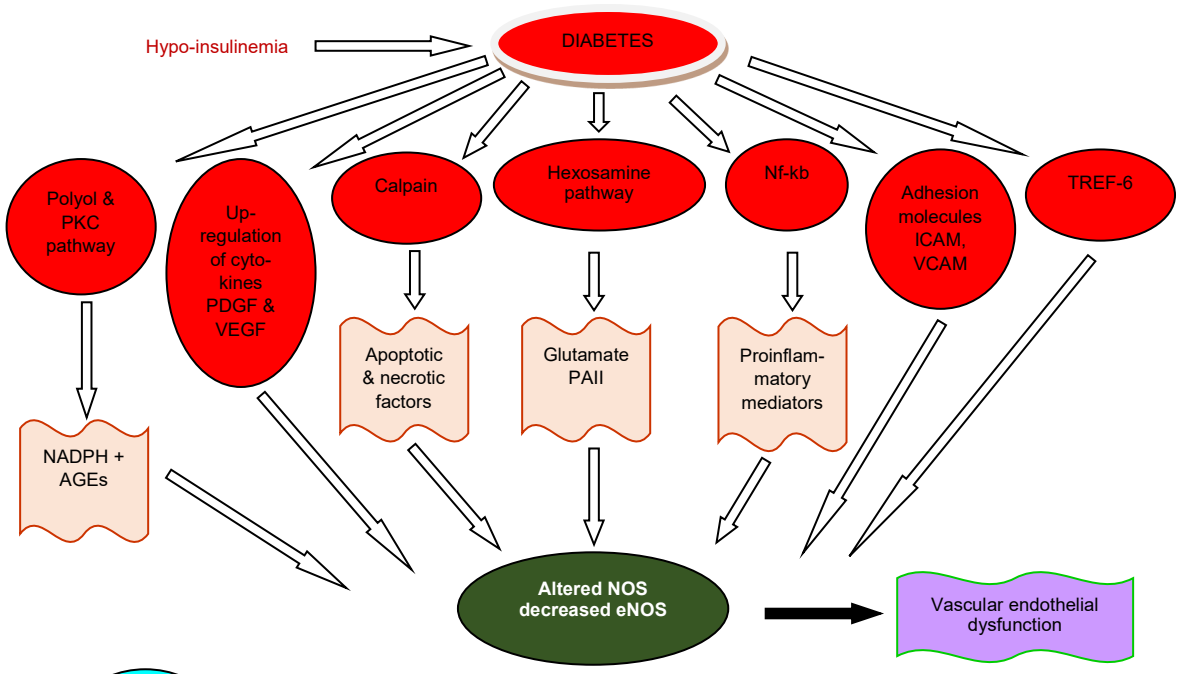


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Mini-Review

# Experimental models for vascular endothelial dysfunction

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Mini-review

# Experimental models for vascular endothelial dysfunction

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## Abstract

Vascular endothelial dysfunction is characterized by apoptosis of endothelial cells, an imbalance between vasoconstrictory and vasodilatory substances, the imbalance between ROS and antioxidants, vascular remodeling, loss of vascular integrity which leads to an increased risk of cardiovascular complications. To date, no therapeutic intervention is available as a promising agent. This may be due to a poor understanding of the underlying mechanism involved in vascular endothelial dysfunction in the pathogenesis. Animal models sharing identical features as that of humans are paramount to understand fundamental physiology, mechanism and to explore new targets for developing therapeutic agents. Thus, it becomes mandatory to re-explore the available animal models for a better understanding of molecular pathways involving vascular endothelial dysfunction. The purpose of this paper is to review different models for vascular endothelial dysfunction to the outlook for developing new drugs to treat vascular endothelial dysfunction.

## Introduction

The vascular endothelium is the innermost lining of the blood vessel. It is a metabolically active layer that tends to release various substances that control vascular relaxation and contraction as well as enzymes that control blood clotting, immune function, and platelet adhesion (Sandoo et al., 2010). It plays a crucial role in maintaining vascular tone, integrity, and free flow of the blood under normal physiology. Destruction or injury in the endothelial layer of arteries leads to create an imbalance between vasoconstriction and vasodilatation factors which complicate vascular endothelial dysfunction and lead to cause various other severe cardiovascular disorders. An increase in free radicle production (ROS/RNS), NADPH oxidase, xanthin-oxidase or decrease in glutathione, no generation is the underlying pathways involved in the pathogenesis of vascular endothelial dysfunction. Regulation of inflammatory mediators such as intracellular adhesion molecule-1, von Willi brand factor, Nf-kb and growth factors like endothelin-1, VEGF, PDGF, OLGF, and ILs mutually affect vascular endothelium (Balakumar et al., 2008a). Atherosclerosis, hypertension, hyperglycemia, and smoking are considered to be the self-governing risk factors and foremost determinants in the progression of vascular endothelial dysfunction (Hadi et al., 2005). To identify the potential pharmacological targets for vascular endothelial dysfunction in different experimental models are designed and employed to induce the vascular endothelial dysfunction. Therefore, this paper aims to review various experimental animal models developed to produce vascular endothelial dysfunction.

## Animal models

Human vascular endothelial dysfunction shares many features which are common with animals. In contrast to direct study on human, animal models are easily manageable, as experimental conditions can be controlled. Vascular and cardiac tissue samples can be taken for detailed biomolecular and histopathological examinations. Mice and rats have long served as the preferred

species for biomedical research due to their anatomical, physiological, and genetic similarity to humans; spontaneous, drug-induced, metabolic alteration related and numerous genetically modified animal model have been developed for improving the understanding of the pathogenesis, prevention, and treatment of vascular abrasion and its comorbidities depend on their validity for representing human forms of vascular endothelial dysfunction. As an alternative, endothelial cells culture has been extensively described in the literature for *in vitro* assessment of oxidative stress, inflammation and proliferation (Le Brocq et al., 2008). The cell culture technique provides a path to explore wide variety of intracellular signalling pathways, with different purposes. However, these processes have restrictions related to phenotypic changes of the cells (Fadini and Avogaro, 2010). Hence, rodent models are preferred models that exhibit progressive vascular endothelial dysfunction is selected for identifying mechanisms involved in vascular endothelial dysfunction and to develop suitable therapeutic substances for its prevention.

## Diabetes-induced

Diabetes mellitus is a pathological group of metabolic disorders characterized by chronic hyperglycemia which is mainly due to impairment of either insulin secretion and insulin action or both causing micro- and macro-vascular complications (Baig et al., 2019). Vascular injury due to uncontrolled hyperglycemia is a key reason behind diabetes associated with cardio- and cerebro-vascular disorders. Diabetes-associated vascular endothelial dysfunction involves multiple signaling pathways including elevation of polyol, protein kinase C, hexosamine, pentose phosphate shunt which are overactive and are involved in diabetes-induced vascular endothelial dysfunction. Diabetes-induced oxidative stress also plays a crucial role in vascular endothelial dysfunction. Though multiple compounds at different doses have been identified, which are correlated with the clinical manifestation of diabetes mellitus-induced vascular endothelial dysfunction and are widely used to identify the potential pharmacological targets for vascular endothelial dysfunction.

### *A) Streptozotocin-induced*

Streptozotocin, a naturally occurring agent obtained from *Streptomyces acromogenes* used to treat cancer of pancreatic islets of Langerhans but is highly toxic to the insulin-producing β-cells of the pancreas resulting in insulin depletion (Balakumar et al., 2008b). Streptozotocin at different doses of 40, 50, 55, 60, or 65 mg/kg intravenous or intraperitoneal route is widely used as an experimental model for inducing vascular endothelial dysfunction (Pieper et al., 1997; Zhu et al., 2011; Yin et al., 2014; Brahmanaidu et al., 2017; Kshirsagar et al., 2017; Azemi et al., 2020; Said, 2020). Within 72 hours of administration, a single dose of streptozotocin produces hyperglycemic effects but vascular endothelial dysfunction can be observed after 4-8 weeks. GLUT-2 present on the pancreatic cell uptakes streptozotocin which results in the alkylation of DNA and triggers activation of PARP, polyol, protein kinase C, hexosamine, pentose phosphate shunt pathways, and overexpression of NOX2, leading to ROS/RNS generation and accumulation in the endothelial lining of blood vessel aggravating vascular endothelial dys-function. The induced vascular endothelial dysfunction is assessed for decreased aortic serum nitrate levels, T-BARS, GSH, NBT levels as well as increased phenylepinephrine-induced pre-contraction (Balakumar et al., 2009; Nie et al., 2019). Vascular endothelial dysfunction induced by intravenous administration of streptozotocin down-regulates the level of eNOS by producing superoxides and peroxynitrites and also alters GCH-1, responsible for $BH_4$ synthesis which leads to the uncoupling of eNOS from its co-factor $BH_4$ and alters NO bioavailability (Oelze et al., 2011).

Streptozotocin in rats (30 mg/kg along with high fat and high sucrose diet), is associated with increased serum von Willibrand factor and decreased acetylcholine-induced relaxation, the content of aortic angiotensin converting enzymes, NOs, and expression of eNOS is decreased which are found responsible for reducing the elasticity of the vessels (Yang et al., 2011). Streptozotocin-induced hyperglycemia (65 mg/kg intraperitoneal) is evaluated *in vivo* and *in vitro* for expression of calpain-1 protein by Western blot. Uncontrolled hyperglycemia persuades hypersecretion of proteolytic enzymes calpain and its isoforms which in turn activates apoptotic and necrotic factors and also alters NOS (Nie et al., 2019). Under streptozotocin-induced hyperglycemia, HAEC (human aortic endothelial cell) is found with the expression of TRAF-6 (Tumor necrosis factor associated factor) and related adhesion factors like ICAM and VCAM. This TRAF-6 protein induces vascular endothelial dysfunction by expressing NFKB and AP-1-dependent signaling pathways (Liu et al., 2018). Transcripting factor AP-1 (activator protein) expression increases under the influence of various stimuli like stress, cytokines, growth factors which are associated with MAPK pathway activation. Streptozotocin is also responsible for decreasing the expression of sirtuin (SIRT-1) which is responsible for maintaining lipid and whole-body cholesterol homeostasis, decreased levels of SIRT-1 are associated with worsening of vascular endothelial dysfunction (Wu et al., 2018; Pal, 2019). Single intraperitoneal administration of streptozotocin not only tends to induce diabetes but also worsens vascular endothelial dysfunction (Ji et al., 2021). C75BL/6 transgenic mice at a single dose of 50 mg/kg intraperitoneal for 5 consecutive days is not only found with elevated oxidative stress, inflammation, and aortic contractility but is also found to decrease SIRT-1 protein (Wu et al., 2018), In addition, BALB/c

transgenic mice with intraperitoneal administration of streptozotocin is reported to up-regulate COX expression which results in increased release of proinflammatory mediators (Nacci et al., 2009).

While in alloxan-induced diabetes, oxidative stress is found to be associated with diabetes-induced complications but not associated with vascular endothelial dysfunction whereas in confirmed diabetic rats' level of eNOS and nNOS is altered and penile erection is reduced when compared to normal rats (Capellini et al., 2010). Despite biochemical alterations, histopathological changes including vacuolization in the endothelium, edema in the tunica adventia, and focal infiltration of tunica media are associated with streptozotocin administration to rats (Adel et al., 2014). Mechanism underlying streptozotocin-induced vascular endothelial dysfunction is depicted in Figure 1 and different doses and animal models for streptozotocin-induced vascular endothelial dysfunction are summarized in Table I.

***B) High fructose diet-induced***

Fructose, a simple ketonic dietary monosaccharide widely used to induce vascular endothelial dysfunction that correlates with clinical features for metabolic abnormalities as it does not only result in hyperglycemia but also alters the lipid profile by enhancing the levels of VLDL. In addition, the uric acid level in the blood is also affected. Thus, play a crucial role in cardiovascular disorders. A decrease in angiogenesis is one of the significant cell losses governed by a high fructose diet (Khitan and Kim, 2013). Fructose at different concentrations of 10, 20, 60, and 65% w/w, has been noted to induce diabetes and associated complications within 8-12 weeks. A high fructose diet to Sprague -Dawley rat is found to have increased plasma triglycerides, cholesterol, fat weight, blood pressure, and decreased glucose tolerance (Babacanoglu et al., 2013; Malakul et al., 2018). In addition, administration of fructose is associated with the increased level of endothelin-1, inflammatory mediators, adhesion molecules in the aorta. Phenylepinephrine and potassium chloride contractility are also exaggerated in rats (El-Bassossy et al., 2014). Furthermore, fructose causes an imbalance between superoxides, peroxynitrites, and antioxidants

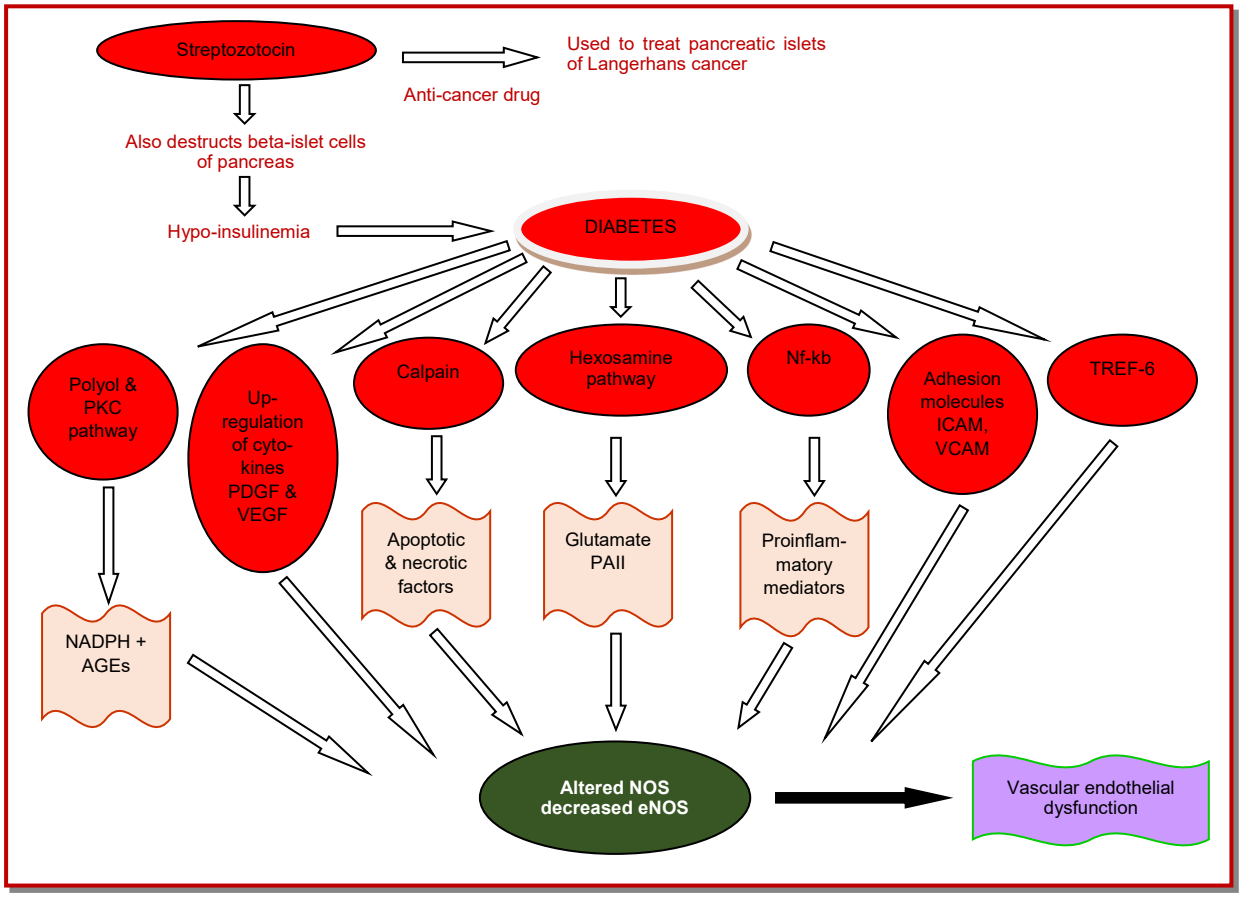


Figure 1. Mechanism underlying streptozotocin-induced vascular endothelial dysfunction.

PKc- protein kinase C signalling pathway; PDGF- platelet derived growth factor; VEGF- vascular endothelial growth factor; NfκB- nuclear factor kappa light chain enhancer of activated B cells; ICAM- intercellular adhesion molecules; VCAM- vascular cell adhesion molecule; TRAF-6- tumor necrosis factor (TNF) receptor associated factor-6; NADPH- nicotinamide adenine dinucleotide phosphate; AGE- advanced glycation end products; NOS- nitric oxide synthetase; NO-nitric oxide

**Table I**

**Streptozotocin-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Observations | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| Male Sprague -Dawley rats | 65 mg/kg, intraperitoneally | single dose | ↑ blood glucose, total cholesterol, triglyceride and LDL<br>impaired acetylcholine-induced relaxation | ↓ aortic and serum NO level | Balakumar et al., 2008b |
| Male Sprague -Dawley rats | 65 mg/kg, intraperitoneally | single dose | ↑ levels of calpain-I, ROS/RNS | ↓ SOD, GSH-px<br>↓ aortic and serum NO level, eNOS | Nie et al., 2019 |
| Male Wistar rats | 60 mg/kg, intraperitoneally | single dose | ↑ oxidative stress | ↓ vasorelaxation induced by acetylcholine in the aortic rings, NO levels<br>↑ eNOS uncoupling | Zhu et al., 2011 |
| Male Wistar rats | 60 mg/kg, intraperitoneally | single dose | ↑ oxidative stress, ↑ endothelin-1 | ↑ eNOS uncoupling | Said et al., 2020 |
| Wistar rats | 60 mg/kg, intravenous | single dose | ↑ NADPH oxidase, Nox-1 and Nox-2<br>↑ eNOS uncoupling due to down-regulation of GCH-1<br>↑ MDA levels | ↑ ROS/RNS<br>Impaired acetylcholine-induced vascular relaxation<br>↓ antioxidant | Oelze et al., 2011 |
| Sprague-Dawley rats | 60 mg/kg, intraperitoneally | single administration | ↑ NADPH oxidase<br>impaired acetylcholine- induced endothelium-dependent relaxation | ↓ eNOS activity<br>↑ ROS/RNS<br>↓ SOD | Ji et al., 2021 |
| Male Wistar rats | 55 mg/kg, intraperitoneally | single dose once a week, for 8 weeks | up-regulation of NADPH oxidase, NOX-2<br>↑ TGF-β<br>↑ phenyl-epinephrine induced contraction | ↓ serum and aortic nitrate/nitrate levels<br>↓ eNOS | Adel et al., 2014 |
| Male Wistar rats | 55 mg/kg, intraperitoneally | single dose once a week for 8 weeks | ↓ arginino succinate synthase, argininosuccinate lyase | ↓ GSH, SOD, GPx, ↓eNOS | Brahmanaidu et al., 2017 |
| Male Sprague -Dawley rats | 55 mg/kg, intravenous | single administration | ↑ norepinephrine-induced vasoconstriction<br>↑ ROS/RNS | Impaired endothelial dependent vasorelaxation<br>↓ SOD | Pieper et al., 1997 |
| Male Wistar rats | 45 mg/kg, intraperitoneally | single administration | ↑ LDL. TG, TC<br>↑ Insulin resistance<br>↑ ROS/RNS/AGE's leading to uncoupling of eNOS from its cofactors | ↓ serum NO level<br>↓ acetylcholine-induced endothelium-dependent relaxation | Kshirsagar et al., 2017 |
| Male Wistar rats | 40 mg/kg, intraperitoneally + high fat diet<br>. | 6 weeks fed with high fat diet followed by single administration | ↑ overexpression of TNF-α, MCP-1, and NF-κB P65<br>↑ LDL, TG, TC<br>↑ LDL oxidation<br>↑ ICAM, VCAM, IL's | ↓ eNOS activity and NO bioavailability<br>↑ ROS/RNS<br>uncoupling of eNOS from its cofactors | Yin et al., 2014 |
| Male Sprague -Dawley rats | 40 mg/kg, intraperitoneally + high fructose diet | 4 weeks fed with high fat diet followed by single administration | ↑ NADPH oxidase<br>↑ eNOS uncoupling<br>↑ AGE's<br>↑ free radicles | ↓ eNOS activity and NO bioavailability<br>Impaired ACh induced endothelium dependent vasorelaxation | Azemi et al., 2020 |
| Rats | 30 mg/kg, streptozotocin intraperitoneally + high fat and high glucose diet | single dose of streptozotocin followed by fed for 1 month | ↑ blood glucose, total cholesterol, triglyceride, low-density lipoprotein<br>↑ vWF<br>↑ phenyl-epinephrine induced contraction<br>↑ MDA levels | ↓ eNOS activity<br>↓ NO bioavailability<br>↑ levels of AGE's<br>↑ uncoupling of eNOS | Yang et al., 2011 |

**Table I**

**Streptozotocin-induced vascular endothelial disorders (cont.)**

| Species | Dose and route | Duration | Observations | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| C57BL/6 mice | 50 mg/kg/ day, intra-peritoneally | once every day for 5 consecutive days | ↑ aortic contractility, p53 hyper-acetylation<br>↓ SIRT1 protein | ↑ oxidative stress, inflam-mation | Wu et al., 2018 |
| C57BL/6 mice | 50 mg/kg/ day, intra-peritoneally | once every day for 5 consecutive days | ↑ COX-2 expression<br>↑ NFκB p56<br>↑ TXA2, PGE2<br>↑ oxidative stress | ↓NO bioavailability<br>↓eNOS activity<br>↓ PPAR-$\gamma$ and AMPK expression | Xu et al., 2019 |
| BALB/c mice | 240 mg/kg, intraperito-neally | single admin-istration | impaired acetylcholine-induced vascular relaxation<br>↑ expression of TNF-α, NFκB<br>↑ PGF-1α | ↑expression of pro-inflammatory signals<br>↑ COX-2 expression | Nacci et al., 2009 |

like SOD, GSH tends to create oxidative stress in the endothelial lining of the blood vessel which leads to impaired vasorelaxation (Kho et al., 2014; Shawky et al., 2014). Regarding vascular structural changes, fructose induces thickening of the endothelial wall due to the release and accumulation of various adhesion molecules, along with hyperplasia (Zhai et al., 2017) (Table II).

Taken together, streptozotocin- and fructose-induced models are widely used for pharmacological evaluation of vasoprotective agents against diabetes-associated vascular endothelial dysfunction. Streptozotocin dose ranges from 50 to 65 mg/kg/day intraperitoneally. Dose selection depends entirely upon the duration of study and frequency of drug administration. Streptozotocin 50 mg/kg/day intraperitoneal is required to administer once daily for 5 consecutive days as compared to a single administration of 65 mg/kg/day intraperitoneally. In case of fructose is employed ranging from 10 to 65% oral solution, again it also depends upon the duration of study as 60 and 65% oral solution of fructose are given for 8 weeks as compared to 10 and 20% oral solution for 12 weeks. However, their effects on biomarkers are the same irrespective of dose. In our opinion, for short-term studies, a high dose is preferred and for chronic studies, small and repetitive doses are preferred. Regarding selection among streptozotocin and fructose, streptozotocin is used for induction of type 1 diabetes, and fructose is preferred for induction of type 2 diabetes.

## Doxorubicin-induced

Doxorubicin (adriamycin) is an anti-cancer drug which belongs to anthracycline class of antibiotic. It is used in treating a wide variety of cancer but due to toxicities like cardiomyopathy and nephropathy, its clinical use is constrained (Carvalho et al., 2009). Excessive generation of mitochondrial ROX impairs endothelial functioning on exposure to doxorubicin. Doxorubicin-treated aortic ring shows abnormal function, induce vascular stress and causes apoptosis of endothelial lining. It also down-regulates the expression of NRF-2 which plays an important role in the protection of vascular endothelial dysfunction (Wang et al., 2015). Doxorubicin 20 mg/kg intraperitoneally on single administration in mouse model decreases the contractile responses to phenylephrine, along with attenuation of relaxant responses to acetylcholine. In addition, decrease in the aortic and serum nitrate, SOD, and GSH levels were observed (Olukman et al., 2009). Administration of doxorubicin (5 mg/kg) for 2-4 weeks is associated with vascular endothelial dysfunction by indirectly altering the level of NO through declining l-arginine (Li et al., 2019). In addition, loss of endothelium function due to marked increase in ROS/RNS is observed in doxorubicin-treated transgenic C57BL/6 mice, as a key role in establishing vascular endothelial dysfunction (Clayton et al., 2020). Doxorubicin not only alters the biochemical mediators but also leads to histopathological changes which include thickening of blood vessels, inflammatory infiltration, loss of vascular integrity, and vacuolization of the endothelial cells when compare to the doxorubicin-untreated aortic block (Li et al., 2019; Table III). To conclude, doxorubicin is used as an experimental tool within the dose ranges from 5 to 20 mg/kg, intraperitoneal, but it's worth mentioning that 5 or 10 mg/kg intraperitoneal is used for genetically engineered models i.e. C57BL/6 mice model. However, for Wistar rats, 20 mg/kg intraperitoneal of doxorubicin is employed to induce vascular endothelial dysfunction. All these show similarities in altered biomarkers.

## Nicotine-induced

Nicotine exposure through cigarette smoking is one of the major factors playing a crucial role in modulating vascular activity leading to the development of endothelium dysfunction which is associated with various life-threatening cardiovascular disorders. Nicotine

**Table II**

**Fructose-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Observations | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| Male Sprague-Dawley rats | 10% orally *ad libitum* | 12 weeks | ↑ blood glucose, total cholesterol, triglyceride and LDL<br>↓ acetylcholine-induced vasorelaxation | ↓ aortic and serum NO level, eNOS, p-eNOS<br>↑ ROS/RNS | Malakul et al., 2018 |
| Male Kunming mice | 20% orally *ad libitum* | 8 weeks | ↑ serum total cholesterol, triglyceride, LDL-C, $TXA_2$ and endothelin-1, AST and ALT thickening of the endothelial wall, accumulation of adhesion molecules and hyperplasia | ↓ HDL-C, $PGI_2$ serum and aortic eNOS levels<br>↓ SOD and GSH | Zhai et al., 2017 |
| Male rats | 65% orally *ad libitum* | 8 weeks | ↑ fat weight, blood pressure, plasma triglyceride, total cholesterol levels, and oral glucose tolerance | ↓ aortic and serum NO level, eNOS, p-Enos<br>increased ROS/RNS | (Kho et al., 2014) |
| Male Wistar rats | 10% orally *ad libitum* | 6 weeks | ↑ serum levels of glucose, insulin, uric acid, TNFα, lipids, AGEs | exaggerated contractility to phenylepinephrine and KCl and impaired relaxation to acetylcholine | El-Bassossy et al., 2014 |
| Male Sprague-Dawley rats | 60% orally *ad libitum* | 8 weeks | ↓ fasting glucose, GSH and MDA levels, serum total cholesterol, LDL-C, C-reactive protein level and LDH | ↓ aortic and serum NO level, eNOS, p-Enos<br>↑ ROS/RNS<br>↑ phenylepinephrine-induce contraction | Shawky et al., 2014 |
| Male rats | 10% and 20%, orally *ad libitum* | 12 weeks | ↑ plasma triglyceride, VLDL, cholesterol, insulin and glucose levels, but not body weights | impaired NO mediated relaxation<br>alters vascular reactivity to insulin, endothelin-1 in conjunction with insulin receptor substrate-1, endothelial nitric oxide synthase, inducible NOS mRNA/ proteins levels in aorta | Babacanoglu et al., 2013 |

**Table III**

**Doxorubicin-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Altered biomarkers | Reference |
|---|---|---|---|---|
| Male C57BL/6 mice model | 10 mg/kg, intraperitoneally | 4 weeks | impairs endothelial function<br>↑ mitochondrial reactive oxygen species (ROS) | Clayton et al., 2020 |
| Wild type male mice C57BL/6 model | 5 mg/kg, intraperitoneally | 2 or 4 weeks | ↓ level of arginine-NO metabolite<br>↑ level of vascular damage<br>↓ vascular relaxation, vascular NO generation<br>↑ blood pressure apoptosis, and oxidative stress | Li et al., 2019 |
| Wistar rats | 20 mg/kg, intraperitoneally | | ↓ NO formation, eNOS and iNOS<br>↓ contractile responses to phenylephrine, but also attenuated the relaxant responses to acetylcholine | Olukman et al., 2009 |
| Adult male Sprague -Dawley rats | 15 mg/kg, intraperitoneally | 14 days | ↑ ROS/RNS<br>↑ MDA<br>↑ expression of inflammatory mediators (ICAM, VCAM, TGF-β, VEGF)<br>↑ apoptosis altered vascular integrity and vascular tone | Wang et al., 2015 |

**Table IV**

**Nicotine-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Pathways | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| Male mice | 2 g/kg/ day, intra-peritoneally | 2 weeks | ↑ NLRP-3 inflammasome<br>↓ ZO-1 and ZO-2 epithelial and endothelial receptors | ↑ cleavage of pro-caspase-1<br>↑ production of IL-1β<br>lysosomal release of cathepsin B | Zhang et al., 2019 |
| Male Sprague-Dawley rats | 0.6 mg/kg, intraperitoneally | 28 days | ↑ oxidative stress, low NO bioavailability<br>↑ accumulation of vascular adhesion molecules vascular wall thickening inflammatory response | loss of vascular integrity<br>↑ vascular remodelling and oxidative stress<br>↑ phenylephrine-induced vasoconstriction | Si et al., 2017 |
| ApoE$^{-/-}$ mice | 0.1 mg/mL, orally *ad libitum* | 12 weeks | ↑ ERK1/2 signalling<br>↑ expression of adhesion molecules ICAM and VCAM promoting adherence of leukocytes<br>↑ NF-κB-dependent expression | larger atherosclerotic plaques loss of vascular integrity<br>↑ vascular remodelling and oxidative stress | Qin et al., 2020 |
| Sprague-Dawley rats with diet-induced obesity | 100 mg/L, orally *ad libitum* | 20 weeks | ↑ TNF α, interleukin 1β,<br>↑ CD36<br>↑ proinflammatory genes<br>↑ NADPH oxidase | ↑ systolic blood pressure, aortic superoxide production<br>↑ impaired endothelial nitric oxide synthase and<br>↓ endothelium-dependent relaxation to acetylcholine | Liu et al., 2017 |
| male Wistar rats | 2 mg/kg/ day, intraperitoneally | 4 weeks | ↑ serum cholesterol, triglycerides and high-density lipoprotein.<br>↓ expression of mRNA for p22phox and endothelial nitric oxide synthase | loss integrity of vascular endothelium<br>↑ serum TBARS<br>↑ aortic superoxide anion concentration | Chakkarwar, 2011 |
| Wistar albino rats | 2 mg/kg/ day, intraperitoneally | 4 weeks | ↑ generation of ROS/RNS<br>↓ acetylcholine-induced endothelium -dependent relaxation | ↓ aortic and serum nitrite/nitrate<br>↑ serum TBARS<br>↑ generation of ROS impairing endothelial integrity | Balakumar et al., 2008c |
| Wistar albino rats | 2 mg/kg/ day, intraperitoneally | 4 weeks | ↓ expression of PPARγ<br>↑ vascular oxidative stress<br>↑ activation of NADPH oxidase<br>↑ xanthine oxidase<br>↑ mononuclear leukocyte adhesion<br>↑ expression of adhesion molecules such as VCAM-1 and ICAM | ↓ expression of eNOS<br>↑ the generation of ROS impairing endothelial integrity | Taneja et al., 2013 |

aggravates multiple inflammatory and metabolic processes. The various vascular endothelial dysfunction is oxidative injury, endothelial damage and dysfunction, enhanced thrombosis, chronic inflammation, hemodynamic stress, adverse effects on blood lipids, insulin resistance and diabetes, reduced oxygen delivery by red blood cells, and arrhythmogenesis. The doses of nicotine are 2 mg/kg (Balakumar et al., 2008c) or 0.6 mg/kg (Si et al., 2017) for 28 days via intraperitoneal route to induce vascular endothelial dysfunction in experimental animal models. The induced vascular endothelial dysfunction is evaluated for various parameters like serum nitrate, aortic nitrate, TBARS, GSH, and SOD levels. Increased expression of TNF-α, interleukin 1β, CD36, $NADPH^+$ are responsible for the production of ROS/RNS which in turn creates a burden on the endothelium of the vessel, which plays a key role in progressing vascular endothelial dysfunction. This oxidative stress induced by nicotine alters the vascular integrity, elasticity of blood vessels and increases phenylepinephrine-induced contraction (Chakkarwar, 2011). Chronic exposure to nicotine causes angiogenesis -mediated inflammation, ischemia, atherosclerosis by binding to α7 nAchR's. In experimental mice model, nicotine is also found responsible for expressing NLRP-3 inflammasomes which in turn cleaves pro-caspase-1 and pro-inflammatory mediators, generating ROS/RNS via down-regulation of special endothelial receptors ZO -1 and ZO-2 which cause macro- and micro-vascular injuries (Zhang et al., 2019). Nicotine also accounts for the up-regulation of ADMA which is an endogenous eNOS inhibitor contributing to increased vasoconstriction (Taneja et al., 2013). Not only endothelial cells but adipocytes have also been reported responsible for vascular injuries as they are found to secrete various inflammatory cytokines (IL-1β, IL-6, TNF-α, NF-κb,)

and adhesion molecules (ICAMS, VCAMS) selectively in the presence of nicotine. Persistent exposure of nicotine acts through pre-ganglionic nerve fibers leading to activation of the sympathetic system, increasing epinephrine levels which ultimately constricts blood vessel, increase cardiac output and creates a burden on the endothelial layer which results in vascular endothelial dysfunction. The endothelial lining of the aortic block of nicotine-treated rats is destructed, and vascular integrity loss is also observed due to the inflammatory infiltration (Si et al., 2017). Concomitant administration of nicotine and a high-fat diet to rats is associated with an increase in diameter and the intense destruction of the endothelial (Liu et al., 2017) suggesting the synergistic effect of nicotine and high-fat diet in the induction of vascular complications. Table IV concludes induction of vascular endothelial dysfunction at different doses of nicotine.

Considering all the studies, it may be concluded that for pharmacological evaluation of vasoprotective agents, nicotine-induced vascular endothelial dysfunction models are widely explored as it provides the clinical relevancy for lifestyle-related vascular endothelial dysfunction. Regarding dose selection of nicotine, it ranges from 0.6 to 2 mg/kg/day that mainly depending upon the frequency of administration and the duration of the study (2-4 weeks; Table IV). Additionally, it also depends upon the species that have been included in the study as Apo E-/- mice established vascular endothelial dysfunction within 12 weeks of orally administering low dose of nicotine (0.1 mg/mL) (Qin et al., 2020). Though a high dose is used for the shorter duration studies, a high dose is associated with mortality. Thus, it may be suggested that small and repetitive doses should be chosen for chronic studies.

## Arsenic-induced

The endothelial cells of the aortic arch of the experimental rats exposed to arsenic-contaminated drinking water for 3 months at the dose of 10 or 50 mg/L are found to be seriously damaged (Guo et al., 2020). The levels of apoptotic factors, vWF, iNOS are elevated, while the level of PEDF (pigment epithelial-derived factor) which helps in maintaining endothelial function is decreased (Guo et al., 2020). Arsenic exposure leads to endothelial cell damage due to the release of apoptotic caspase-3. The detrimental role of arsenic is further supported by a study that reported that after 90 days of consecutive exposure to 100 ppm arsenic through drinking water, rats are found to have increased levels of adhesion molecules, cytokines, and inflammatory mediators while reduced levels of eNOS, iNOS mRNA expression, NO production which leads to vascular endothelial dysfunction-associated cardiovascular disorders (Kesavan et al., 2014). Arsenic has also been noted to be involved in the activation of NADPH oxidase that tends to generate ROS/RNS in the blood vessels ultimately destroying its innermost layer (Ellinsworth, 2015). The detrimental role of arsenic has been further confirmed by the studies stating that exposure to arsenic for 2 weeks at a dose of 1.5 mg/kg relatively increases the expression of TNF-α, ROS/RNS which worsens the vascular endothelial dysfunction (Kaur et al., 2010; Jyoti et al., 2016). Histopathology studies from Wistar rats exposed to arsenic revealed that arsenic exposure is associated with agglutination of erythrocytes followed by infiltration of mononuclear cells, cytoplasmic swelling along morphological changes in nuclei of aortic endothelial cells (Guo et al., 2019b). Observed data from different studies in Table V express that exposure to arsenic at different doses ranging from 2-50 mg/L for 3-6 months via drinking water is a widely used model for chronic study while the exposure through 100 mg/L arsenic in drinking water for 90 consecutive days is an alternative study with relevant alterations in biomarkers. As high doses selected for short-term studies are associated with increased mortality. Therefore, low doses selected for long-term chronic studies are preferred to reduce the mortality and toxicity index. Administration of 1.5 mg/kg of arsenic through intraperitoneal route for 2 weeks is preferred for short-term study.

## Bisphenol-A-induced

An industrial chemical, bisphenol-A, is an endocrine imitating chemical that hampers the normal physiology. Bisphenol-A exposure to the animal (Wild-type CD1 mice) is associated with increased oxidative stress and it contributes to worsening vascular endothelial functioning by mediating the release of inflammatory mediators, up-regulating M-1 macrophage, and activating CAM-KII. Activation of CAM-KII is further associated with the release of apoptotic and necrotic factors which damages endothelial cells of blood vessels (Reventun et al., 2020). Thus, bisphenol-A is considered an important factor in the induction and progression of vascular endothelial dysfunction. Experimental rat model reveals that administered of bisphenol-A 0.1 mg/kg/day for 60 consecutive days, inhibits acetylcholine-induced relaxation and also associated with NOS and COX blockage, increased levels of NADP+ with elevating the level of ROS/RNS resulting in impaired endothelial function. The model also states the involvement of prostanoids, increase levels of which results in vasoconstriction (Friques et al., 2020). It is worthwhile to note that administration of bisphenol-A through contaminated drinking water (4 nM to 400 μM) for 30 days to CD11 mice is found to increase Ang-II expression which uncouples eNOS from its co-factors like FMN, BH-4, promoting oxidative stress. Wistar rats exposed to bisphenol-A at a dose of 35 mg/kg via oral route for long-term study (60 days) decrease acetylcholine-induced relaxation by increasing oxidative stress and lipid peroxidation (Rameshrad et al., 2018). In addition, the concentration of calcium/calmodulin-

**Table V**

**Arsenic-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Pathways | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| Male Wistar rats | 2, 10, and 50 mg/L, orally *ad libitum* | 3-6 months | ↓ pigment epithelium-derived factor<br>↑ protein levels of Fas, FasL, P53, and phospho-p38<br>↑ ROS/RNS | ↓ serum nitric oxide, von Willebrand factor, and nitric oxide synthase | Guo et al., 2019a; Guo et al., 2020 |
| Male Wistar rats | 100 mg/L, orally *ad libitum* | 90 consecutive days | ↓ acetylcholine induced relaxation, aortic eNOS at the levels, NO production<br>↑ production of pro-inflammatory mediators (IL-1β, IL-6, MCP-1, VCAM, ICAM) and serum C-reactive protein | ↑ phenylephrine<br>↑ eNOS and iNOS mRNA expression<br>↓ NO bioavailability and production | Kesavan et al., 2014 |
| Wistar rats of either sex | 1.5 mg/kg/ day, intraperitoneally | 2 weeks | ↑ TNF-α<br>↓ l-arginine converting enzyme | ↑ oxidative stress, TBARS abrogated acetylcholine-induced vasorelaxation<br>↓ serum nitrite/ nitrate concentration, glutathione level | Kaur et al., 2010 |
| Wistar rats of either sex | 1.5 mg/kg/ day, intraperitoneally | 2 weeks | ↑ TNF-α<br>↓ l-arginine converting enzyme | ↑ oxidative stress, super oxides<br>↓ eNOS expression,<br>↓ serum nitrite/ nitrate concentration, glutathione level | Jyoti et al., 2016 |

dependent protein kinase II α in 10 nM bisphenol-A-treated CD11 mice increases in aortic endothelial cells when assessed by microarray analysis which increases the generation of ROS/RNS (Saura et al., 2014). To summarize, bisphenol-A is continuously administered for at least 4 weeks to induce vascular endothelial dysfunction, which may be extended to 16 weeks depending upon the species to species and route of administration. Wild-type CD1 mice exposed to different doses ranging from 4 nM to 400 µM of bisphenol-A in the drinking shows the clinical signs of vascular endothelial dysfunction after 30 days. On the other hand, 35 mg/kg/day orally for 2 months induced vascular endothelial dysfunction in Wistar rats, suggesting that a low dose is sufficient for genetically modified mice as compared to Wistar rats (Table VI).

## Hypertension

Hypertension is one of the independent contributing risk factors for increasing vascular endothelial dysfunction and associated vital organ dysfunction in the initial stages, while the progressed vascular endothelial dysfunction plays a crucial role in worsening hypertension therefore, it has been stated that hypertension and vascular endothelial dysfunction are interlinked. Hypertension alters the normal physiological balance between vasorelaxant and vasoconstrictor factors and stimulates the vascular endothelial cells to release the vasoactive component like Ang-II, inflammatory cytokines, adhesion molecules (CAM, VCAM) resulting in vasoconstriction, thrombosis, and coagulation. A study revealed that hypertension, as well as vascular endothelial dysfunction, is responsible for promoting atherosclerosis. The formation of atherosclerotic plaques interferes with the free flow of the blood (Taddei et al., 2001).

### *A) Deoxycorticosterone induced*

Deoxycorticosterone acetate is a mineralocorticoid that maintains electrolyte and fluid balance. It is used experimentally in animals to induce hypertension (Schenk and McNeill, 1992). Intramuscular administration of deoxycorticosterone on day 1 (20 mg/kg), day 14 (10 mg/kg) to dog (Ueno et al., 1988) shows results similar to that of rat exposed to deoxycorticosterone subcutaneously at the dose of 50 mg/kg twice a week (Basso et al., 1985). Deoxycorticosterone subcutaneous injection at 20 (Han et al., 2019) or 50 mg/kg (Niazi et al., 2020) has shown hypertension-induced vascular endothelial dysfunction by decreasing eNOS bioavailability and altered vascular tone (Kubacka et al., 2019). Deoxycorticosterone-induced hypertension is associated with vascular endothelial dysfunction by the generation of ROS/RNS in the blood vessels that hampers the normal physiological balance between vasoconstrictory and vasodilatory factors. In addition, administration of deoxycorticosterone is associated with increased expression of Ang-II and NADPH oxidase.

Mitochondrial SIRT-3 and SIRT-6 are deacetylase proteins that are expressed in cardiomyocytes and play a crucial role in regulating metabolic and antioxidant function. Interestingly, in deoxycorticosterone-induced

**Table VI**

**Bisphenol A-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Pathways | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| Wild-type CD1 mice | ≤50 mg/kg/ day (a low dose) ethanol dissolved-BPA in drinking water | 4, 8, and 16 weeks | ↑ expression of inflammatory cytokines, TNF-α, RIP-3, caspase-I activating CAM-KII<br>↓ PARP expression | ↑ oxidative stress<br>↓ eNOS expression, TBARS abrogated acetylcholine-induced vasorelaxation<br>↑ lipid peroxidation, decreased glutathione (GSH) levels | Reventun et al., 2020 |
| Male albino Wistar rats | 35 mg/kg/ day orally | 60 days | ↑ vascular cell adhesion molecule<br>↑ lipid peroxidation | ↑ ROS/RNS, oxidative stress<br>↓ acetylcholine-induced relaxation | Rameshrad et al., 2018 |
| Male Wistar rats | 0.1 mg/kg/ day orally | 60 days | ↑ ROS, NADPH oxidase<br>↑ prostanoids-mediated vasoconstriction | ↓ NO<br>impaired acetylcholine-induced relaxation | Friques et al., 2020 |
| Wild-type CD1 mice | 4 nM to 400 µM in drinking water | 30 days | ↑ arterial angiotensin II<br>↑ NADPH oxidase | impairment of acetylcholine relaxation<br>significant superoxide and peroxynitrite accumulation | Saura et al., 2014 |

hypertensive mice, the expression of SIRT-3 and SIRT-6 is noted to be depleted which elevates SOD2 acetylation, caspase-I, Nf-KB activity, VCAM, ICAM and MCP1 levels that are responsible for ROS/RNS genaration leading to vascular endothelial dysfunction and associated cardiovascular disorders (Dikalova et al., 2020). A novel blood pressure regulator GATA-5 also expressed through SIRT-6 by inhibiting Nkx-3 transcription. Hypertension induced by deoxycorticosterone is also associated with increased systolic blood pressure, decreased NO levels in plasma and increased endoplasmic reticulum stress which elevates GRP78, IP3R1 and EGFR levels (Guo et al., 2019a).

***B) Monocrotaline-induced***

Monocrotaline is a macrocyclic pyrazolidine alkaloid obtained from *Crotalaria spectabilis a*ssociated with proliferative vasculitis, remodelling of pulmonary vessels, endothelial dysfunction and oxidative stress. A single administration (60 mg/kg) of monocrotaline induces pulmonary vascular syndrome in animal model within 2-3 weeks (Li et al., 2014). Monocrotaline directly contributes to causing pulmonary hypertension by increasing the ROS/RNS generation and accumulation which results in vascular endothelial dysfunction (Steven et al., 2017). Monocrotaline-induced vascular endothelial dysfunction on a single administration via subcutaneous route influences the normal physiology and acts by decreasing the levels of PPAR-γ, PI3K-Akt, and elevating the levels of inflammatory cytokines, Ang-II and adhesion molecules. These pathways, directly and indirectly, mediates the generation and accumulation of ROS/RNS which tends to decrease acetylcholine-induced vasorelaxation and alters the bioavailability of eNOS leading to the loss of vascular tone, integrity and free flow of the blood (Li et al., 2014). Monocrotaline is responsible for apoptosis of the endothelial cells as it decreases the anti-apoptotic factors (Sahara et al., 2012).

***C) Ethinyl estradiol-induced***

Ethinyl estradiol is one of the major components found in birth controlling pills but is also associated with the risk of the blood clot which can further lead to cardiovascular disease including stroke, hypertension and high cholesterol, which stimulates endothelial cells to release vasoactive components (Balakumar et al., 2007). The combined pill increases the blood pressure, uric acid, C-reactive proteins, PAI-1 and activates the expression of RAAS (angiotensin converting enzyme, ATR's, Ang-II) as well as increases the level of pro-inflammatory mediators. A study stated that the ethinyl estradiol-treated experimental model reverses the relaxation to acetylcholine. The risk of hypertension in ethinyl estradiol-treated rats is accompanied by endothelial degradations and elevated levels of proinflammatory factors and RAAS (Olatunji et al., 2016). The administration of ethinyl estradiol is also considered as a factor for premature atherosclerosis, thrombogenesis and causes change in the endothelial structure and its function.

***D) Spontaneous hypertensive rats***

Spontaneous hypertensive rat is the genetic laboratory hypertensive animal model prepared by inbreeding of Wistar-Kyoto rats with elevated blood pressure. The spontaneous hypertensive rat is characterized by various vascular disorders. Spontaneous hypertensive rat tends to increase the expression of angiotensin and mediates the release of inflammatory factors worsening the integrity of the vascular endothelial altering the

**Table VII**

**Deoxycorticosterone-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Pathways | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| Male Wistar rats | 50 mg/kg in corn oil, subcutaneous | once a week for 5 weeks | COX and NO blockage<br>↑ prostanoid secretion<br>↑ NADPH oxidase subunit p22 | ↑ phenylepinephrine-induced contraction<br>↓ serum/aortic NOS and eNOS | Niazi et al., 2020 |
| Male Wistar albino rats | 20 mg/kg, subcutaneous | 12 weeks Twice a week | ↑ GRP78, $IP_3R1$ and EGFR,<br>↑ expressions of SERCA2 and Bcl2in vessels | ↓ serum/aortic NOS and eNOS, acetyl-induced vasorelaxation | Han et al., 2019 |
| Male Wistar rats | 20 mg/kg in olive oil, subcutaneous | twice weekly for 12 weeks. | ↑ interleukin 6, C-reactive protein, inflammation mediators | ↑ inflammatory cell infiltration, fibrosis and arteriosclerotic alterations<br>↓ endothelial integrity, NOS activity and bioavailability | Kubacka et al., 2019 |
| Dog | day 1 (20 mg/kg, intramuscular) day 14 (10 mg/kg, intramuscular) | Day 1 and day 14 | altered Ang-II, vasopressin activity tachycardia | failed vasodilatory responses<br>↑ intracellular $Ca^{+2}$ | Ueno et al., 1988 |
| Male Wistar rats | 50 mg/kg subcutaneous | Twice a week | altered Ang-II, vasopressin activity tachycardia | failed vasodilatory responses<br>↑ intracellular $Ca^{+2}$ | Basso et al., 1985 |

vascular tone. Spontaneous hypertensive rat is governed by increase ROS/RNS production while it is reported to show altered vasorelaxation towards acetylcholine (Chi et al., 2017).

Taken together, it may be concluded that deoxycorticosterone and monocrotaline are gaining more attention by researchers for the induction of vascular endothelial dysfunction in males. Both models are associated with increased ROS generation and reduction in NO level. However, deoxycorticosterone-induced vascular endothelial dysfunction has been correlated with the slow pathogenetic mechanism as deoxycorticosterone (20 mg/kg, subcutaneously) administration once weekly took 12 weeks to induce vascular endothelial dysfunction. On another hand, a single intravenous injection of monocrotaline in doses ranging from (30-60 mg/kg) takes 2-6 weeks to induce experimental vascular endothelial dysfunction. Among hypertension-associated vascular endothelial dysfunction models, ethinyl estradiol in combination with norgestrel is considered as the gold standard for evaluation of vasoprotective potential of an agent against vascular endothelial dysfunction in females. For short-term studies, especially where the agent is evaluated for its potential to modulate the RAAS pathway, spontaneous hypertensive rat is preferred (Table VII to IX).

## PM-2.5 induced

Tiny and fine inhalable elements of diameter <2.5 µm present in air is one of the most common and most dangerous air pollutants responsible for causing severe health problems including vascular complications. Acute and chronic exposure to PM-2.5 for short or long duration through inhalation not only affects lungs but after reaching to blood vessels can cause vascular endothelial dysfunction and can lead to cardiovascular disorders. PM-2.5 increases the vascular permeability and can alter the barrier function of blood vessels. A study demonstrated that PM-2.5 instigates the release of Ang-II, angiotensin converting enzyme and AT-1R in the blood vessels which subsequently increases oxidative stress (ROS/RNS) by enhancing the release of pro-inflammatory mediators (Qimuge et al., 2019). In addition, PM-2.5 exposed animal model reveals a negative co-relation between eNOS, acetylcholine and TNF-α; as the levels of TNFα in PM-2.5-induced animals are elevated while the acetylcholine-induced vasorelaxation is altered due to the decreased levels of eNOS. It is worthwhile to note that PM-2.5 associated accumulation of adhesion molecule in the blood vessels creates an imbalance between vasoconstricting and vasorelaxing factors (Liang et al., 2019) that further worsen the vascular integrity. Further, PM-2.5 exposure induces the expression of NRF-2, HO-1 that enhances the oxidative stress ultimately, alters the blood vessel integrity. Moreover, eosin and hematoxylin staining of rat aorta revealed that PM-2.5 also increases vascular permeability resulting in interstitial edema and vascular injuries due to the accumulation of inflammatory cells. A decrease in the aortic lumen diameter and an increase in thickness of the endothelial layer is also observed in PM 2.5-treated rat aorta (Dai et al., 2017). Summarized data (Table X) suggest that intratracheal instillation of PM-2.5 from 1.8-16.2 mg/kg is in practice to induce experimental vascular endothelial dysfunction. Intra-

**Table VIII**

**Monocrotaline-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Pathways | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| Male Wistar rats | 30, 40 and 60 mg/kg single intravenous | 2, 4 and 6 weeks i.e. 14th, 28th and 43rd day | ↑ endothelial dysfunction<br>↑ ROS/RNS<br>↑ endothelin-1 activity<br>↑ pulmonary wall thickening | ↓ NO's<br>↑ uncoupling of eNOS<br>↑ adhesion molecules (ICAMS/VCAMS) | Steven et al., 2017 |
| Male Sprague Dawley rat | 60 mg/kg single subcutaneous | single-dose | ↓ expression of PPARγ<br>↓ PI3K-Akt<br>↓ eNOS | ↓ vasorelaxation<br>↑ vascular remodelling<br>↑ ROS/RNS<br>↓ acetylcholine-induced endothelium-dependent vasorelaxation of pulmonary arteries | Li et al., 2014 |
| Sprague-Dawley rats | 60 mg/kg | | ↑ RVSP<br>↑ up-regulation of caspase-III<br>↓ PI3K/Akt<br>↓ anti-apoptotic factors (BCl-2) | Down-regulated eNOS expression<br>↑ ROS | Sahara et al., 2012 |

**Table IX**

**Ethinyl estradiol-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Pathways | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| Female Sprague-Dawley rats | combination of 0.1 µg ethinylestradiol and 1.0 µg norgestrel orally<br>1.0 µg ethinylestradiol and 10.0 µg norgestrel | 6 weeks/day | ↑ ACE, Ang-II and AT-R expression<br>↑ contractile responses to phenylephrine, impaired acetylcholine-induced relaxation<br>↓ eNOS and NO activity | ↑ hypertension<br>↑ uric acid, C-reactive protein<br>↑ PAI-1 | Olatunji et al., 2016 |

tracheal instillation of PM-2.5 from 5.4 mg/kg to Sprague-Dawley rat seems to be the most preferable model.

## Monosodium glutamate-induced

Monosodium glutamate (ajinomotto) acts as a neurotransmitter that affects the physiology of the body, leading to various disorders (Niaz et al., 2018). Excessive consumption of ajinomotto is associated with vascular endothelial dysfunction by increasing the oxidative stress along with significant elevation of matrix metaloprotinase-1 and endothelin-1 levels (Abo Zeid et al., 2020). Injurious role of monosodium glutamate is confirmed as triglycerides, total cholesterol, LDL-C, TNF-α are found to be increased while NO levels are decreased in serum and aorta tissue homogenate. Decreased expression of Akt, PI3K, and $PGI_2$ are also found to be the governing factors of vascular endothelial dysfunction (Leao et al., 2019). Excessive consumption of monosodium glutamate is reported to increase the ROS generation building the stress on vascular endothelial by inducing the release of inflammatory cytokines that finally resulting in induction and progression of vascular endothelial dysfunction (Lobato et al., 2011). Monosodium glutamate-treated rat aortic endothelium showed the thickening of tunica media along with deposition of fats that reduces the lumen of the aorta. In addition, increased inflammatory mediators are also observed (Abo Zeid et al., 2020). Complied data from different studies in Table XI reflect no variation in dose of monosodium glutamate to induce vascular endothelial dysfunction irrespective of age of the animals. All the models showed common pathogenetic pathways for vascular endothelial dysfunction induction and progression.

## Uric acid-induced

Excessive level of uric acid to severe disorders like gout, diabetes, kidney stones, etc (Fathallah-Shaykh et al., 2013) along with vascular endothelial dysfunction, which is one of the most serious condition which can lead to different cardiovascular disorders. Hyperuricemia is often associated with increased oxidative stress, lipid peroxidation and decreased levels of SOD, nitric oxides, glutathione, thus play a crucial role in induction and progression of vascular endothelial dysfunction. In addition, uric acid-treated animal model reveals that hyperuricemia is also associate with elevation of increased inflammatory mediators, chemokines,

cytokines and adhesion molecules like VCAM (Oyabambi et al., 2020). Further, the expression of various pathways like Nf-Kb, TNF-α enhanced due to accumulation of uric acid. Moreover, uric acid exposure also activates the RAAS system which increases the expression of Ang-II, angiotensin converting enzyme and angiotensin II receptor type 1 which contributes in causing vascular endothelial dysfunction (Balakumar et al., 2008c). Animal species, dose & route, duration along with the associated pathways have been summarized in Table XII.

## Homocysteine-induced

Excessive levels of homocysteine, an independent risk factor, contributing to cause vascular endothelial dysfunction and is also associated with cardiovascular system disorders (Moretti and Caruso, 2019; Esse et al., 2019). Homocysteine quenches the level of NO by reducing the bioavailability and uncoupling NOS from its co-factors (Kumar et al., 2017; Ji et al., 2021). Homocysteine-induced vascular endothelial disorder is associated with increased oxidative stress which further instigates the release of pro-inflammatory, apoptotic and pro-thrombotic factors while down-regulates VEGF, p-tyr-VEGFR2 and phospho-focal adhesion kinase (Tyr397) (Lan et al., 2011; Wang et al., 2019). In addition, phenylepinephrine-induced vasoconstriction and vWf is also noted to be increased. Hyperhomocysteinemia also tends to increase the expression of Ero-1 (endoplasmic reticullum oxidoreductin) which tends to trigger oxidative stress by mediating the generation of ROS/RNS. Homocysteine increases apoptosis by enhancing the release of caspase-3 and Bax (Ren et al., 2016). Animal species, dose & route, duration along with the associated pathways have been summarized in Table XIII.

## High fat diet-induced

Obesity is a widespread metabolic disorder which serves as a risk factor for complicating various micro- and macro-vascular diseases. Different studies stated that consumption of high fat diet is associated with altered NO's function as a result leading to vascular endothelial dysfunction. It is considered as the major cause of morbidity and mortality (Zhao et al., 2020). Increased level of LDL's and v-LDLs in the blood vessels leads to the formation of atherosclerotic

**Table X**

**PM 2.5-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Pathways | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| Sprague-Dawley rats | 1.8, 5.4 and 16.2 mg/kg intratracheal instillation | every 3 days for 30 days | ↑ expression of JAK/STAT signalling pathway<br>↑ expression of TLR4/p38/NF-κB pathway<br>↑ arterial thrombus formation | ↑ inflammatory mediators (IL-6, VCAM, ICAM, MCP, CRP)<br>disruptive fibrinolysis | Liang et al., 2019 |
| Sprague-Dawley rats | | once every 3 days for 28 days | ↑ phosphorylation of STAT3<br>up-regulation of miR-21<br>inhibits TIMP3/MMP9 signalling | alteration in the barrier function of the vascular endothelium | Dai et al., 2017 |

**Table XI**

**Monosodium glutamate-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Pathways | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| New born Wister Albino rats | 4 mg/g orally | 14 days | ↑ expression of endothelin-1<br>↑ expression of MMP-1<br>↑ ROS/RNS<br>↑ NADPH oxidase<br>↑ NOX-4 expression | ↓ eNOS and NO activity<br>↑ inflammatory response<br>↑ VSMC | Abo Zeid et al., 2020 |
| New born Wistar rats | 4.0 g/kg subcutaneous injection | second to the sixth day after birth | ↑ levels of LDL's, vLDL's and triglycerides<br>↑ ROS/RNS<br>↑ phenylepinephrine-induced vasoconstriction | ↓ eNOS levels<br>loss integrity of vascular endothelium | Lobato et al., 2011 |
| Male Wistar rats | 4.0 g/kg subcutaneous injection | first 5 days | ↓ Akt/ PI-3K expression<br>↑ ROS/RNS<br>↑ cholesterol<br>↑ thrombosis, endothelin-1<br>↑ platelet adhesion and aggregation | ↓ acetylcholine-induced vasorelaxation<br>↓ expression of $PGI_2$, NO | Leao et al., 2019 |

**Table XII**

**Uric acid-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Pathways | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| Male Wistar rats | 150 mg/kg/ day intraperitonially | 3 weeks | ↑ generation of ROS/RNS<br>↓ acetylcholine-induced endothelium-dependent relaxation<br>↑ activation of NADPH oxidase<br>↑ xanthine oxidase<br>up-regulate the expression of various fibroblast growth factor, tumor necrosis factor and plasminogen activator inhibitor-1<br>↑ mononuclear leukocyte adhesion<br>↑ expression of adhesion molecules such as VCAM-1 and ICAM | ↓ aortic and serum nitrite/nitrate,<br>↑ aortic superoxide anion generation,<br>↓ acetylcholine-induced endothelium-dependent relaxation<br>impairing endothelial integrity, inducing vascular oxidative stress<br>↑ serum TBARS<br>down-regulating the expression of eNOS<br>impairing endothelial integrity | Balakumar et al., 2008c; |
| Female Wistar rats | High salt feed consisting of 8 % NaCl– plasma uric acid level rise to 15 U/mL | 6 weeks | ↑ expression of adhesion molecules such as VCAM-1 and ICAM | ↓ aortic and serum nitrite/nitrate,<br>↑ aortic superoxide anion generation | Oyabambi et al., 2020 |

plaques which interfere with the free flow of blood. High concentration of fat is responsible for stimulating the vascular endothelial cells to release vasoactive substances, thrombotic factors and decreases vasodilatory substances (NO, $PGI_2$). High cholesterol increases the lipid peroxidation of LDL, which tends to increase the oxidative stress by generating large amounts of ROS/RNS, alters the expression of PI3K-Akt, eNOS and SIRT pathways which leads to the loss of vascular tone, integrity, arginase activity (Huang et al., 2018). Male CDH5 CreERT2 Ftoflox/flox mice fed on 60% high fat diet for the duration of 8 weeks showed obesity with increased level of cholesterol which directly and indirectly lead to increase the oxidation of LDL's or vLDL's which gets accumulated in the blood vessels resulting in the formation of atherosclerotic plaques and narrowing the lumen of the blood vessels (Kruger et al., 2020). Apart from the mice or rats, male hybrid flanders rabbits is employed to induce obesity and subsequent vascular endothelial dysfunction with standard rabbit chow added with 18% of fats (10% corn oil + 8% lard) for 6 weeks (Alarcon et al., 2018). Numerous studies used different percentage of fat to induce obesity with different composition, suggesting that there is huge scope to formulate their high fat diet to induce obesity related vascular endothelial dysfunction.

## Glucocorticoid-induced

Glucocorticoid (play crucial role on metabolism and glucocorticoid analogues like prednisolone, dexamethasone) is also widely employed for reducing the inflammation, fight certain cancers, autoimmune disorders but due to their severe effects on vasculature the use is limited (Yang and Zhang, 2004). Excessive levels of glucocorticoids are associated with vasoconstriction, altered capillary permeability, Atherosclerosis and increased blood pressure i.e. hypertension which overall results in vascular endothelial dysfunction (Ross and Linch, 1982). Glucocorticoids has been noted to increase the superoxide levels which led to the uncoupling of eNOS from its co-factors resulting in NO's dysfunctioning (Akaike and Matsumoto, 2007). In addition, mice treated with dexamethasone (0.1-3 mg/kg) revealed that glucocorticoid involves down-regulation of eNOS, down-regulation of cationic amino acid transporter-1, and 3) and generation of reactive oxygen species for induction of vascular abrasion (Schafer et al., 2005). It is worthwhile to note that endothelium-dependent vasodilation in response to acetylcholine (0.1-10 μM) is reduced by dexamethasone in a dose-dependent fashion (Schafer et al., 2005).

## Hypochlorite-induced

Hypochlorite is chlorine ox-anion widely explored for it disinfectant and exposure to hypochlorite can cause skin or eye irritation, severe injuries, burns etc. Hypochlorite itself is a superoxide mediator which is responsible for inducing oxidative stress (Radovits et al., 2013). This generated oxidative stress is responsible for uncoupling the eNOS from its co-factors altering the NO's bioavailability. A study also stated that hypochlorite intensifies the NADPH oxidase activity leading to overproduction of ROS/RNS molecules which creates an imbalance between antioxidant and free radicle resulting in vascular endothelial dysfunction (Tian et al., 2017). Pre-treatment with hypochlorite results in impaired endothelial-dependent vasorelaxation and acetylcholine-induced vasorelaxation and increases the formation of atherosclerotic plaques

**Table XIII**

**Homocysteine-induced vascular endothelial disorders**

| Species | Dose and route | Duration | Pathways | Altered biomarkers | Reference |
|---|---|---|---|---|---|
| Male Sprague Dawley rats | 3% methionine (w/w) in feed | 8 weeks | ↑ endothelin-1, Ang-II<br>↑ TXA-2<br>↓ PGI-2<br>↑ expression of RAAS | rapid decomposition of NO<br>↑ thrombosis<br>inhibition of NO production and eNOS | Ji et al., 2020 |
| Male Wistar rats | L-methionine 1.7 g/kg/day, orally | 32 days | ↑ phenylepinephrine-induced constriction<br>↑ ROS/ RNS<br>↓ NO bioavailability<br>↓ SOD, GSH. CAT | attenuated vasorelaxation<br>impaired eNOS activity<br>↑ TBARS | Kumar et al., 2017 |
| Male Sprague-Dawley rats | L-methionine 1 g/kg/day intragastricly | 4 weeks | ↓ PI3K, Akt<br>↑ oxidative stress<br>↓ NO bioavailability | down-regulating the expression of eNOS<br>impairing endothelial integrity | Lan et al., 2011 |
| Male Sprague-Dawley rats | L-methionine by 1 g/kg/ day intra-gastricly | 4 weeks | ↑ apoptotic factors, and apoptosis of endothelial cells<br>↑ caspase-3 , bax<br>↓ Akt expression | ↑ vascular injury<br>impaired endothelium-derived vasorelaxation<br>↓ eNOS activity<br>↓ NO bioavailability | Ren et al., 2016 |

(Ding et al., 2014). Isolated aorta of rat exposed to hypochlorite at the dose of 100-400 µM is found to be associated with decreased acetylcholine-induced vasorelaxation. This alteration in vascular tone is due to eNOS uncoupling and decreased the NO bioavailability (Tian et al., 2017). In addition, the exposed aorta is found to associated with decreased PARP expression, increased lipid peroxidation, release of inflammatory mediators and increased DNA damage resulting in worsening of vascular endothelial dysfunction (Radovits et al., 2013).

## Endotoxin-induced

Lipopolysaccharide is one of the most common endotoxins formed by covalent bond between lipid A and polysaccharide. It is found in the outer layer of the Gram negative bacteria (Lynn and Golenbock, 1992). Endotoxins stimulate the endothelial cells to activate fibroblasts (Echeverría et al., 2014) and enhance the release of pro-inflammatory cytokines, and increase oxidative stress burst which result in causing fever, inflammation, aseptic shock and death. Lipo-polysaccharide (1 mg/kg intraperitoneal once) to mice is noted to be associated with increased mRNA levels of IL-6, and IL-8 (Huang et al., 2019). While single intravenous administration of *E. coli* 15 mg/kg established vascular endothelial dysfunction in rats within 6 hours (Balakumar et al., 2007). Activation of fibroblast leads to endothelial fibrosis which tends to alter the NO's bioavailability leading to vascular endothelial dysfunction especially pulmonary vascular injury (Chuaiphichai et al., 2016; Huang et al., 2019).

## Overectomy/estrogen deficiency-induced

Oestrogen, an endocrine hormone has been noted to regulate eNOS activity which helps to release sufficient amount of NO for the vasodilation of the vessel. Low levels of oestrogen in post-menopausal female is one of the causes for increased risk of cardiovascular disease. Low levels of oestrogen up-regulates RAAS system which results in hypertension and is also responsible for atherosclerotic plaque formation while it also increases the concentration of free radicles which activates endothelial cells to cause vascular endothelial dysfunction (Wassmann et al., 2001). Thus, to establish the correlation between the hypertension, oestrogen deficiency associated vascular endothelial dysfunction, overiectomized spontaneously hypertensive female rats are considered to be gold standard and are widely used to evaluate the therapeutic agents for their potential to manage the vascular complications in menopausal condition.

## Conclusion

To understand complex pathogenesis and to develop a new therapeutic alternative for treating vascular endothelial dysfunction animal models are widely used. Continued utilization of these experimental models simulating human vascular endothelial dysfunction, particularly those that combine other clinically relevant comorbidities like obesity, nicotine intake, hyperuricemia or hypercholesterolemia, may open a new vista in development of effective strategies to address the vascular complications. Nevertheless, a restrained methodology is mandatory while experimental findings in these models are extrapolated to human vascular endothelial dysfunction.

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## Conflict of Interest

Authors declare no conflict of interest

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