HSP70 Expression and its Role in Preeclamptic Stress

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Preeclampsia, a hypertensive pregnancy-specific disorder, has long been analyzed for its association with cellular stress. It still remains one of the most serious complications of pregnancy. It is a multi-system disorder that affects maternal vascular function and fetal growth. The physiopathology of preeclampsia is still unclear, but an imbalance between reactive oxygen species (ROS) and antioxidants, appears to be an important contributing factor. Oxidative stress has been increasingly postulated as a major contributor to endothelial dysfunction in preeclampsia (PE). The ROS promotes lipid oxidation and are known to induce stress proteins, such as hemeoxygenase 1 (HO-1) and heat-shock protein 70 (HSP70). Embryonic and placental cells are highly sensitive to oxidative stress due to their proliferative nature. Endothelial cell dysfunction is suggested to be a part of wider maternal inflammatory reaction responsible for the clinical syndrome of preeclampsia. Part of the dysfunction in endothelial cell and trophoblast is attributed to oxidative stress developed during pregnancy. The disequilibrium in compensatory antioxidant control is proposed as a causative mechanism in the pathophysiology of preeclampsia. HSP70 acts as the secondary line of defense in systems with compromised antioxidant function. This article reviews the differential expression of HSP70 and the effect of mint-tea therapy to modulate preeclamptic oxidative damage.

Keywords: Preeclampsia, HSP70, Stress, Mint-tea, Ureaplasma urealyticum

Introduction

Preeclampsia is a pregnancy-specific multi-system disorder characterized by the development of hypertension (>140/90 mm Hg) and proteinuria (>0.3 g/dL) after 20 weeks of gestation and remission of these signs after placental removal. According to the World Health Organization, preeclampsia is a major cause of both maternal and fetal morbidity and mortality. This syndrome is associated with decreased utero-placental perfusion, increased trophoblast cell death and generalized activation of maternal endothelial cells and is one of the major indications for elective premature delivery. It affects around 6–12% of all pregnancies of which most are primigravida. A genetic association of this disorder is also been established.

Preeclampsia is characterized by oliguria, xanthine oxidase, sialic acid, creatinine, uric acid, lactate dehydrogenase, aspartate transaminase, alterations in the serum and local edema. It is considered severe, if one or more of the following criteria are present: Blood pressure of 160 mm Hg systolic or higher and 110 mm Hg diastolic or higher on two occasions at least 6 h apart while the patient is on bed rest, proteinuria of 5 g or higher in a 24 h urine specimen or 3+ or greater on two random urine samples collected at least 4 h apart, or oliguria of less than 500 mL in 24 h. The severe cases may also be accompanied by cerebral edema, vasospasm, microinfarcts and hemorrhages. These complications may lead to the development of eclamptic convulsions. Increased platelet reactivity, elevated von Willebrand factor and an imbalance of the prostacyclin–thromboxane ratio, which may lead to disseminated intravascular coagulation are also associated with preeclampsia. Increased xanthine oxidase activity is used as a biomarker for preeclampsia. Hyperuremia demonstrated by increased uric acid levels is assessed to confirm the
increased xanthine oxidase activity. The increased levels of cholesterol, triglycerides, free fatty acids, phospholipids, LDL, VLDL and sdLDL confirm the dyslipidemia occurring during preeclampsia. The essential HDL levels remain decreased in cord blood.

The pathophysiology of this syndrome remains unclear. It is believed that an incomplete trophoblast invasion in the placenta retains the arteries spiral, leading to maternal and fetal complications. A recent study has clearly shown that the placentation in preeclampsia is compromised in the first trimester by maternal and fetal immune dysregulation, abnormal decidualization or both, thereby impairing trophoblast invasion. Some of the placental abnormalities in preeclampsia include immune maladaptation and deficient implantation, which leads to the failure of the physiological remodeling of decidual vessels and improper placental vascular development.

Preeclamptic delivery is also associated with preterm birth which is the ultimate result of several different pathways that culminate in the initiation of labor on or before 37 weeks of gestation. The preeclamptic pregnancy is the most studied among pregnancy-specific complications due to their strong association with stress and infection, which can result into endothelial dysfunction and various further complications. This exerts the requirement of pre and post-partum care and treatment to the mother and fetus. The available remedy and its inability to tolerate preeclamptic complication enforce the requirement of a natural alternative medicine for the management and treatment of preeclampsia and the associated complications.

**Preeclamptic stress**

Preeclampsia has been associated with increased oxidative stress. Reactive oxygen species (ROS) play an important role in placental cellular growth, differentiation, apoptosis and in events which are of critical importance in determining the outcome of pregnancy. A relatively hypoxic placenta due to an inadequate uteroplacental circulation is thought to release placenta-derived factor(s) into the systemic maternal circulation. These factors are proposed to mediate disturbance of the maternal endothelium and result in the clinical manifestation of preeclampsia, such as hypertension and proteinuria. Exposure to a range of non-physiologically significant concentrations of ROS or reactive nitrogen species (RNS) that induce oxidative stress results in the oxidative damage of cellular lipids, proteins, DNA and RNA irreversibly. This results in the accumulation of their oxidized end products and stimulation of responses such as repair, adaptation or transformation.

Lipid peroxidation is a process, where polyunsaturated fatty acids and other lipids are oxidized by intermediate free radicals to form conjugated dienes, lipid hydroperoxides, malondialdehyde (MDA) and many other products. Many reports suggest the association between increased lipid peroxidation and preeclampsia. Evidence of increased oxidative lipid derivatives in the decidual placental tissues has been observed in women with established preeclampsia. We have also reported that in preeclampsia, an uncontrolled lipid peroxidation may occur in placenta and impair normal endothelial cell function. Lipid peroxidation results in reduction of Ca²⁺/ATPase activity, which in turn increases cytosolic Ca²⁺ concentration which might be in part responsible for some of the symptoms of this disease.

Placental increase in lipid peroxidation under conditions of preeclampsia is also determined by the changes in isoprostane level in the circulation. Isoprostane, a lipid peroxidation product and a prostaglandin-like compound is produced in vivo by free radical peroxidation of arachidonic acid. A new index of oxidative stress, the breath methylated alkane contour (BMAC) and their monomethylated derivatives are also increased under conditions of preeclampsia. A significant increase in the level of lipid hydroperoxides under conditions of preeclampsia is well documented. Lipid hydroperoxides function in normal physiology by regulating enzymes and redox-sensitive genes. Circulating lipid hydroperoxides are damaging to endothelial cells in vitro. Elevated levels of oxidative lipid derivatives and conjugated diene in the maternal circulation have also been reported. An increase in placental conjugated diene is demonstrated in the placental tissue and endothelial cell. The involvement of stress in preeclampsia and alternatively an effective role of oxidative stress in preeclamptic etiopathogenesis are well documented. Increasing incidence of preeclampsia is observed in pregnancies afflicted with asthma, a state in which the concentration of available CO₂ for use by the placenta is reduced by vascular perfusion.

Increased nitrosylation of proteins and other signs of oxidative stress have been shown in placental tissue in preeclampsia. The ROS and RNS
developed under conditions of preeclampsia may result in oxidation of amino acid residues on proteins forming protein carbonyl (PC) and causes cellular damage. A significant increase in plasma levels of PC is reported. An association has also been found between increased PC levels in placenta and the chance of concurrent hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome. Measure of the levels of nitrate and nitrite reflects the degree of RNS in preeclampsia. The hypertensive state in preeclampsia leads to the release of vasodilators like nitric oxide (NO), a gaseous molecule with a very short half-life (nearly 4 s), which rapidly gets converted into its metabolites nitrite (NO$_2$) and nitrate (NO$_3$).

The preeclamptic plasma contains increased levels of nitrate and nitrite, while the urine levels of nitrate and nitrite are noted to be increased in few reports and decreased in few studies, suggesting the systemic levels of NO during preeclampsia. The local increase in the vasodilator NO of preeclamptic patients has been confirmed by an increase in nitrate and nitrite levels in the amniotic fluid and placental tissue. The increase in NO level leads to microscopic lesions in placenta, inhibiting nutrient supply to the growing fetus, causing growth restriction and preterm birth (unpublished data). Reports also indicate a direct association between the severity of preeclampsia and nitrite/nitrate levels.

Cellular oxidative stress transcends to the mitochondria. Mitochondrial oxidative stress results in mitochondrial membrane swelling, loss of mitochondrial cristae and increasing mitochondrial number in the affected cell. Evidence suggests that a mitochondrial defect might cause the impairment of differentiation and invasion of trophoblast, leading to preeclampsia. A decreased mitochondrial ATP level observed in preeclampsia may lead to decrease in the activity of mitochondrial membrane-bound ATPases like total ATPase, Na$^+$/K$^+$-ATPase, Ca$^{2+}$-ATPase and Mg$^{2+}$-ATPase. Such altered mitochondrial bioenergetics along with the free radical mediated protein damage acts as primary mediators of the mitochondrial respiratory enzyme complex damage.

The increased ROS generation in preeclampsia has also been correlated with the expression of NADPH oxidase, a phagocytic enzyme. An increased concentration of TNFα observed in conditions of preeclampsia has an effect on the coenzyme Q, which further leads to superoxide formation. Reports suggest that deficiency in riboflavin might also be a possible risk for preeclampsia. Deficiency of riboflavin-derived cofactors FAD and FAM (mononucleotide) could contribute to the established pathophysiologic changes, including mitochondrial dysfunction-enhanced oxidative stress and disturbances in NO release. Conversely, chronic increase in NO level stimulates mitochondrial biogenesis in diverse cell types. Mitochondrial stress causes an increase in MDA, PC, nitrite, nitrate and reduced dichlorofluorescein (DCFH) with a corresponding decrease in the activity of superoxide dismutase (SOD), Gpx and glutathione redox ratio (GRR) in preeclamptic placenta. Lipid peroxidation in the mitochondria stimulated by NADPH and Fe$^{3+}$ is found to be reduced by the addition of SOD.

The delicate balance of oxidative control by antioxidant protein is crucial to the healthy progression of pregnancy, while disequilibrium in compensatory antioxidant control is proposed as a causative mechanism in the pathophysiology of preeclampsia. Antioxidant defense in preeclampsia is comparatively weak either due to the uncontrolled increase in oxidative stress or due to its improper functioning under an abnormally stressed status. Evidence suggests that radical scavenging SOD is increasingly consumed by the increased lipid peroxidation in conditions of preeclampsia. SOD catalyzes the conversion of two superoxide molecules to H$_2$O$_2$ and oxygen, in order to provide an adaptive oxidative stress-specific response. The increased MDA and Cu$^{2+}$ levels along with decreased SOD and Zn$^{2+}$ levels suggest a decrease in Cu-Zn SOD (cytoplasmic and nuclear) and Mn SOD (mitochondrial) levels during preeclampsia. Catalase (CAT), a homotetrameric ferriheme-containing enzyme decomposes H$_2$O$_2$ to water and oxygen. It is localized mainly in peroxisomes, but may also be detected in the cytoplasm and mitochondria. A reduced CAT activity has been observed under conditions of preeclampsia.

As glutathione level is increased or its synthesis might be disturbed in preeclampsia, it might affect the pathophysiology of preeclampsia. Glutathione, abundant in most cells represent the major low molecular weight antioxidant redox recycling thiol in mammalian cells and plays a central role in the cellular defense against oxidative damage. It provides the cell with a reducing environment, in addition to maintaining the proteins in a reduced state, which...
dynamically regulates the protein function. It is an important substrate for glutathione peroxidase (GPx) and glutathione-S transferase (GST) and also quenches free radicals. GPx, a selenium-dependent enzyme decomposes $\text{H}_2\text{O}_2$ and various hydro- and lipid peroxides. The classical form of GPx is cellular and dispersed throughout the cytoplasm, but GPx activity is also found in mitochondria, while its extracellular form is genetically distinct from cellular GPx.

GPx is deficient in placental tissue from preeclamptic women. The reduction of GPx levels corresponding to the increased lipid peroxidation induces the production of prostaglandin H synthase, resulting in increased serum thromboxane, another indicator of preeclampsia. GST is an antioxidant capable of reducing hydroperoxides. Maternal levels of soluble antioxidants, e.g., vitamin E, vitamin A, β-carotene, glutathione and red blood cell lysate thiols are lower in women with preeclampsia than in normal pregnancies. A decrease in GPx and vitamin E levels is reported in the serum of preeclamptic patients. Changes in the ratio of intracellular reduced and oxidized forms of glutathione (GSH/GSSG) can affect signaling pathways that participate in various physiological responses from cell proliferation to gene expression and apoptosis. This glutathione redox cycle represents one of the important defense mechanisms against oxidative stress in an enzyme-coupled manner. We have also demonstrated that the ratio of GSH: GSSG is significantly lower in preeclamptic than the normotensive subjects.

A decreased ferric-reducing ability of plasma (FRAP) levels (i.e.) total antioxidant capacity has been demonstrated in decidual and placental tissue in women with severe preeclampsia compared to normal pregnant controls. On the contrary, in preeclamptic patients an increase in FRAP levels in the circulation has also been reported. The FRAP assay has the restriction that it measures mainly the antioxidant capacity of water-soluble antioxidants (uric acid, vitamin C and bilirubin) and to a lesser extent that of hydrophobic components (vitamin E) and sulfhydryl groups of proteins. A decrease in the total peroxyl radical trapping antioxidant parameter (TRAP) and ascorbic acid corresponding to an increase in lipid peroxidation in preeclamptic subjects is also reported. A low level of the total antioxidant capacity (TAC), combined measure of cellular antioxidant representing the balance between oxidative stress and neutralizing systems in preeclamptic subjects has been demonstrated.

**Preeclamptic infection**

Preeclamptic patients are at high risk of infection, due to reduced immunological status. Preeclampsia has been attributed to a breakdown in maternal immune tolerance to foreign placental antigens. A previous study has clearly demonstrated a reduced immunological status with respect to preeclampsia through various danger signals, making the host more susceptible to infection. Infection in preeclampsia may lead to low fetal birth weight, chronic lung disease of the fetus and various other complications. Altered tissue cytokine production (increased TNF-α, decreased IL-6 and IL-8) has also been reported in the preeclamptic placenta as a marker of inflammation.

Infection has a dominant role in complicating the pregnancy. Primary or reactivated adenoassociated virus-2 (AAV2) infection early in pregnancy is associated with adverse reproductive outcomes like placental dysfunction, including preeclampsia, stillbirth and spontaneous preterm delivery. Preeclampsia, generally considered as a metabolic disorder may also result as an outcome of infectious severity. Urinary tract infection and periodontal disease during pregnancy are associated with an increased risk of preeclampsia. Women who are seronegative to viral agents may acquire primary viral infection in pregnancy and subsequently be at increased risk of preeclampsia. Past infection with Cryptoplasma pneumoniae could be another risk factor for preeclampsia. Women with preeclampsia/eclampsia may probably involve in the induction of apoptosis, following Parvovirus B19 infection. Antibodies to Chlamydia and Cytomegalovirus are higher in early onset preeclamptic patients. Increase in urogenital infection in preeclamptic pregnancy may reflect the higher rates of underlying renal disease and placental bed abnormalities occurring in preeclampsia.

In preeclampsia, the bacterial Ureaplasma urealyticum and Gardenella vaginalis infections have been observed through symptoms like bacteriuria. The perfuse colonization of U. urealyticum and their subsequent infection is associated in addition to preeclampsia with non-gonococcal urethritis, post-partum endometritis, still birth, bronchopulmonary displasia (BPD) and premature birth. Association of preeclampsia and U. urealyticum is not completely apprehended. The difficulty in U. urealyticum...
detection is primarily due to its complex growth requirement which makes its cultivation difficult. The commensal nature of U. urealyticum in the vagina further creates difficulty in analyzing its infection. U. urealyticum, also called the T-(tiny) mycoplasma is a prokaryotic cell-wall lacking member of the class mollicutes. Being one of the smallest organisms, it is associated with the mucosa, residing predominantly in the respiratory and urogenital tract\(^8\). A novel tool for easy detection of U. urealyticum using MTT assay is demonstrated\(^8\).

U. urealyticum and/or Mycoplasma hominis cultures (and/or DNA polymerase chain reaction) of the placental membranes and amniotic fluid have been consistently associated with histologic chorioamnionitis, preterm birth and adverse perinatal outcomes\(^8\). U. urealyticum colonization in the vagina during pregnancy is increasingly associated with premature rupture of membrane (PROM) and fetal complications like respiratory distress syndrome, intraventricular hemorrhage, bronchopulmonary dysplasia, very low birth weight infants etc\(^8\). The involvement of U. urealyticum in preeclamptic placental stress alteration has been clearly demonstrated\(^8\). Similarly, phospholipase A and C of U. urealyticum may lead to the loss of membrane integrity and increase its permeability by changing the membrane lipid composition\(^8\).

**Endothelial cell dysfunction in preeclampsia**

Evidence of endothelial dysfunction (ED) as an early event in preeclampsia suggests that it is a possible cause and not a result of this pregnancy-specific disorder. The vascular endothelium has many important functions, including control of smooth muscle tone through release of vasocostrictor and vasodilatory substances, regulation of anticoagulation, antiplatelet and fibrinolysis functions via release of different soluble factors\(^7\). The release of these factors from the placenta in response to ischemia results in ED of the maternal circulation\(^8\).

Lipids have also been implicated in mediating ED in preeclampsia. In women who develop preeclampsia, plasma triglyceride levels are significantly elevated as early as 10 weeks of gestation compared with those in normal pregnant women. Fatty acids contribute to ED by serving as substrate for lipid peroxidation. Lipid peroxides are also significantly increased in plasma from women with preeclampsia\(^8\). Therefore, the generation of free radicals, lipid peroxides and ROS may be an important mechanism for ED in preeclampsia\(^1\).

Oxidative stress may cause ED which may lead to hypertension by reduced release of vasodilating agent, such as NO\(^9\). Preeclampsia is associated with an impairment of endothelium-dependent relaxation in maternal resistance arteries\(^9\). A decrease in urinary excretion of NO metabolites is also reported in preeclampsia\(^9\). The dysfunctional endothelial cells undergo activation and produce leukocyte-endothelial adhesion molecules which mediate the adherence of inflammatory cells. These inflammatory cells enable production of cytokines like IL-6, IL-1 receptor antagonist and increased levels of IL-2. These circulating endothelial adhesion molecules are also used as diagnostic markers of risk for development of preeclampsia\(^93\). Soluble plasma constituents though are responsible for preeclamptic complications are unlikely to be involved in the systemic endothelial cell activation in preeclampsia\(^94\). Many endothelial changes of potential relevance to preeclampsia are induced by lipid peroxidation in experimental systems\(^95,96\). Recently, an increase in cellular fibronectin levels in plasma has been used as a marker to determine ED in preeclamptic women\(^97\). The ED during preeclampsia is assessed non-invasively by measuring the flow-mediated vasodilation of the radial artery with high resolution ultrasound. The reduction in flow-mediated vasodilation in preeclampsia is also correlated with plasma fibronectin\(^99\). Serum-soluble VCAM-1\(^93\) is elevated in blood and urine of preeclamptic women\(^87\).

In preeclampsia, the H\(_2\)O\(_2\) generated may have dual action on placental activity and acts not only as a cytotoxic mediator, but also as a signaling molecule able to induce human chorionic gonadotrophin (hCG) secretion. hCG may be a protective antioxidant released by the placenta to counter low oxidative stress challenge\(^99\). Reports also suggest the role of hCG in promoting the secretion of vascular endothelial growth factor (VEGF), an essential angiogenic factor that exhibits protective role in preeclampsia\(^100\).

**Heat shock protein and its savior mechanism**

Activation of stress system helps the organism to overcome the influence of stressors and therefore, postpones all functions that may interfere with the chance of the individual to survive\(^101\). To maintain the homeostasis under oxidative stress, the cells apart from the induction of antioxidant enzymes produce high level of stress proteins or heat shock proteins (HSPs), which protect them against the damage\(^102\). HSPs
are both stress response proteins and constitutively expressed intracellular chaperones. These proteins are involved in various aspects of protein metabolism and are essential for regulating cellular homeostasis and promoting cell survival. The involvement of HSP in a multitude of intracellular action makes them as central coordinators in deciding the fate of the cell. HSP acts as an antioxidant in maintaining cellular redox homeostasis. HSPs are found to inhibit intracellular ROS level and increase the glutathione level. The coordinated activities of the HSPs, thus modulate multiple events within apoptotic pathways to help sustain cell survival, following damaging stimuli.

HSP70 are multi-gene families that range in molecular mass from 10 to 150 kDa and are found in all major cellular compartments. Extensive studies have revealed three major families of HSPs: low-molecular mass HSPs (16-47 kDa), HSP70 (68-73 kDa) and HSP90 (85-90 kDa). The active phosphorylated HSP27 oligomer stabilizes cytoskeletal components and thereby favors cell survival. HSP27-mediated suppression of BH3 (Bc12-homology domain) interacting domain death agonist protein (Bid) translocation to the mitochondria correlates with an inhibition of cytochrome c release from the mitochondria and thus remains anti-apoptotic.

The HSP70 is essential for protein folding, translocation across cellular compartments, assembling and maintaining multi-protein complexes in active states and preventing self-association. It also directs misfolded and short-lived proteins to destruction by the proteasome in order to maintain protein homeostasis. In human cells, HSP70 is cell cycle-regulated and is found to be upregulated to assist in the protein refolding under stressed condition by their enhanced peptide-binding ability and peptide complex stability. Studies on elevated HSP70 levels in the serum and placental tissue and placental endothelial cells of preeclamptic patients reveal their role in oxidative defense. Russo et al. first observed that depletion of cellular reduced glutathione (GSH) results in thermotolerance and concomitant synthesis of HSP70 through the activation of HSF. An increase in HSP70 during preeclampsia with respect to U. urealyticum infection is also reported. A recent study has also shown genotype specificity in HSP70 expression under conditions of preeclampsia.

The mitochondrial HSP70 (HSP75) is essential for driving preproteins across the mitochondrial membrane into the matrix. This process is essential for maintaining mitochondrial membrane potential that constitutes the motor unit of mitochondrial protein import machinery. Recently, we have reported that mtHSP70 increases in response to an increased mitochondrial oxidative and nitrate stress along with a decreased antioxidant status during preeclampsia. The decrease in the activity of mitochondrial respiratory enzyme complexes act as a primary stimuli in inducing the expression of mtHSP70. 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. The mtHSP70 can suppress mitochondrial ROS production through stabilizing cytochrome c and other important components of electron transport chain and in addition through enhancing mitochondrial antioxidant mechanisms.

Key antioxidants such as Gpx are decreased in placential tissue from preeclamptic subjects, adding to the oxidative stress in these tissues which may lead to increased apoptosis and even necrosis. Diminished or poor placental function in preeclampsia also increases the chance of apoptosis. Function of HSPs encompass an anti-apoptotic role that can, but does not always depend upon their chaperoning ability. HSP70 blocks heat-induced and NO-induced apoptosis primarily by inhibiting BCL-2-associated X protein (Bax) activation and thereby preventing the release of proapoptotic factors like cytochrome c from mitochondria. It also inhibits events, leading to mitochondrial membrane permeabilization in stressed cells and thereby controls the decision to die, but does not interfere with cell death after this event has occurred.

HSP70 can function alone to inhibit apoptosis, while cooperative interaction with their designated co-chaperone molecules is likely to enhance their anti-apoptotic activities. It can inhibit the formation of apoptosome during the process of apoptosis by their specific interaction with apoptotic proteins. Though elevated expression of HSP70 can block the death process, an extreme increase in HSP70 expression can either favor cell death or reduce its growth. This might further increase the maternal and fetal complications, which emphasizes the need for appropriate therapy.

**Natural therapeutic intervention**

A significant decrease in several important antioxidants, such as vitamin E, β-carotene, ascorbic...
acid and glutathione along with the decrease in free iron binding activity has been demonstrated in preeclamptic patients. Vitamin E is capable of affecting cytokine signaling in placental trophoblasts and maternal immune effector cells, both in early and late human pregnancies. Reduced incidence of preeclampsia in women at risk is reported to be treated with antioxidants like vitamin C and E. The potential benefit of vitamin C and E supplementation to prevent preeclampsia in women with clinical risk factor on the contrary is reported to be smaller than estimated. The effectiveness of low-dose aspirin for preventing preeclampsia might relate to inhibition of lipid peroxide formation as well as inhibition of thromboxane. Magnesium sulphate administered to treat increased cytosolic Ca concentration due to reduced Ca/ATPase activity also reduces preeclamptic complication

The importance of nutrient balance in pregnant women is increasingly being recognized. Increased sodium intake can result in placental oxidative stress by the end of gestation, indicating that placental oxidative stress plays an important role in the onset of preeclampsia. Thus, a balanced diet constituting natural source of antioxidants may protect the cells from free radical mediated damage. Tea (Camellia sinensis), is the most popular beverage in the world. Black tea made from the mild oxidation of green tea leaves amounts to the 80% of the world tea production. Tea is rich in catechin and is a powerful antioxidant capable of rapid reduction of superoxide radical and alkyl peroxyl radicals. It possesses high antioxidant properties and protects human cells from the adverse effect of ROS. Phenolic compounds in green and black tea (catechin, flavanol, theaflavin) inhibit cytochrome P-450 MFO (mixed-function oxidase) and enhances the phase II enzyme GST. Apart from the polyphenols and flavonols, tea also contains theophylline and theobromine in small concentrations which aid in the reduction of preeclampsia. A reduction in the occurrence of preeclampsia among pregnant asthmatic women treated with theophylline has been reported. Theobromine is a myocardial stimulant as well as a vasodilator; it increases heartbeat and also dilates blood vessels causing a reduced blood pressure. Mint (Mentha spicata) is used and valued as an aromatic herb for thousands of years. It is considered as stimulant, carminative, antispasmodic, stomachic and diuretic. It contains twenty flavonoid aglycones and is a good source of a number of micro-minerals like Mn, Cu, Zn and Se with potent antioxidant properties. The polyphenols found in mint act as scavenger to prevent cellular damage. Mint leaves have powerful anti-angiogenic effect, further supporting their use in treatment of preeclampsia. Mint leaves protect tissues from superoxide by stimulating antioxidative enzymatic system. The flavonoids from the mint leaves increase the activity of enzymes like SOD and CAT. The protective effect of mint extract on H2O2-induced damage of human lymphocytes is also reported. The presence of rosmarinic acid along with other compounds in M. spicata may be correlated with its oxidative DNA damage protecting activity. It is also beneficial in the treatment of allergic diseases. Mint tea is one of the most commonly used herbal teas and is a folk remedy for a variety of gastrointestinal problems. A greater DPPH and NO radical scavenging activity in mint extract, increased reducing power in tea extract, a significant hydroxyl and superoxide anion radical scavenging effect in tea with mint extract is also reported. The catechin and tannin present in the tea interfere with iron absorption in the stomach, but mint extract contains provitamin enabling the iron to be available in soluble state, thereby increasing its absorption. Thus, a combination of black tea and mint extract may have more beneficial effect than administered alone.

Selenium (Se) deprivation is able to modulate the endogenous expression of key antioxidant proteins, leading to a state of placental oxidative stress, resulting in physiological changes in pregnant rats similar to those seen during human preeclampsia. There is evidence that Se is particularly important in the nutrition of pregnant women. Significant correlation between preeclampsia and decreased Se status prior to pregnancy has been reported. Considering preeclampsia is associated with significant reduction in Se status, it can be hypothesized that reduction in antioxidant function is linked to Se deficiency. It is possible to regulate the activity of GPx through the modulation of Se, as its activity depends on Se availability. Small quantities of Se added to cell culture medium significantly increases the cellular expression and activity of GPx. Se supplementation could be a simple applicable method of alleviating placental oxidative stress in women suffering from preeclampsia. Mint, tea and mint-tea extracts contain most of the essential minerals (Na, Mg, K, Cr, Fe, Co, ...
Cu, Zn and Se) in adequate amounts and also show an antibacterial activity (a property of its phenolic compound). The mint-tea extract also has an effect in reducing the lipid peroxidation and improving the antioxidant status in preeclamptic patients\(^\text{18}\).

Tea and mint contain low amount of polyamines\(^\text{157,158}\). Polyamines are effective antioxidants playing a role in controlling ROS-mediated damage\(^\text{159}\) and exert both protective and pro-apoptotic effects, depending on the cellular context and pro-apoptotic stimuli\(^\text{160}\). However, a decrease in the anti-apoptotic protein HSP70 expression is observed when the placental endothelial cells are incubated with tea and mint extracts (unpublished data), indicating their protective effect under conditions of preeclampsia. Taken together, the unregulated oxidative stress could induce differential expression of HSPs, which in turn may play distinct roles in the cellular defense. Targeting HSPs, therefore, may provide novel tools for treatment of many diseases\(^\text{160}\), including preeclampsia.

**Conclusion**

Elevated expression of HSP70 can block the death processes\(^\text{127}\), while an extreme increase in HSP70 expression can favor cell death or reduce its growth\(^\text{160}\). Thus, once when adequate amount of HSP70 is synthesized, transcription of hsp70 gene must be rapidly attenuated to favor cell survival. Studies have shown a decrease in expression of HSP70, when placental endothelial cells are incubated with tea and mint extracts (results not shown), which could be due to the minimal amount of polyamines, which are known to exert both protective and pro-apoptotic effects\(^\text{161}\). Thus, mint and tea apart from being an effective antioxidant during stressed condition like preeclampsia can also favor cell survival.

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