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Mitochondrial HSP70 Attenuates ATPase Activity In Pre-eclamptic Trophoblast Mitochondria With VER-155008 Treatments.

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ABSTRACT

Preeclampsia is a hypertensive disorder associated with deficient trophoblast invasion. During hypoxia, trophoblast fails to replace the endothelial cells. Molecular chaperones of HSP70 protect cells from stress. VER-155008 is an inhibitor of HSP70 and act as an ATP competitive inhibitor for ATPase activity. These are used for various applications in particular to emphasis the importance of HSP70. Hence the present study aims to analyze the effect of mitochondrial HSP70 in normotensive and preeclamptic trophoblast mitochondria with and without incubation of VER-155008. The stress status was assessed by the level of lipid peroxides (LPO), nitrite (NO_2^-), catalase (CAT), total antioxidant capacity (TAC) and ATPase. A significant increase in the level of LPO and NO_2^- along with decrease in the level of CAT, TAC and ATPase in preeclamptic trophoblast mitochondria was observed, whereas VER-155008 incubation moderately alters the stress and antioxidant status. The level of HSP70 was inhibited by 31% at 10 µM of VER-155008 for 3 hours. An alteration in the expression of HSP70 by VER-155008 causes redox imbalance and attenuates the ATPase activity. These changes in the preeclamptic condition indicate that HSP70 may be one of the key factors to maintain cell homeostasis under stress condition.

Keywords: Antioxidant, HSP70, Oxidative stress, Preeclampsia, VER-155008

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INTRODUCTION

Preeclampsia is a life-threatening disease that occurs exclusively in pregnant women during 20th week of gestation.[1] The symptoms are hypertension (140/90 mmHg) and proteinuria (>300mg/day), leading to maternal and perinatal morbidity and mortality, complicating at least 9-11% of pregnancies.[2] It is characterized by defensive placentation, impaired trophoblast invasion, endothelial cell dysfunction and increased vasoconstriction which may lead to the premature birth, low birth weight babies and fetal growth restriction.[3] The human placenta plays a major role in embryonic development, particularly in regulating the transport of ions, nutrients and immunoglobulin's from the maternal system to the fetal circulation. Trophoblasts are specialized cells of the placenta that play an important role in embryo implantation and interaction with the decasualized maternal uterus.[4] The trophoblast replaces the endothelial cells in the uterine spiral arteries and remodels the uterine vessels for maternal vasoconstriction. During preeclampsia impairment of trophoblast invasion occurs which in turn leads to altered uteroplacental blood flow from maternal to the fetal circulation and finally results in failure of vascular invasion.[5]

Mitochondria are ''energy mediator" of cells and this may be responsible for the consumption of oxygen and ATP generation. In mitochondria 2–3% of free electrons may leak out from the mitochondrial respiratory enzymes, to form the reactive oxygen species.[6] These are involved in major tasks such as signalling, cellular differentiation, cell cycle and cell growth.[7] They also play a vital role in the sustainment of pregnancy and neonatal maturation through the regulation of metabolic activity and ATP production.[8] Production of high energy protein molecules such as ATP is generated by mitochondrial respiratory chain which consists of four respiratory complexes (complex I-IV) to catalyze the electron transfer via the oxidative phosphorylation. In normal condition, generation of free radicals from mitochondria would be quenched by antioxidants. During stress condition free radicals will be overwhelmed and this inturn leads to antioxidant insufficiency, enormous production of ADP molecules, reduced output of ATP molecules, which can promote the free radicals from the mitochondria and it may subsequently affect the trophoblast cell, then its leads to the dysfunction of trophoblastic cells in the placenta.[9] Pregnancy itself is a physiological condition in which women are more prone to oxidative stress, which results in an imbalance between the prooxidants-antioxidant levels.[10] However the oxidative status of placental trophoblast mitochondria are not understood clearly, which is the major intracellular organelle responsible for energy production and free radicals generation.

Oxidative stress is a state of hypoxia/ischemia which may promote the recurrent pregnancy loss due to existence of unpaired electron which may attack the phospholipids of cell membranes and react with polyunsaturated fatty acids to form lipid peroxides resulting in cellular damage.[11] Conjugated diene, lipid peroxide, lipid hydroperoxides and hydroxynonenal are predominant markers of lipid peroxidation which further cause the oxidative damage of cells and tissues. The overproduction of free radicals is involved in various pathological cellular processes. It has been reported that the interaction of nitric oxide (NO) with polyphenolic antioxidant is highly relevant in physiological and pathological cellular mechanisms.[12] NO mediated nitrative stress plays an important role in the pathogenesis of preeclampsia. NO can enhance reactive oxygen species (ROS) toxicity due to its rapid reaction to form peroxynitrite ($ONOO^-$). Peroxynitrite is a potentially harmful ROS as it causes nitrosylation of tyrosine residues leading to changes in protein conformation and its inactivation. The resultant placental oxidative state may contribute to the elevation of maternal blood pressure, proteinuria, platelet dysfunction and the level of thromboxane, prostacyclin in preeclampsia.[13]

Organisms are challenged by a multitude of stresses throughout their lifespan from both internal and external environments which cause irreversible damage to proteins that in turn could impair cellular processes.[14] To ameliorate these stresses, living system has evolved a variety of strategies to repair the damage and/or eliminate the damaged components. Heat shock proteins (HSPs), a class of molecular chaperones are upregulated in oxidatively damaged cells which play a major role to sustain the cell in the hypoxic condition and for the maintenance of protein homeostasis.[15,16] The expression of heat shock proteins concise the constitutive and inducible form. The constitutive form of HSPs is exposed to the unstressed conditions which regulate protein folding, translocation, assembly and disassembly of protein oligomers, degradation of aged proteins. Inducible form of HSPs plays an important role in defense mechanisms against oxidative stress, thereby preventing stress induced protein aggregation and restoring normal protein folding for the maintenance of cellular homeostasis.[17] HSPs are classified broadly into six

major families namely HSP40 (J-proteins), HSP60 (chaperonins), HSP70, HSP90, HSP100 (Clp proteins) and small heat shock proteins HSP32 (HO-1).

HSP70 is a stress-inducible protein that plays an important role in defense mechanisms against agents that promote oxidative injury, thereby preventing stress induced protein aggregation and restoring normal protein folding.[18] HSP70 can function alone to inhibit apoptosis while cooperative interaction with their designated co-chaperone molecules is likely to enhance their antiapoptotic activities.[19] In contrast, inhibition of HSP70 function using the selective HSP70 inhibitor VER-155008, an inhibitor that targets the ATPase-binding domain of HSP70, prevented HSP70-mediated improvements in ATP levels in oxidative stress.[20] HSP70 contains two domains namely N-terminal ATPase domain and C-terminal substrate binding domain. The N-terminal ATPase domain of HSP70 can facilitates the release of client protein degradation and ATP hydrolysis. C-terminal peptide binding domain is responsible for the release of substrates which requires the binding of ATP to HSP70.[21] VER-155008 is an ATP/adenosine analogue which binds to the nucleotide binding site of HSP70 arrests the nucleotide binding domain (NBD) in a half-open conformation, which does not allow the complete closure to the central cleft of NBD. VER-155008 acts as an ATP competitive inhibitor for ATPase followed by the inhibition of chaperon activity.[22, 23] **(Figure 1)** In this context, the present study aims to investigate the cytoprotective role of mitochondrial HSP70 by using VER-155008 in mitochondria of preeclamptic placental trophoblast.

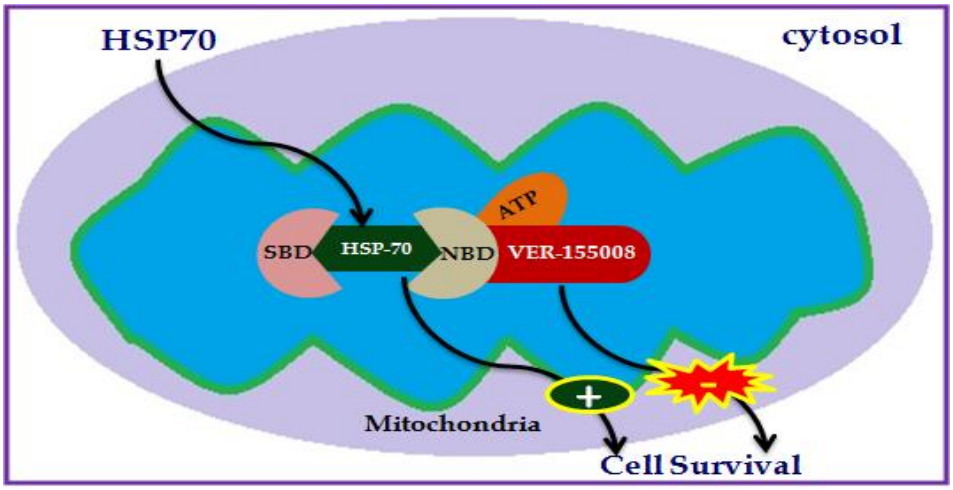

Figure 1: Schematic diagram illustrates the role of HSP70. HSP70 are highly expressed in the stress conditions for maintaining cell homeostasis. HSP70 contains two domains namely nucleotide binding domain (NBD) and substrate binding domain (SBD). VER155008 binds to NBD of HSP70 and it act as an ATP competitive inhibitor which inhibits the chaperon activity.

MATERIALS AND METHODS

Selection of subjects

Patient registered in a public sector hospital in Chennai were enrolled in this study. Clearance was obtained from Institute Ethical Committee (IEC/S/BWC/609/2014) prior to the commencement of study and the informed consent was received from all the subjects. Placenta was collected from both normal (n=17) and preeclamptic (n=17) pregnant women in the age group of 20-40 years, post-delivery. Patients with preeclampsia were defined on the basis of the following laboratory criteria: blood pressure >140/90 mmHg but <160/110 mmHg, proteinuria >300 mg/L and xanthine oxidase activity of approximately 2.6 units/ mg protein.[24] Patients with severe preeclampsia and other severe maternal complications were excluded from the study.

Isolation of placental Trophoblast

Third-trimester villous trophoblast cells, which were used for comparison, were isolated from term placentas by the method of Douglas and King.[25] Human term placenta from the preeclampsia and

normotensive subjects were obtained immediately after delivery in accordance with the established guidelines of the institutional ethical committee along with the informed consent of the patient. Briefly, placental villi were cut and thoroughly washed to remove blood. Thereafter, they were incubated four times in a enzyme digestion medium composed of HBSS, containing trypsin and deoxyribonuclease for 30 min at 37ºC in a water bath with continuous shaking. The dispersed cells were layered on top of a discontinuous 5–70% Percoll gradient, and centrifuged for 25 min at 507 Xg. The intermediate layers (density between 1.048 & 1.062) containing cytotrophoblast cells were removed and washed and following trophoblast isolation, cells were seeded at a density of approximately 1.6×106 cells per plate in a 5 mL cell culture plate. The complete culture medium, constituted of M199, 2mm glutamine & 10% FBS. All the experiments were performed within a day of trophoblast isolation in-order to overrule the influence of cultivation process.

Cell viability by MTT assay

The viability of placental trophoblast cells was determined using the MTT assay (Mosmann [26]). The trophoblast cells were resuspended in PBS buffer and serially diluted to a concentration of 200 mg of protein per mL of suspension using the same buffer system. One hundred mL of the dilutions were plated out into the wells of a microtiter plate in duplicate. In the control wells, PBS alone was incubated to provide the blank for absorbance readings. Ten mL of MTT reagent was added to each well, including controls. After incubation for 1 h, the visualized purple precipitate (formazan product) was solubilised with 1 mL acidic isopropanol. The plates were covered and left in the dark for 4 h at room temperature. Absorbance was measured at 560 nm. The relative viability was calculated by dividing the optical density of sample by the optical density of control well having the PBS buffer solution with MTT reagent, and multiplying by 100.

Isolation of trophoblastic mitochondria

The mitochondria were isolated according to procedure given by Zhang et al.[27]. Isolated trophoblast cells were resuspended in ice-cold RSB buffer (10 mmol/L NaCl, 1.5 mmol/L Mgcl2, and 10 mmol/L Tris-HCL, pH 7.5) to swell for 5 to 10 minutes followed by homogenization. Cell lysates were added with 2.5X MS buffer (525 mmol/L mannitol, 175 mmol/L sucrose, 12.5 mmol/L Tris-HCl, pH 7.5, and 2.5 mmol/L ethylenediaminetetraacetic acid, pH 7.5) to a final concentration of 1X MS followed by centrifugation at 1300 Xg for 5 minutes to remove nuclei, unbroken cells and large membrane fragments. The supernatant was centrifuged at 17,000 Xg for 15 mins followed by 2X of washing with 1X MS buffer. The resultant pellet containing the mitochondrial fraction was suspended in 0.25M sucrose solution (pH 7.4) and homogenized for one minute, which was then used for further studies.

Marker enzymes (succinate dehydrogenase)

Succinate dehydrogenase activity was assayed by the method of Green and Narahara.[28] The specific activity was expressed as the rate of change in OD/min/mg protein of mitochondria.

Estimation of protein

The protein concentration was estimated by the method of Bradford [29] the use of bovine serum albumin as the standard. The result was expressed as mg protein/g placental trophoblast. The lysate was used for the estimation of the following parameters.

VER-155008 incubation

VER-155008 solution was prepared by dissolving VER-155008 (Sigma- Aldrich) in water. Then 200 µL of VER-155008 solution (10µM, 20µM and 30µM) was added to the respective sample tubes (containing 100µg of protein / 200µL of sample (both normal and preeclampsia) and the tubes were incubated for 180mts, 240mts and 300mts at 37 ºC. Following the incubation, cell viability was assessed in all the tubes and the effective concentration at the time having effective efficiency was utilized for the quantification of oxidant-antioxidants.

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Estimation of lipid peroxide

Lipid peroxide analysis was determined by the method of Ohkawa et al. [30]. The lipid peroxide content was expressed as nanomoles of MDA/ mg of protein.

Estimation of nitrite

Nitrite was estimated by the method of Yokoi et al. [31]. The nitrite content was expressed as nanomoles of nitrite/ mg of protein.

Assay of catalase

Catalase activity was measured by the method of Beer et al. [32]. The activity of the enzyme was expressed as units of CAT/min/mg protein.

Estimation of total antioxidant capacity

Total antioxidant capacity (TAC) analysis was performed by the method of Prieto et al. [33]. The total antioxidant activity was expressed as Trolox equivalent in mmol/ L.

Measurement of ATPase

The ATPase activity was determined by Anderson and Murphy method.[34] The activity of ATPase enzymes was expressed as units/min/mg of protein.

Statistical analysis

Data were analyzed using statistical software package version 7.0. Student's t-test was used to ascertain the significance of variations between normotensive and preeclamptic trophoblast mitochondria. All data were presented as mean ± SD of 17 samples. Differences were considered significant at $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS

Cell viability

The viability of placental trophoblast was assessed by MTT assay which represents the % difference. Viability of preeclamptic trophoblast was decreased by 19% ($p<0.05$) when compared to normotensive trophoblast (**Figure 2**).

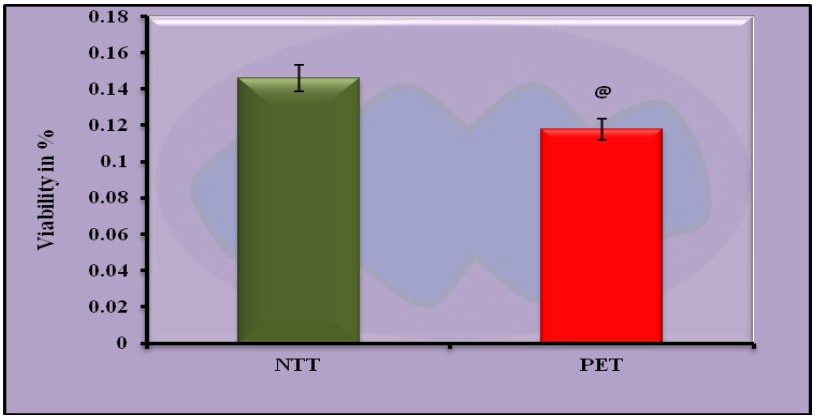

Figure 2: Cell viability of normotensive and preeclamptic placenta. Values are expressed as mean ± SD (n=17)

Where, NTT-Normotensive Trophoblastic; PTT-Preeclamptic Trophoblast
[@]p<0.05 When compared with normotensive placental trophoblast

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Mitochondrial marker enzymes (Succinate dehydrogenase)

The level of succinate dehydrogenase was significantly decreased by 45% ($p<0.001$) in preeclamptic trophoblast mitochondria when compared to normotensive trophoblast mitochondria (**Figure 3**).

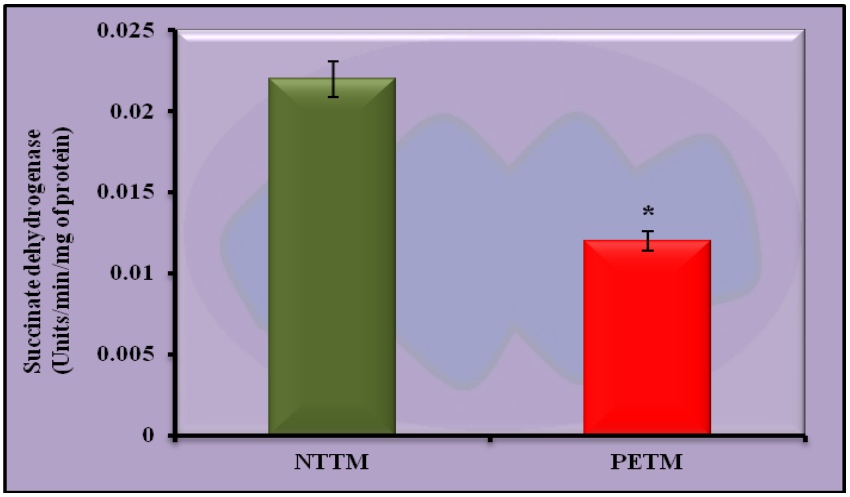

Figure 3: Mitochondrial marker enzymes in normotensive and preeclamptic placental trophoblast mitochondria. Values are expressed as mean ± SD (n=17)

Where, NTTM-Normotensive Trophoblastic Mitochondria; PTTM-Preeclamptic Trophoblast Mitochondria
*p<0.001 when compared with normotensive trophoblast mitochondria

Effect of VER-155008 on cell viability

The standardization of trophoblast mitochondria incubated with VER-155008 in various concentrations 10µM, 20µM and 30µM at different time intervals (180mts, 240mts and 300mts) for both groups was observed in **Figure 4**. The terminal reaction of these period indicated that 10µM of VER-155008 incubated for 180mts (3 hours) was more effective to study the changes of placental trophoblast mitochondria.

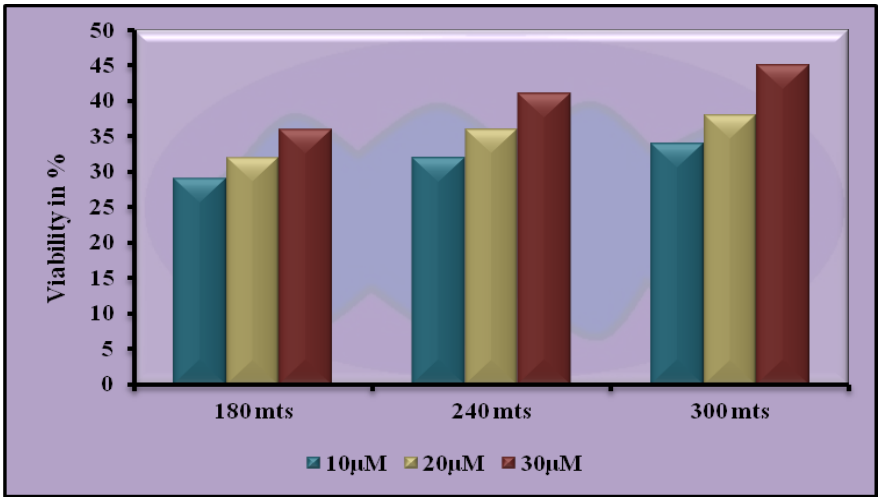

Figure 4: Effect of VER-155008 on cell viability at different time with varying concentrations in mitochondria of normotensive and preeclamptic placental trophoblast. Values are expressed as mean ± SD (n=17)

Figure 4 represents the % difference of placental trophoblast viability in various concentrations of VER-155008 (10µM, 20µM and 30µM) at different time intervals (180mts, 240mts and 300mts). Standardization of VER-155008 concentration and incubation period in our lab indicated that 10µM of VER-155008 incubated for 180mts was more effective to study the placental changes.

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Effect of VER-155008 on LPO

Figure 5 described the level of LPO in mitochondria of normotensive and preeclamptic placental trophoblast with and without incubation of VER-155008. The level of LPO was significantly increased by 42% in mitochondria of preeclamptic placental trophoblast than normotensive group without incubation of VER-155008. After incubation with VER-155008, the level of LPO was significantly increased by 59% in mitochondria of preeclamptic placental trophoblast than normotensive group.

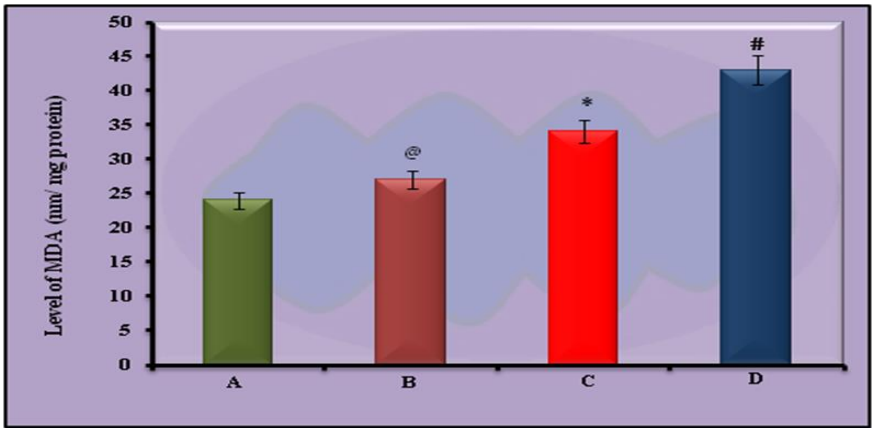

Figure 5: Effect of VER-155008 on LPO in mitochondria of normotensive and preeclamptic placental trophoblast. Values are expressed as mean ± SD (n=17)

Where, A- Normotensive trophoblast mitochondria; B- Normotensive trophoblast mitochondria with VER-155008 incubation; C- Preeclamptic trophoblast mitochondria; D- Preeclamptic trophoblast mitochondria with VER-155008 incubation.

@p<0.05 when compared with normotensive trophoblast mitochondria without VER-155008 incubation

*p<0.001 when compared with normotensive trophoblast mitochondria without VER-155008 incubation

#p<0.01 when compared with preeclamptic trophoblast mitochondria without VER-155008 incubation

Effect of VER-155008 on NO_2^-

The level of NO_2^- in mitochondria of normotensive and preeclamptic placental trophoblast with and without incubation of VER-155008 was found in **Figure 6**. The level of NO_2^- was significantly increased by 43% in mitochondria of preeclamptic placental trophoblast than normotensive group without incubation of VER-155008. After incubation with VER-155008, the level of NO_2^- was significantly increased by 58% in mitochondria of preeclamptic placental trophoblast than normotensive trophoblast mitochondria.

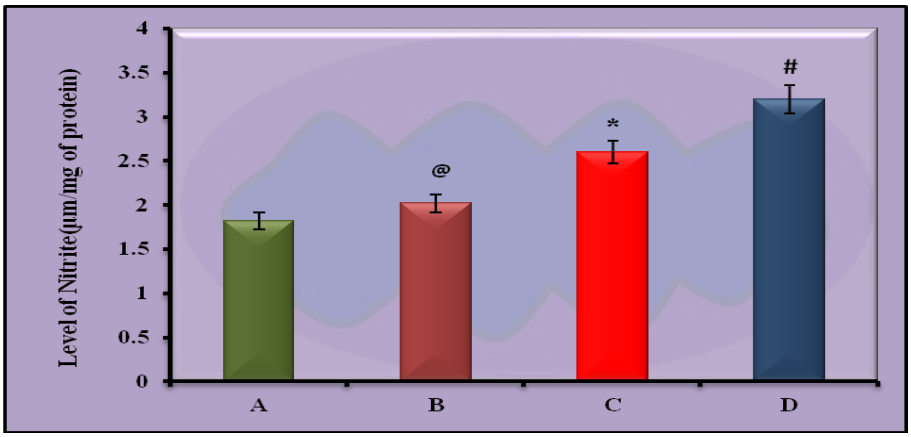

Figure 6: Effect of VER-155008 on NO_2^- in mitochondria of normotensive and preeclamptic placental trophoblast. Values are expressed as mean ± SD (n=17)

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Where, A- Normotensive trophoblast mitochondria; B- Normotensive trophoblast mitochondria with VER-155008 incubation; C- Preeclamptic trophoblast mitochondria; D- Preeclamptic trophoblast mitochondria with VER-155008 incubation.
Values are represents mean ± SD (for 20 samples in each group)
[@]p<0.05 when compared with normotensive trophoblast mitochondria without VER-155008 incubation
*p<0.001 when compared with normotensive trophoblast mitochondria without VER-155008 incubation
[#]p<0.01 when compared with preeclamptic trophoblast mitochondria without VER-155008 incubation

Effect of VER-155008 on CAT

The level of CAT in mitochondria of normotensive and preeclamptic placental trophoblast with and without incubation of VER-155008 was mentioned in **Figure 7**. The level of CAT was significantly decreased by 27% in mitochondria of preeclamptic placental trophoblast than normotensive group without incubation of VER-155008. After incubation with VER-155008, the level of CAT was significantly decreased by 36% in mitochondria of preeclamptic placental trophoblast than normotensive trophoblast mitochondria.

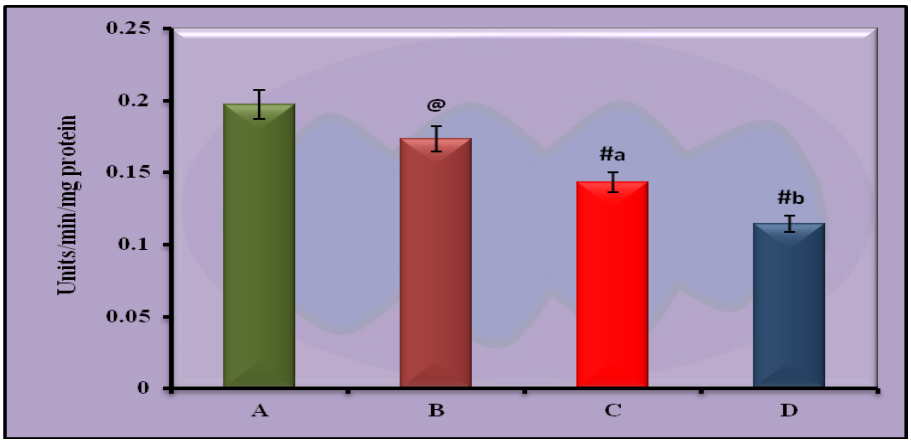

Figure 7: Effect of VER-155008 on CAT in mitochondria of normotensive and preeclamptic placental trophoblast. Values are expressed as mean ± SD (n=17)

Where, A- Normotensive trophoblast mitochondria; B- Normotensive trophoblast mitochondria with VER-155008 incubation; C- Preeclamptic trophoblast mitochondria; D- Preeclamptic trophoblast mitochondria with VER-155008 incubation.
Values are represents mean ± SD (for 20 samples in each group)
[@]p<0.05 when compared with normotensive trophoblast mitochondria without VER-155008 incubation
[#a]p<0.01 when compared with normotensive trophoblast mitochondria without VER-155008 incubation
[#b]p<0.01 when compared with preeclamptic trophoblast mitochondria without VER-155008 incubation

Effect of VER-155008 on TAC

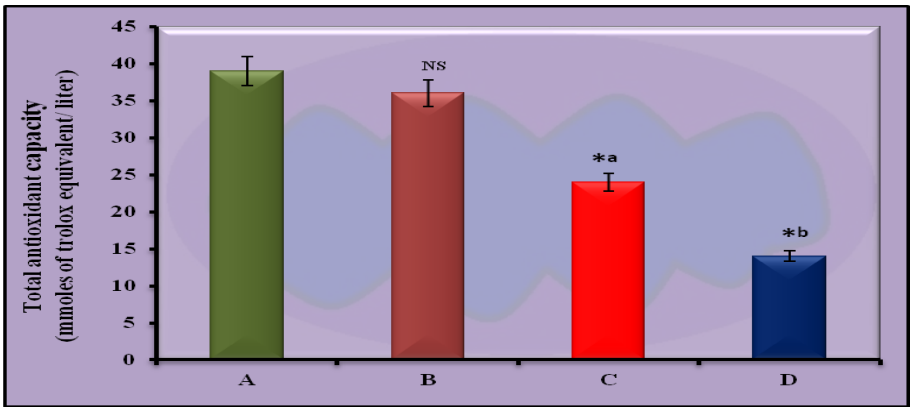

Figure 8: Effect of VER-155008 on TAC in mitochondria of normotensive and preeclamptic placental trophoblast. Values are expressed as mean ± SD (n=17)

Where, A- Normotensive trophoblast mitochondria; B- Normotensive trophoblast mitochondria with VER-155008 incubation; C- Preeclamptic trophoblast mitochondria; D- Preeclamptic trophoblast mitochondria with VER-155008 incubation.
Values are represents mean ± SD (for 20 samples in each group)
NS- Non significant when compared with normotensive trophoblast mitochondria without VER-155008 incubation
*[a]p<0.001 when compared with normotensive trophoblast mitochondria without VER-155008 incubation
*[b]p<0.001 when compared with preeclamptic trophoblast mitochondria without VER-155008 incubation

Figure 8 noticed the total antioxidant capacity in mitochondria of normotensive and preeclamptic placental trophoblast with and without incubation of VER-155008. The level of TAC was significantly decreased by 38% in mitochondria of preeclamptic placental trophoblast than normotensive group without incubation of VER-155008. After incubation with VER-155008, the level of TAC was significantly decreased by 61% in mitochondria of preeclamptic placental trophoblast than normotensive trophoblast mitochondria.

Effect of VER-155008 on ATPase activity

The level of ATPase in mitochondria of normotensive and preeclamptic placental trophoblast with and without incubation of VER-155008 was cited in **Figure 9**. The level of ATPase was significantly decreased by 50% in mitochondria of preeclamptic placental trophoblast than normotensive group without incubation of VER-155008. After incubation with VER-155008, the level of ATPase was significantly decreased by 58% in mitochondria of preeclamptic placental trophoblast than normotensive trophoblast mitochondria.

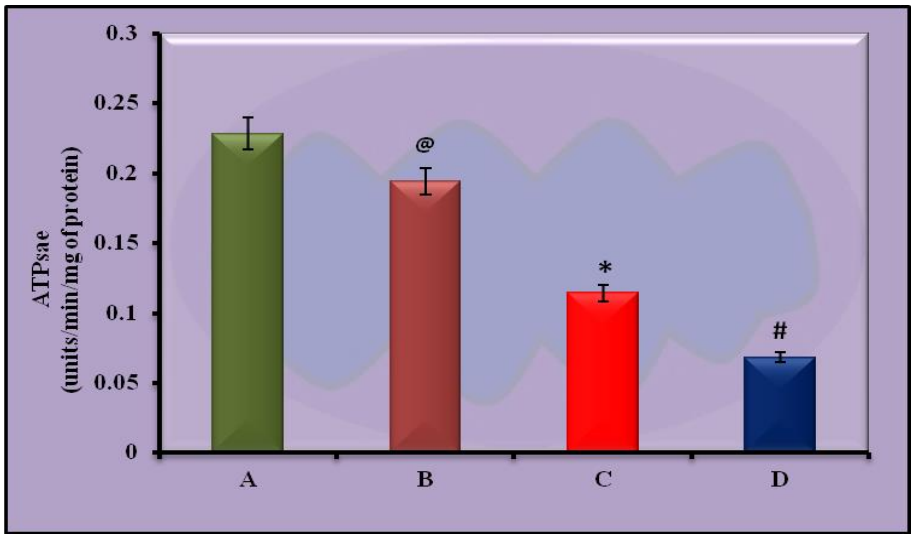

Figure 9: Effect of VER-155008 on ATPase activity in mitochondria of normotensive and preeclamptic placental trophoblast. Values are expressed as mean ± SD (n=17)

Where, A- Normotensive trophoblast mitochondria; B- Normotensive trophoblast mitochondria with VER-155008 incubation; C- Preeclamptic trophoblast mitochondria; D- Preeclamptic trophoblast mitochondria with VER-155008 incubation.
Values are represents mean ± SD (for 20 samples in each group)
[@]p<0.05 when compared with normotensive trophoblast mitochondria without VER-155008 incubation
*p<0.001 when compared with normotensive trophoblast mitochondria without VER-155008 incubation
[#]p<0.01 when compared with preeclamptic trophoblast mitochondria without VER-155008 incubation

Effect of VER-155008 on mtHSP70

The level of mtHSP70 in mitochondria of normotensive and preeclamptic placental trophoblast with and without incubation of VER-155008 was detected in **Figure 10**. The level of mtHSP70 was significantly increased by 33% in mitochondria of preeclamptic placental trophoblast than normotensive group without incubation of VER-155008. After incubation with VER-155008, the level of mtHSP70 was increased by 2% in mitochondria of preeclamptic placental trophoblast than normotensive trophoblast mitochondria. The present result demonstrates that the level of HSP70 was inhibited by 31%.

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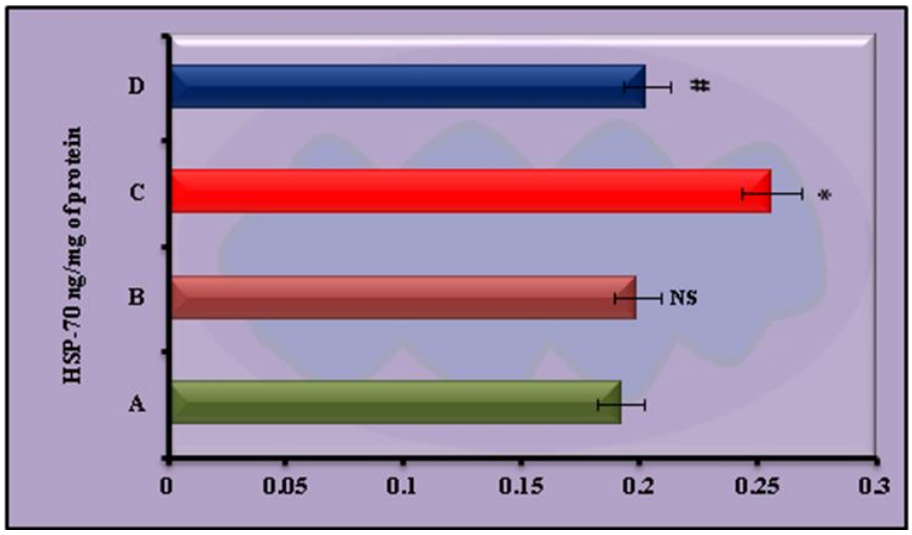

Figure 10: Effect of VER-155008 on HSP70 in mitochondria of normotensive and preeclamptic placental trophoblast. Values are expressed as mean ± SD (n=17)

Where, A- Normotensive trophoblast mitochondria; B- Normotensive trophoblast mitochondria with VER-155008 incubation; C- Preeclamptic trophoblast mitochondria; D- Preeclamptic trophoblast mitochondria with VER-155008 incubation.
Values are represents mean ± SD (for 20 samples in each group)
NS- Non significant when compared with normotensive trophoblast mitochondria without VER-155008 incubation
#p<0.05 when compared with preeclamptic trophoblast mitochondria without VER-155008 incubation
**p<0.001 when compared with normotensive trophoblast mitochondria without VER-155008 incubation

DISCUSSION

Preeclampsia is a unique syndrome of human pregnancy; it is more often associated with placental abnormalities, such as improper trophoblast invasion, vasoconstriction, poor villous development and endothelial cell dysfunction.[35] Proper invasion is critical for placental and fetal development and its dysregulation results in a considerable spectrum of pregnancy abnormalities. Shallow invasion has been implicated in intra-uterine growth restriction (IUGR) [36, 37] and early-onset pre-eclampsia.[38]

Emerging evidence shows that preeclampsia is a major cause for increased oxidative and nitrative stress which commenced the damage of placental cells.[39] Lukyanova et al., [40] demonstrated that mitochondrial marker enzyme succinate dehydrogenase is sensitive to hypoxia. Similarly, decreased level of mitochondrial marker enzyme in preeclamptic condition was observed in the present study. Oxidative stress plays a pivotal role in pathogenesis of preeclampsia which is implied in the increased production of lipid peroxides. Earlier studies have established that MDA was significantly increased in placental tissue.[41] In this present study, the levels of lipid peroxide are also significantly increased in the preeclamptic trophoblast mitochondria compared with normotensive trophoblast mitochondria. The elevation of MDA is due to overproduction of ROS generated in preeclampsia.[42] Concomitantly nitrative stress is a condition which occurs by the production of highly reactive nitrogen containing chemicals such as nitrous oxide, nitric oxide and peroxynitrite. The end product of nitrative stress affects the vasodilation in the spiral artery which alters the protein structure interfering with normal body functions.[43] The overproduction of this free radical is involved in various pathological cellular processes. Subsequently it may involve in the dysfunction of the mitochondria. These are important factors in a range of human diseases due to their influence in cell metabolism.

Antioxidant defenses in preeclampsia are comparatively weak, either due to the uncontrolled increase in oxidative stress or due to their improper function under this condition of abnormal stress. The developing embryo can generate both intracellular and extracellular ROS, thus requiring effective antioxidant activity suggesting that SOD, which plays major role in the conversion of superoxide anion to hydrogen peroxide.[44] This result has also been confirmed by our previous analysis of endothelial cell mitochondria.[17] Buhimschi and Weiner[45] have established that catalase activity clearly decreases in pregnancy induced

hypertension (PIH) placenta in comparison to normal placenta. In our study, the level of catalase and total antioxidant capacity were decreased in mitochondria of preeclamptic placental trophoblast when compared to mitochondria of normotensive placental trophoblast.

Mitochondria are major sites for free radicals generation and to produce the harmful oxidant formed by the reaction between superoxide and nitric oxide. These may act as major sources of NO-dependent dysfunction in mitochondria through their oxidizing and nitrating properties. It also inhibits respiratory complexes I, II, and IV.[46] The decrease in enzyme activity in preeclamptic villous trophoblasts may be closely associated with the pathogenesis of preeclampsia. Decrease in intracellular ATP concentrations would result in disturbance in mitochondria energetics. This leads to impairment of ATP synthesis, which in turn affects mitochondrial membrane integrity and a decreased ATPase level.[47] Grazyna et al., [20] have demonstrated that the overexpression of inducible HSP70 (iHSP70) had no effect on ATP content in non-injured RPTCs (renal proximal tubular cells), but it reduced the decreases in ATP content and promoted ATP recovery after oxidant exposure. In contrast, inhibition of HSP70 function using the selective HSP70 inhibitor VER-155008, an inhibitor that targets the ATPase-binding domain of HSP70, prevented iHSP70-mediated improvements in ATP levels in injured RPTCs. The results from the present study also showed that the mtHSP70 ATPase significantly decreased in mitochondria of preeclamptic trophoblast than normotensive trophoblast mitochondria thus may lead to reduced ATP levels. The depletion of ATP is a proteotoxic stress that may lead to dysfunction, destabilization and aggregation of many cellular proteins.[48]

The first line of defense for oxidative stress is not sufficient enough to combat with the generated stress in trophoblast mitochondria. Thus HSPs, a group of chaperones act as secondary line of defense whose induction is altered in response to oxidative stress as they are involved in maintenance of the cell homeostasis. HSP acts as an antioxidant in maintaining the cellular redox homeostasis by enhanced peptide binding ability and peptide complex stability under oxidative stress conditions.[49] The stress induced HSP70 is an ATP-dependent molecular chaperone that plays a key role in refolding misfolded proteins and promoting cell survival under stress. HSP70 can function alone to inhibit apoptosis, while cooperative interaction with their designated co-chaperone molecules is likely to enhance their anti-apoptotic activities.[19] The mitochondrial HSP70 (Hsp75) is crucial for driving preproteins across the mitochondrial membrane into the matrix. This process is necessary for maintaining mitochondrial membrane potential that constitutes the motor unit of mitochondrial protein import machinery.[50] In our study, the expression of mtHSP70 increased in mitochondria of preeclamptic placental trophoblast than normotensive placental trophoblast. Increase in the levels of mtHSP70 is also associated with the prevention of NO-dependent increase in cellular free iron from the mitochondrial respiratory complexes, thereby maintaining their integrity.[51] The mtHSP70 can suppress mitochondrial ROS production through stabilizing cytochrome c and other important components of the electron transport chain, and in addition, through enhancing mitochondrial antioxidant mechanisms.[52, 17]

The housekeeping function of HSP70 is inhibited by VER-155008 which binds to the nucleotide binding site of HSP70.[53] The concentration of VER-155008 may play an important for client protein degradation via an HSP70-dependent mechanism.[54] Schlecht et al., [53] demostrated that VER-155008 inhibited the HSP70 activity. Sharma et al., [55] reported that HSP70 inhibition curtailed the enhanced cell survival. In our work, the cell viability was decreased after VER-155008 incubation. Sekihara et al., [56] demonstrated that decreased cell viability could be due to loss of membrane permeabilization. In our study, ROS production peaked in three hours after the addition of VER-155008, results indicating that VER-155008 leads to oxidative damage. The cellular stress in turn initiates the unfolded protein response, but this fails to restore cell homeostasis so proteins aggregate and cells die.[57] In our study, the level of HSP70 was inhibited by 31% after incubation of VER-155008. The data suggests that VER-155008 leads to apoptosis by inhibiting the defensive effect of HSP70. VER-155008 can be useful for the determination of cellular survival pathway mediated by HSP70 in placental trophoblast mitochondria. Results observed on HSP70 inhibition by VER-155008 emphasize that HSP70 is crucial for cell survival under stress condition.

CONCLUSION

In this study we investigated the cytoprotective role of HSP70 expression in placental trophoblast mitochondria. HSP70 exhibits crucial functions in the maintenance of cell homeostasis under stress condition. In addition, the inhibitory effect of VER-155008 in preeclamptic trophoblast mitochondria is reported for first time in our laboratory. An alteration in the expression of HSP70 by VER-155008 inhibitor causes oxidant-

antioxidant imbalance and attenuates the ATPase activity. These changes in the preeclamptic condition indicate that HSP70 may be one of the key factors to maintain cell homeostasis under stress condition.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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