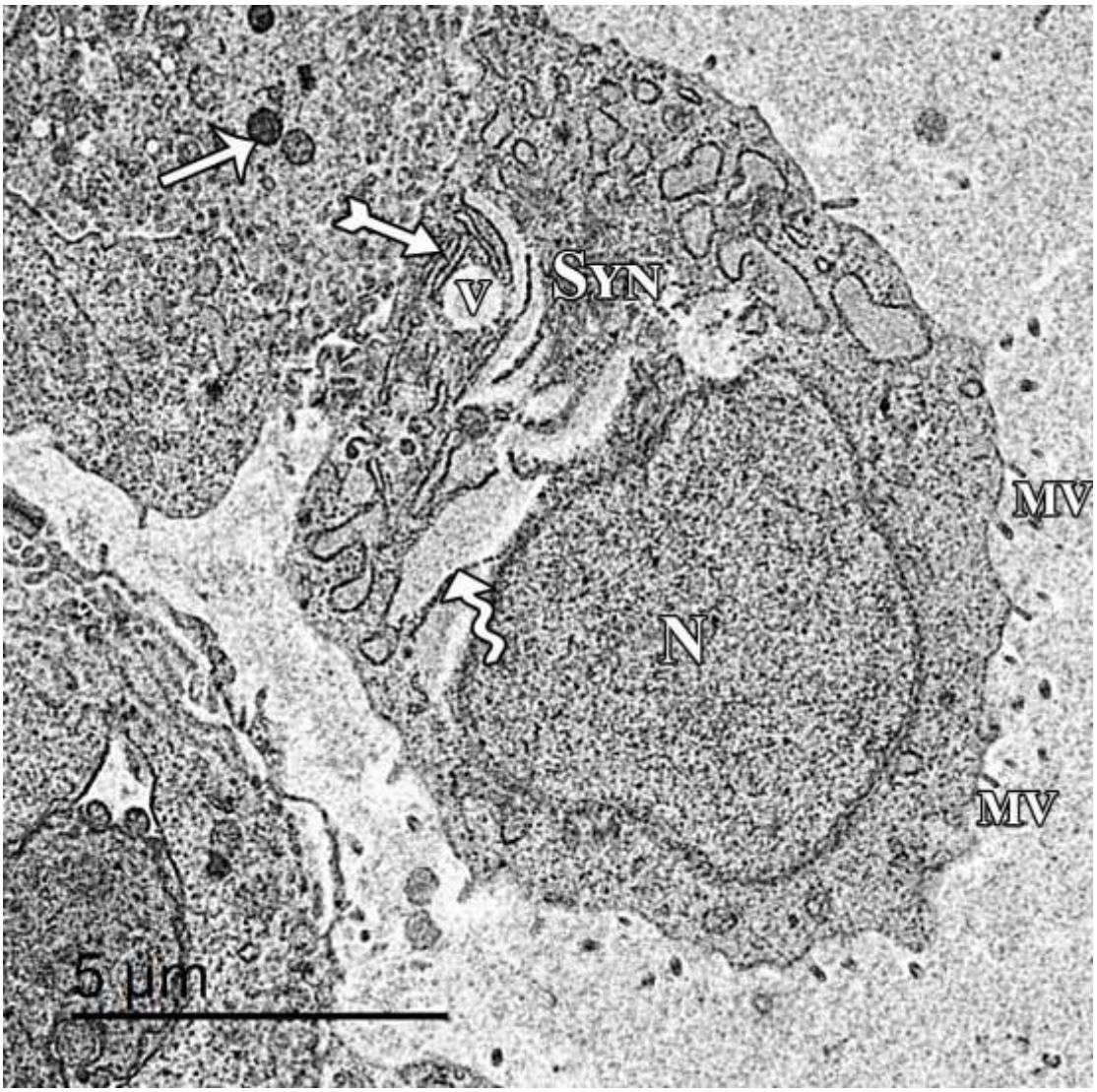

Fig. (11): A transmission electron photomicrograph of placenta of ART group. SYN: syncytiotrophoblast, N: nucleus, V: vacuole, MV: microvilli, arrow: mitochondria, wavy arrow: dilated rER, forked arrow: normal rER.

Discussion

Preeclampsia is a persistent hypertensive gestational disease marked by high blood pressure and proteinuria during the second or third trimester of gastation. It is considered as one of the most threatening complications of pregnancy. At the cellular level, PE has largely been associated with the release of free radicals by the placenta. Oxidative stress was considered as a major factor in the pathophysiology of PE (Aouache et al., 2018a).

In the current study, administration of L-NAME, a nitric oxide synthase (NOS) inhibitor, in rats showed significant increase in the MABP in PE group when compared to control group. These findings were in consistent with multiple previous studies by (Granger et al., 2001a, Gilbert et al., 2008, Matsubara et al., 2010a, Ma et al., 2011, Hariharan et al., 2017, FATHY and NADINE, 2018, Shu et al., 2018). This increase in the MABP in PE group could be explained by the potent competitive inhibitory effect of L-NAME on NOS reducing NO synthesis leading to vasoconstriction (Wei et al., 1999). This also leads to increase the adhesion molecules expression with subsequent acceleration of the inflammation in systemic vasculature and causing endothelial dysfunction with utero-placental perfusion failure (Matsubara et al., 2010b). Moreover, The reduction in NO synthesis increase in ET-1 as NO was known to be a potent inhibitor of ET-1 synthesis (Murphy et al., 2010). L-NAME decreases prostacyclin and increases thromboxane A2 with more vasoconstriction, oxidative stress and hypertension (Granger et al., 2001b).

In the present study, ART treatment from GD 7 till end of gestation period showed non significance decrease of blood pressure when compared to PE diseased group. The choice of ART in this model of hypertension was based on its anti-inflammatory and antioxidant properties. In addition, ART is a scavenger of reactive oxygen species and oxidative stress which play a role in the etiology of hypertension. This was in agreement with earlier research by (Touyz, 2004).

The primary substrate for vascular NO production is L-arginine. The level of L-arginine in eight Artemisia subgenus plants was discovered to be 5 to 10 times higher in arginine (Gokce, 2004, Brisibe et al., 2009). Plants of the Artemisia genus are high in potassium and low in sodium. Potassium is a crucial blood pressure regulator. Increased potassium causes endothelial cells to enlarge and changes their stiffness (Fenardji et al., 1974, Krishna, 1990).

There is significant increase in 24h urinary protein in PE group when compared to normal control group. This could be attributed to the vascular endothelial dysfunction that leads to change in renal hemodynamics decreasing renal excretory function, leading to hypertension and proteinuria. In addition, L-NAME caused glomerulosclerosis, tubulo-interstitial fibrosis, and tubular atrophy associated with a deteriorated glomerular filtration rate and increased urinary protein excretion. This was in agreement with previous researches (Ikeda et al., 2009, Polichnowski et al., 2011).

In the current study, ART showed a significant decrease in proteinuria in comparison to PE group. This result coincides with previous studies by (Hou et al., 2011, Hou et al., 2012, Li et al., 2015, Xia et al., 2020). ART was proved to attenuate podocyte effacement and fusion via nephrin and podocin regulation and decrease the shedding of podocyte so, reduce glomerular permeability and improve proteinuria (Wu et al., 2014, Xia et al., 2020). ART also reduced level circulating antibodies, as well as by the reduced immune complex deposition, reversed podocyte injuries, and attenuated tubulointerstitial fibrosis in the kidneys (Li et al., 2015).

In the current study, L-NAME administration resulted in increased placental tissue oxidative damage represented by increased MDA, increased iNOS, and reduced activities of the protective antioxidant GSH in placental tissues of PE group in comparison to control group. This in accordance with the results of the (Orhan et al., 2003, Aouache et al., 2018a, Sljivancanin Jakovljevic et al., 2019).

Placental MDA is often used in as a marker of oxidative stress and is generated as a byproduct of the peroxidation and breakdown of polyunsaturated fatty acids in cells as result of placental hypoxia and necrosis (Uchida et al., 1999).

Placental glutathione plays a role in both the direct repair of oxidative DNA damage and the prevention of apoptosis caused by ROS or cytokines. It also scavenges a variety of ROS, including O2–•, OH•, peroxyl nitrite, and lipid hydroperoxides (Schoots et al., 2018).

ART treated group showed significant increase in GSH level and significant decrease in placental MDA and placental tissues iNOS levels in comparison to PE group. This was also reported by (Vega-Rodríguez et al., 2015, Ittarat et al., 2003, Wang et al., 2021). ART can elicit broad-spectrum inhibitory effect on iNOS reducing the production of the inflammatory cytokine. This was in accordance with (Li et al., 2013).

The antioxidant effect of ART was previously reported in AKI mice. It could ameliorate oxidative stress by restoring malonyl dialdehyde (MDA), nitric oxide (NO), glutathione peroxidase (GSH), catalase (CAT), and superoxide dismutase (SOD) activity in the kidney (An et al., 2017, Liu et al., 2019). ART was found to enhance NADPH oxidation which catalyze the production of GSH in rat liver, kidney, and intestinal cytosols, as well as its affinity purified glutathione transferases; GSTs (Mukanganyama et al., 2001).

Histopathological examination of placenta of PE group stained with H&E showed alteration in its histological structure compared with control group represented by thinning and degeneration of glycogen cells of tropospongium together with narrowing of maternal blood sinusoids and deposition of hyaline material in their lumen. This could be attributed to the vasoconstriction caused by inhibition of NOS leading to decrease NO production. This caused decreased placental perfusion that lead to fetoplacental ischemia. Necrotic placental tissues may be the source of the hyaline material appeared in maternal sinusoids. This was also reported by (Furukawa et al., 2011, FATHY and NADINE, 2018, Mohammed et al., 2018, Shu et al., 2018, Wilson et al., 2020). The necrotic placental micro-fragments present in the maternal circulation is thought to exacerbate oxidative stress markers which results in endothelial dysfunction and increase blood pressure which further exacerbates PE and the systemic inflammatory response (Sedeek et al., 2008, Negi et al., 2014).

Ultrastructural study of placenta of control rats showed that trophoblastic cells contained some mitochondria and profiles of rER with numerous microvilli projecting from its surface into maternal blood sinusoids. These microvilli play an important role in the fetal-maternal exchange. Similar findings were previously described by Selim et al. (2013)

Morphological changes were observed in placenta of PE group. Trophoblastic cells contained numerous vacuoles, dilated rER and dense small mitochondria. Oxidative phosphorylation is a highly efficient process that occurs in mitochondria through which cells use enzymes to oxidize nutrients, thereby releasing energy which is used to form ATP. Oxidative stress increased activity of placental mitochondria, enhancing oxidative phosphorylation. This results in overproduction of ROS that leads to a biological molecular damage of trophoblastic cells. This was also reported by (Vangrieken et al., 2020, Abdel Salam et al., 2015, Belkacemi et al., 2007).

There was loss of integrity of the trophoblastic cell membrane attributed to increase levels of 4-hydroxylnonenal (marker of lipid peroxidation) and nitro tyrosine in the syncytiotrophoblast layer because of oxidative stress which led to interaction of reactive oxygen species with polyunsaturated fatty acids of membrane lipids. This resulted in the production of aldehydes such as malonaldehyde; MDA (Esterbauer et al., 1991).

These trophoblastic cytoplasmic changes are in accordance with (Belkacemi et al., 2007, Abdel Salam et al., 2015) who related these changes to hypoxia-reoxygenation injury leading to apoptosis. The cytoplasmic vacuoles observed in the cytoplasm of syncytiotrophoblast can be explained by reduced activity of ATPase due to

mitochondrial injury, which results in failure of the sodium pump mechanism responsible for water and electrolyte control; as a result, cells accumulate water, which causes vacuolar degeneration.

The present study is the first study to declare the effect of ART in structure of placenta of PE rats. In this study, histological structure in ART treated group showed improvement in the structural changes in trophoblastic cells. Cytoplasm contained normal mitochondria, and rER, however, some dilated rER and few vacuoles were still seen. These results were in consistence with previous studies (Muehlenbachs et al., 2012, Marder et al., 2016).

This agreed with previous studies (Selim et al., 2013, Bodnar et al., 2014, Megahed and Abd Al-Aleem, 2015, Hutabarat et al., 2018) which describing the protective role of ART in PE and underling mechanisms alleviating disease.

Conclusion: We found that the administration of L-NAME during the pregnancy created a preeclamptic like syndrome. ART treated rats showed improvement in oxidative stress parameters and proteinuria when compared to their respective preeclamptic group, however, no marked improvement was observed on the blood pressure of rats. Partial structural improvement as a regard of histological examination by L.M. and ultrastructure study by E.M which is better than PE group but less than control group.

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