RESEARCH PAPER

Endothelial Protective Effects of a Polyherbal Formulation Against Diabetes- and Nicotine-Induced Vascular Dysfunction: Evidence from Functional and Biochemical Studies in Rats

Parkhi Rastogi[1], Gunjan Singh[2*]

[1]School of Pharmacy, Sharda University Greater Noida, U.P, INDIA, 201310, ORCID -0000-0003-0980-8295.

[2]School of Pharmacy, Sharda University Greater Noida, U.P, INDIA, 201310.*

Email:gunjan.singh2@sharda.ac.in, ORCID: 0000-0001-5973-9270

ABSTRACT

Introduction: Vascular endothelial dysfunction (VED) is a key factor in cardiovascular disease development, characterized by impaired blood flow regulation and reduced oxygen delivery. In diabetes, VED is primarily driven by decreased nitric oxide (NO) bioavailability and increased oxidative stress. This study evaluated the vasoprotective potential of a polyherbal extract (PHE) comprising Vitis vinifera, Allium sativum, Gymnemasylvestre, and Murrayakoenigii against metabolic and toxin-induced endothelial dysfunction.

Methods: Sixty male Wistar rats were divided into ten groups, including normal control, disease control (diabetic and/or nicotine-induced), atorvastatin-treated (30 mg/kg), and PHE-treated groups (100 and 400 mg/kg). Diabetes was induced using streptozotocin (60 mg/kg, i.p.), while endothelial dysfunction was induced using nicotine (2 mg/kg/day, i.p.) for four days. Endothelial function was assessed using acetylcholine and sodium nitroprusside in isolated aorta. Biochemical parameters included blood glucose, lipid profile, nitrite/nitrate levels, TBARS, and superoxide anions.

Results: Disease groups showed significant endothelial dysfunction, hyperglycemia, dyslipidemia, reduced NO levels, and elevated oxidative stress. PHE treatment significantly improved endothelium-dependent vasorelaxation, increased nitrite/nitrate levels, reduced oxidative stress markers, and normalized metabolic parameters. The higher dose (400 mg/kg) demonstrated effects comparable to atorvastatin.

Discussion & Conclusion:

The study highlights oxidative stress and NO depletion as central mechanisms in VED. The polyherbal formulation exerted strong antioxidant and vasoprotective effects through multi-target actions, including glycemic and lipid regulation. Its comparable efficacy to atorvastatin suggests that PHE may serve as a promising alternative or adjunct therapy for managing endothelial dysfunction associated with diabetes and toxic insults.

Keywords: Endothelial dysfunction;Streptozotocin-induceddiabetes;Aortic ring reactivity;Lipid modulation; Cardiovascular protection;Nitric oxide bioavailability.

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INTRODUCTION

Cardiovascular disease is still the leading cause of death worldwide, with a particular focus on vascular endothelial dysfunction (VED) due to early onset of atherosclerosis and related vascular complications. The vascular endothelium regulates vascular homeostasis via control of nitric oxide (NO) production, control of vascular tone, inflammatory signalling, and oxidative balance. Endothelial dysfunction is characterised by impaired endothelial-dependent retrograde flow through the use of axis-based, endothelial-controlled vasodilatory response and little or no response to pro-inflammatory or reactive oxygen species (ROS) and little or no NObioavailability.Diabetes (one of the most recognised metabolic contributors) damages endothelium. An example of the experimental induction of diabetes through STZ is a commonly used model for hyperglycaemia in producing vascular damage through excessive production of ROS, lipid peroxidation, and impairment of endothelium-dependent retrograde flow to vasodilation [1, 2].Chronic nicotine use, one of the most recognised cardiovascular risk factors, produces vascular endothelial dysfunction via increased sympathetic activity, increased oxidative stress, and decreased acetylcholine-mediated NO release [3, 4].While there are many differences in diabetes caused by STZ and nicotine, oxidative stresses result in commonality between both diabetic mice and those receiving chronic nicotine treatment, resulting in impaired vascular function and endothelium injury [5]Pharmacological agents such as atorvastatin exhibit endothelial protective properties that extend beyond lowering lipids; provide an increase in NO bioavailability and decrease oxidative stress; long-term

treatment may be inadequate due to the multiplicity of mechanisms for vascular injury [6,7], leading to an increased interest in the use of phytotherapeutic approaches to target the causes of vascular injury caused by hyperglycaemia, dyslipidaemia, oxidative stress, and endothelium dysfunction.

Bioactive phytochemical components found in Vitis vinifera (grape), Allium sativum (garlic), Gymnemasylvestre (gymnema), Murrayakoenigii (curry leaf) have shown to exhibit antioxidant, antidiabetic, antihyperlipidemic, and vascular protective properties when tested in experimental models [8]. However, although each individual plant extract is promising, there has not been much scientific evidence to support their combined effect with respect to their use as a standardized polyherbal formulation for endothelial dysfunction.

Thus, to address this issue the present study was conducted in order to evaluate the protective effects of a polyherbal extract (PHE) that consists of equal parts of four medicinal plants on endothelium (vessel lining) injury in Wistar rats that occurred due to both STZ (streptozotocin) and nicotine administration [9]. An integrated approach to evaluating the mechanism of action for the potential use of a multi-targeted phytotherapeutic concept for the prevention of both metabolic and toxin-induced vascular endothelium injury was employed through assessing the isolated aortic ring reactivity for both endothelial dependent (endothelium-dependent, relaxant; EDR) and endothelial independent (endothelium-independent, relaxant; EIR) vasorelaxation via an in vitro experiment; and measuring nitrite/nitrate levels, oxidative stress markers, fasting blood glucose and lipid profile parameters; for all experiments, in all rats before administration as well as after the rats were placed into the different experimental groups (four groups)[10].

MATERIAL AND METHODS

Ethical Approval

Approval of the IAEC of Subharti Medical College was obtained 120G/PO/RE/S/08/CCSEA/24-07 for the animal studies and CCSEA guidelines were followed throughout the experiment.

Animal

Sixty Male Albino Wistar rats, 6-8 weeks, 150-180 g, were obtained from the Animal House of Subharti Medical Collegeand housed at 25 ±5 °C, and 60 ±10% humidity in a well-ventilated animal house under a 12-hour light dark-cycle. The animals were housed in separate cages and had free access to water and food.

Acute Toxicity Study

Equal quantity of plants extracts of *Vitis vinifera, Allium sativum, Gymnema Sylvestre* and *Murrayakoenigii*were mixed (1:1:1:1 ratio) andAcute toxicity under the guidelines of OECD 423 was performed (11). Animals were administered with different doses of 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg.

Induction of VED

Streptozotocin Induction Model of Diabetes mellitus

The diabetic rat model was developed by a single intraperitoneal (i.p.) injection of streptozotocin (STZ) (60 mg/kg) freshly dissolved in sterile sodium citrate buffer (0.1 M, pH 4.5). One week later, tail blood samples were obtained from each rat for fasting blood glucose measurements using glucometer. Rats with 250 mg/dl or greater fasting blood glucose levels were considered as diabetic rats and included in the study. (12)(13)(14).

Nicotine Model for induction of VED

Administration of nicotine at 2 mg/kg/day, intra peritoneal for four weeks causes vascular endothelial dysfunction (15)(3). This administration produced VED in rats by attenuating acetylcholine-induced endothelium-dependent relaxation in the isolated aortic ring preparation, decreasing aortic and serum nitrite/nitrate concentration, impairing endothelial integrity, and inducing vascular oxidative stress (16).

Intervention: Poly Herbal Extract (PHE)

Provision of PHEof*Vitis vinifera, Allium sativum, Gymnema Sylvestre* and*Murrayakoenigii* in ratio of 1:1:1:1begins since the diagnosis of diabetes mellitus and will be given for four weeks at two different doses of i.e. 100 and 400mg/kg/day.

However, these dose range were lies between previous studies over these plants in different pre-clinical evaluations. For examples; *Vitis vinifera* 500/400 mg/kg/day for 28 days in doxorubicin hydrochloride induced cardiorenal injury (17). And 78/ 156/ 312 mg/kg/day for 14 days of *Allium sativum* extract for alterations in cellular integrity, and the expression of enolase-2 and GFAP in the prefrontal cortex(18). And *Gymnemasylvestre*100 and 200 mg/kg/day for 5 days administered orally to evaluate the anti-ulcer activity in NSAIDs and pylorus ligation-induced rat models (19). As well as 200 mg/kg/day for 14 days orally of aqueous and methanolic extract of *Murrayakoenigii* leaves for HFFD induced hyperlipidemia in rats(20). For Atorvastatin, the nicotine treated (2 mg kg−1 day−1, i.p., 4 weeks) rats received atorvastatin (30 mg kg−1 day−1, p.o.) in a study which evaluated effect of benfotiamine, a thiamine derivative, in nicotine and uric acid-induced vascular endothelial dysfunction (VED) in rats. (5).After treatment with PHE for 4 weeks, all rats were fasted overnight and anaesthetized. Blood samples were drawn via intracardiac puncture, centrifuged to obtain plasma, and used to assess fasting plasma glucose (FPG). Thoracic aortas from the rats were immediately cut into rings 3 mm in width for the subsequent vascular function experiments. The remaining aortas were stored at −80 °C for future processing.

Table1: Animal Groups and Study Protocol

Group	Group Name	Intervention	Animal Allotted
1	Normal Control	Normal without any Intervention	06
2	Positive Control (ATS)	Atorvastatin 30 mg/kg/day p.o for 28 days	06
3	Negative Control (STZ)	Streptozotocin 60 mg/kg once via i.p	06
4	Standard (STZ+ATS)	Streptozotocin 60 mg/kg once via i.p + Atorvastatin 30 mg/kg/day p.o for 28 days	06
5	Test I (STZ + Dose I)	Streptozotocin 60 mg/kg once via i.p + PHE 100 mg/kg/day for 28 days via gavage	06
6	Test II (STZ + Dose II)	Streptozotocin 60 mg/kg once via i.p + PHE 400 mg/kg/day for 28 days via gavage	06
7	Negative Control (Nicotine)	Nicotine 2 mg/kg/day via i.p for 28 days	06
8	Standard (Nicotine +ATS)	Nicotine 2 mg/kg/day via i.p for 28 days + Atorvastatin 30 mg/kg/day p.o for 28 days	06
9	Test I (Nicotine + Dose I)	Nicotine 2 mg/kg/day via i.p for 28 days + PHE 100 mg/kg/day for 28 days via gavage	06
10	Test II (Nicotine + Dose II)	Nicotine 2 mg/kg/day via i.p for 28 days + PHE 400 mg/kg/day for 28 days via gavage	06
Total Animals			60

Estimation of Lipid Profile

The serum levels of triglyceride (TGL), total cholesterol (TC) and high-density lipoprotein-cholesterol (HDLC) were determined spectrophotometrically, using enzymatic colorimetric assay kits (Randox, Northern Ireland) while low-density lipoprotein cholesterol (LDLC), very low-density lipoprotein cholesterol (VLDLC) and antiartherogenic index (AAI) were calculated. Animals were fasted for 12-16 hours before blood samples were obtained. About two milliliters of blood was collected from the tail vein of each rat into an ice-cold centrifuge tubes. The blood samples were centrifuged in a Denley BS400 centrifuge (England) at 5000 R.P.M for 5-minutes. The supernatant (serum) collected was assayed for the serum levels of TGL, TC and HDL-C using the Randox Biochemical kits while LDL-C and VLDL-C were calculated (21).

Estimation of serum or aortic nitrite/nitrate concentration

The serum or aortic nitrite/nitrate concentration was estimated spectrophotometrically using the Greiss method. The standard graph of sodium nitrite (0.5–40 μM) was plotted to calculate concentration of serum nitrite/nitrate (μM) and aortic nitrite/nitrate (μM/mg of protein) (22)(23). The protein concentration in homogenized aortic preparation was estimated by Lowry's method(24)(25).

Estimation of serum TBARS & Superoxide anion

Estimation of serum thiobarbituric acid reactive substances (TBARS) The serum concentration of TBARS was estimated spectrophotometrically to assess oxidative stress. A standard graph using 1, 1, 3, 3 tetramethoxypropane (1–50 μM) was plotted to calculate the concentration of TBARS (12)(13)(16).Estimation of superoxide anion generation was estimated spectrophotometrically by using nitroblutetrazolium (NBT) (12)(13)(26).

Aortic ring preparation

Vascular reactivity will be studied by aortic ring preparation method (27)(28). In brief, aortic rings were suspended between 2 steel hooks in jacketed organ chambers with 10 mL Krebs solution (mM, NaCl 118.5, KCl 4.7, CaCl2 1.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25.0, and glucose 11.0) aerated with carbogen and kept at 37 ℃ for 1 hour. Force transducers were used to detect isometric tension changes, which were recorded using a polygraph (Nanjing MedEase Science and Technology Co., China). After pre-contraction with 10−6 M phenylephrine (α1-adrenergic receptor agonist), cumulative relaxation-response curves to 10−8–10−4 M acetylcholine and 10−10–10−6 M sodium nitroprusside were recorded, respectively. Relaxant responses induced by acetylcholine and sodium nitroprusside were calculated as a percentage of the response to phenylephrine.

Mercaptopropionylglycine (MPG), a powerful superoxide anion scavenger, is able to enhance superoxide anion-mediated nitric oxide (NO) oxidative inactivation in aortic rings (29). In the present experiment, the response to 10−8–10−4 M acetylcholine was re-tested in aortic rings previously incubated with 10 μM MPG. The difference between acetylcholine-induced maximum relaxation values

obtained with and without the presence of MPG was taken to represent the superoxide anion-mediated NO oxidative inactivation.

Statistical analysis

Data were presented in the mean ± standard deviation and analyzed by ANOVA test. The p value < 0.05 was considered statistically significant.

Acute oral toxicity study

No mortality was found and the equal amount of PHE was found to be safe up to 2000mg/kg.

Group	Dose	Mortality					Result
		0.5 Hrs	2.0 Hrs	4.0 Hrs	7 Days	14 days	
1	5 mg/g	0	0	0	0	0	
2	50 mg/g	0	0	0	0	0	
3	300 mg/g	0	0	0	0	0	
4	2000 mg/g	0	0	0	0	0	(Safe)

In this toxicity study (the 14 days test), we check whether the animals (rodents) developedtoxicity at a dose of 2000mg/kg. If there is no toxicity, we consider the LD50 to be >2000mg/kg.Taking this assumption, we calculate 1/10 of 2000mg/kg as an effective dose foradministration: 200mg/kg. And, to get comparative doses, we take half and double of 200mg/kg(100mg/kg & 400mg/kg). This technique is common in many journal articles conductingactivitytests on plant extracts without any appropriate references

Effect of PHE on Body Weight

STZ and Nicotine cause significant reduction in body weight which were normalized by PHEadministration in dose dependent manner.

Table2: Body Weight Difference after PHE administration

Group	Group Name	Initial (g)	Final (g)
G 1	Normal Control	143±3.67	145±2.45
G 2	Positive Control (ATS)	141±4.34	142±3.65
G 3	Negative Control (STZ)	146±3.76	121±5.56
G 4	Standard (STZ+ATS)	119±5.56	120±2.36
G 5	Test I (STZ + Dose I)	122±4.54	128±3.45
G 6	Test II (STZ + Dose II)	123±3.27	134±4.61
G 7	Negative Control (Nicotine)	142±2.40	124±2.30
G 8	Standard (Nicotine +ATS)	144±3.20	125±2.11
G 9	Test I (Nicotine + Dose I)	143±4.65	128±2.53
G 10	Test II (Nicotine + Dose II)	145±2.30	133±2.43

Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), #P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), ##P<0.05 *vs.* Negative Con rats (G7).

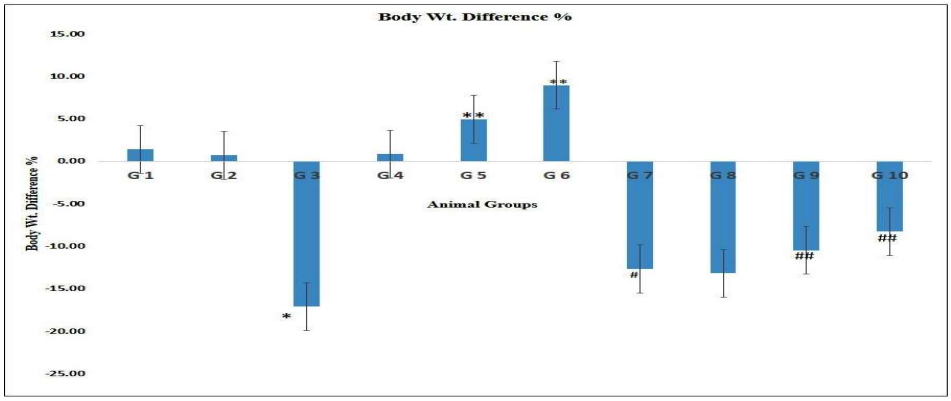

Figure1: Effect of PHE on Body Weight.

Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

Fasting Blood Glucose

STZ and Nicotine cause significant increment in Fasting

Blood Glucose which were significantly reduced by PHE administration in dose dependent manner.

Table: Fasting Blood Glucose after 4-week study

Group	Group Name	Fasting Blood Glucose (mg/dL) After 4 Week Study
G 1	Normal Control	93±1.21
G 2	Positive Control (ATS)	101±1.31*
G 3	Negative Control (STZ)	317±3.51*
G 4	Standard (STZ+ATS)	214±4.32**
G 5	Test I (STZ + Dose I)	187±6.34**
G 6	Test II (STZ + Dose II)	139±4.85**
G 7	Negative Control (Nicotine)	268±5.50#
G 8	Standard (Nicotine +ATS)	253±4.06##
G 9	Test I (Nicotine + Dose I)	189±3.25##
G 10	Test II (Nicotine + Dose II)	128±5.71##

Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

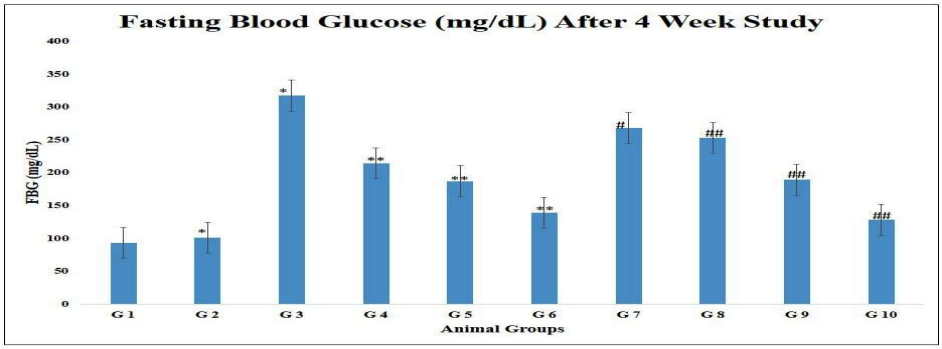

Figure2: Fasting Blood Glucose (mg/dL).

Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

Effects of PHE (Poly Herbal Extract) on vascular relaxation responses to acetylcholine and sodium nitroprusside

As shown in Figure, the results of the aortic ring experiments revealed that the addition of acetylcholine after pre-contraction with phenylephrine induced relaxation responses in all groups of rats in a concentration-dependent manner. Acetylcholine-evoked endothelium-dependent relaxation was obviously decreased in the STZ rats compared with the Con rats. Treatment with PHE significantly ameliorated the impairment of the endothelial-dependent vasodilator response. Furthermore,no differences were observed between the four groups in terms of endothelium-independent vasorelaxation induced by sodium nitroprusside (Figure 2B).

Table3: pD2 values of agonists in aortae from rats in study groups

Group	Group Name	*Phenylephrine*	*Acetylcholine*	*Sodium nitroprusside*
G 1	Normal Control	6.08±0.11	6.79±0.13	8.21±0.06
G 2	Positive Control (ATS)	5.87±0.09*	6.98±0.11*	8.23±0.02*
G 3	Negative Control (STZ)	6.37±0.13*	6.21±0.21*	8.13±0.05*
G 4	Standard (STZ+ATS)	5.77±0.05**	6.61±0.06**	8.18±0.03**
G 5	Test I (STZ + Dose I)	6.11±0.21**	6.46±0.04**	8.14±0.02**
G 6	Test II (STZ + Dose II)	5.89±0.05**	6.68±0.05**	8.15±0.13**
G 7	Negative Control (Nicotine)	7.03±0.21#	5.68±0.07#	8.15±0.03#
G 8	Standard (Nicotine +ATS)	6.86±0.11##	5.97±0.03##	8.17±0.01##
G 9	Test I (Nicotine + Dose I)	6.89±0.05##	5.75±0.02##	8.15±0.13##
G 10	Test II (Nicotine + Dose II)	6.47±0.13##	5.67±0.03##	8.20±0.21##

Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

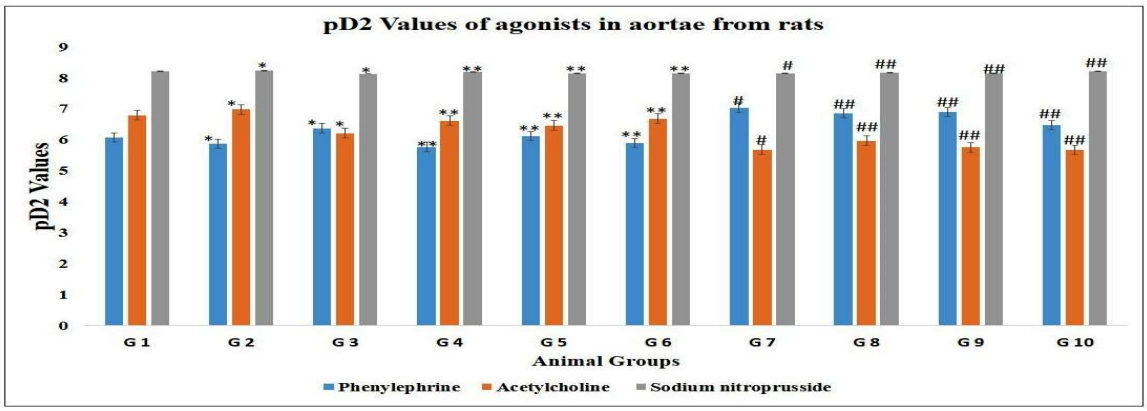

Figure3: pD2 Value of Agonist in Aortae.
Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

Table4: Effect of STZ, Nicotine, and Atorvastatin on pD_2 Values with Mechanistic Insights

Drug	Effect, Normal Value and Mechanism (STZ)	Effect, Normal Value and Mechanism (Nicotine; Sympathomimetic)	Effect, Normal Value and Mechanism (Atorvastatin)
Phenylephrine	**Increased, ~6.5,** Endothelial dysfunction reduces NO → heightened vasoconstriction via α_1-receptors	**Increased, ~6.5,** Stimulates sympathetic ganglia → ↑ NE release → ↑ α_1-receptor response	**Decreased**, ~7.0, Improves NO bioavailability → blunts α_1-mediated vasoconstriction
Acetylcholine	**Decreased**, ~7.2, Damaged endothelium → impaired ACh-induced NO release → ↓ vasodilation	**Decreased**, ~7.2, Sympathetic dominance suppresses parasympathetic ACh effect	**Increased**, ~6.3, Restores endothelial function → enhances ACh-induced NO-mediated vasodilation
Sodium Nitroprusside	**Slight**↓, ~7.4, Oxidative stress scavenges NO → slightly reduced vasodilatory effect	↔ **No Change**, ~7.4, SNP acts directly on smooth muscle → unaffected by autonomic tone	↔ **Slight** ↑, ~7.1 Antioxidant action preserves NO activity → slight enhancement

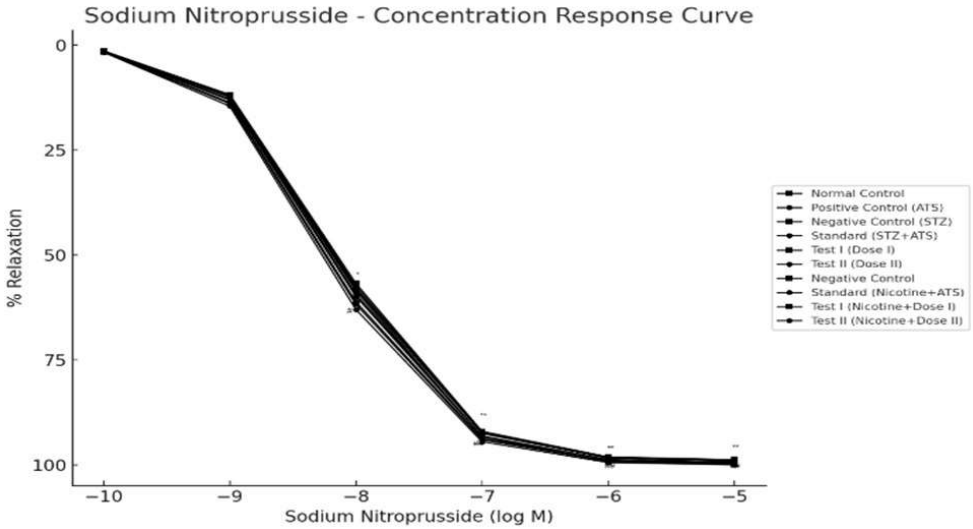

Figure 4: Acetylcholine concentration–response curve.
Data are expressed as mean ± SEM. *P<0.05 vs Control (G1);**P<0.05 vs STZ control (G3); #P<0.05 vs Control (G1);##P<0.05 vs Nicotine control (G7).**

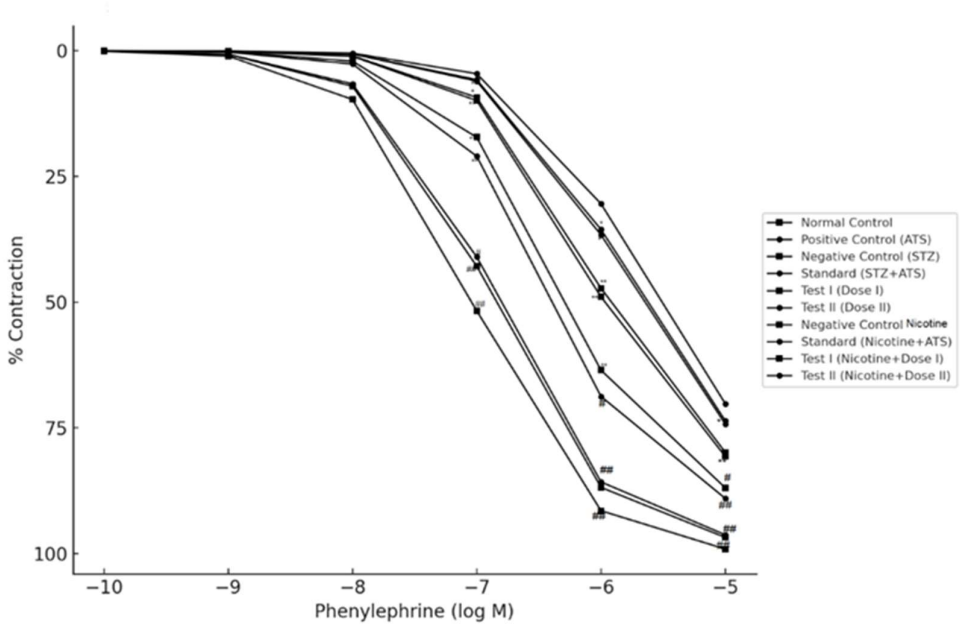

Figure5: AcetylcholineConcentration Response Curve.
Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

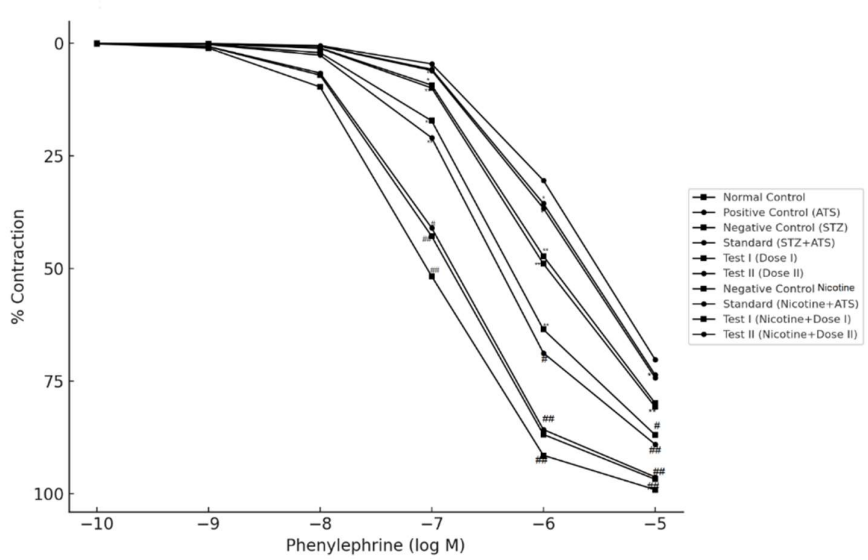

Figure6: Phenylephrine Contraction Response Curve.
Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

Effect of pharmacological interventions on serum and aortic nitrite/nitrate concentration

The serum and aortic concentration of nitrite/nitrate was noted to be reduced in STZ and nicotine administered rats when compared with normal rats. However, treatment with Atorvastatin and PHE significantly attenuated nicotine induced decrease in serum and aortic nitrite/nitrate concentration.

Effect of pharmacological interventions on serum TBARS and aortic superoxide anion generation

The increase in serum TBARS concentration and aortic superoxide anion generation was noted in rats administered with STZ and nicotine. However, treatment with Atorvastatin and PHE significantly attenuated STZ and nicotine-induced increase in serum TBARS concentration and aortic superoxide anion generation.

Table5: Effect of PHE on serum and aortic nitrite/nitrate, Serum TBARS, and Reduced NBT

Group	Group Name	Serum nitrite/nitrate (µM)	Aortic nitrite/nitrate (µM/mg of protein)	Serum TBARS (µM)	Reduced nitrobluetetrazolium (NBT) (pmol/min/mg)
G 1	Normal Control	12±0.12	16±0.16	3.7±0.13	16±0.23
G 2	Positive Control (ATS)	14±0.15*	17±0.23*	3.9±0.05*	16±0.83*
G 3	Negative Control (STZ)	7±0.42*	7±0.13*	9.3±0.04*	23±0.38*
G 4	Standard (STZ+ATS)	11±0.14**	13±0.24**	4.67±0.11**	17±0.36**
G 5	Test I (STZ + Dose I)	9±0.51**	9±0.16**	6.15±0.13**	20±0.48**
G 6	Test II (STZ + Dose II)	10±0.23**	12±0.34**	4.20±0.21**	17±0.67**
G 7	Negative Control (Nicotine)	6±0.13$^{\#}$	6±0.22$^{\#}$	8.89±0.05$^{\#}$	24±0.45$^{\#}$
G 8	Standard (Nicotine +ATS)	10±0.52$^{\#\#}$	13±0.23$^{\#\#}$	4.03±0.11$^{\#\#}$	18±0.34$^{\#\#}$
G 9	Test I (Nicotine + Dose I)	8±0.13$^{\#\#}$	10±0.43$^{\#\#}$	5.86±0.31$^{\#\#}$	21±0.38$^{\#\#}$
G 10	Test II (Nicotine + Dose II)	10±0.12$^{\#\#}$	13±0.16$^{\#\#}$	4.19±0.15$^{\#\#}$	17±0.19$^{\#\#}$

Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), $^{\#}$P<0.05 *vs.* Con rats (G1), $^{\#\#}$P<0.05 *vs.* Negative Con rats (G7).

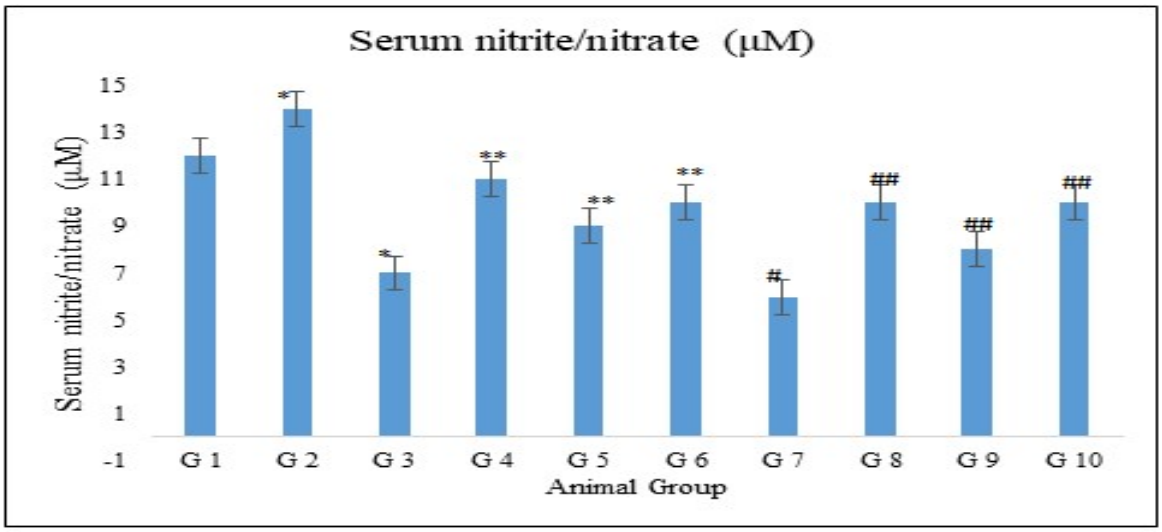

Figure7: Serum nitrite/nitrate (µM) concentration.

Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), $^{\#}$P<0.05 *vs.* Con rats (G1), $^{\#\#}$P<0.05 *vs.* Negative Con rats (G7).

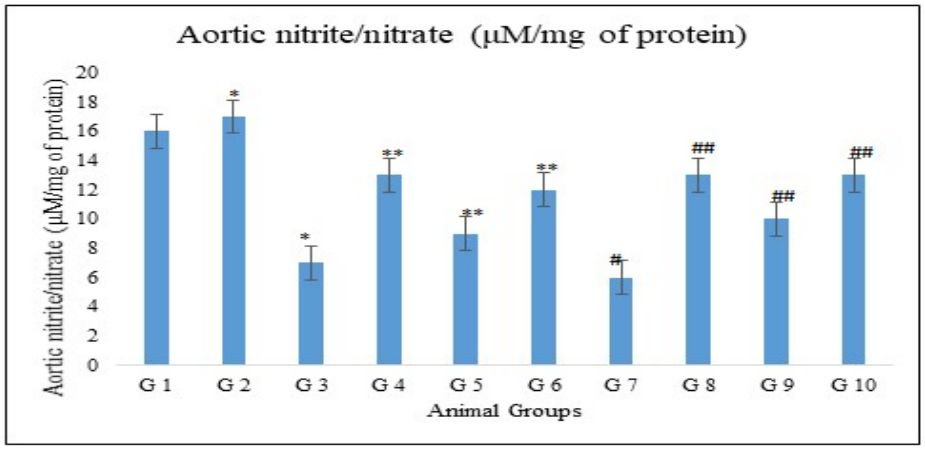

Figure8: Aortic nitrite/nitrate (µM/mg of protein).
Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

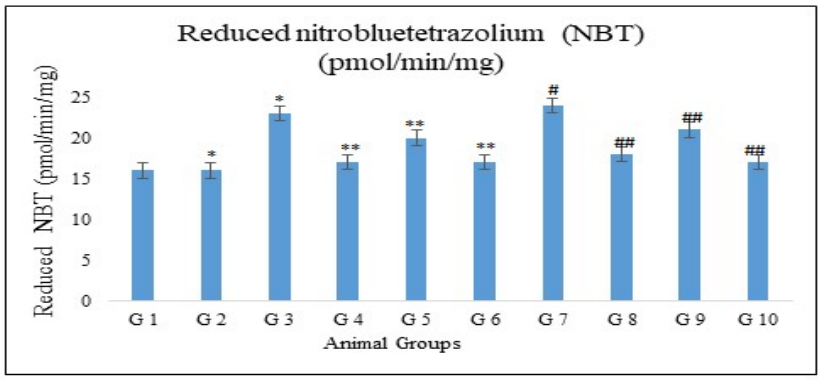

Figure9: Reduced Nitrobluetetrazolium (NBT) (pmol/min/mg).
Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

Effect of pharmacological interventions on serum lipid concentration

The significant increase in serum concentration of total cholesterol and triglycerides and decrease in HDL were noted in rats with endothelial dysfunction when compared with normal rats. However, treatment with Atorvastatin and PHE significantly recover the STZ and nicotine-induced alterations in serum lipids.

Table6: Effect of PHE on Lipid Profile

Group	Group Name	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
G 1	Normal Control	95±4.04	78±2.08	41±2.32	18±2.11	49±1.23
G 2	Positive Control (ATS)	89±3.05*	69±3.21*	46±3.15*	16±1.88*	41±2.34*
G 3	Negative Control (STZ)	146±3.42*	148±2.32*	32±3.05*	29±2.24*	107±1.79*
G 4	Standard (STZ+ATS)	113±5.11**	85±4.01**	39±2.16**	21±1.42**	56±2.32**
G 5	Test I (STZ + Dose I)	128±4.53**	113±2.12**	35±3.11**	23±3.32**	71±3.11**
G 6	Test II (STZ + Dose II)	108±5.23**	83±3.04**	39±2.31**	21±1.34**	53±2.30**
G 7	Negative Control (Nicotine)	150±5.11#	151±2.12#	31±2.15#	28±3.23#	109±3.57#
G 8	Standard (Nicotine +ATS)	118±4.53##	89±3.13##	40±1.05##	17±2.24##	57±3.43##
G 9	Test I (Nicotine + Dose I)	132±5.08##	116±2.34##	36±1.51##	22±1.81##	75±2.11##
G 10	Test II (Nicotine + Dose II)	109±3.07##	91±3.11##	39±2.25##	19±2.12##	59±2.45##

Lipid Profile: TC, TG, HDL, VLDL, and LDL (mg/dL). Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

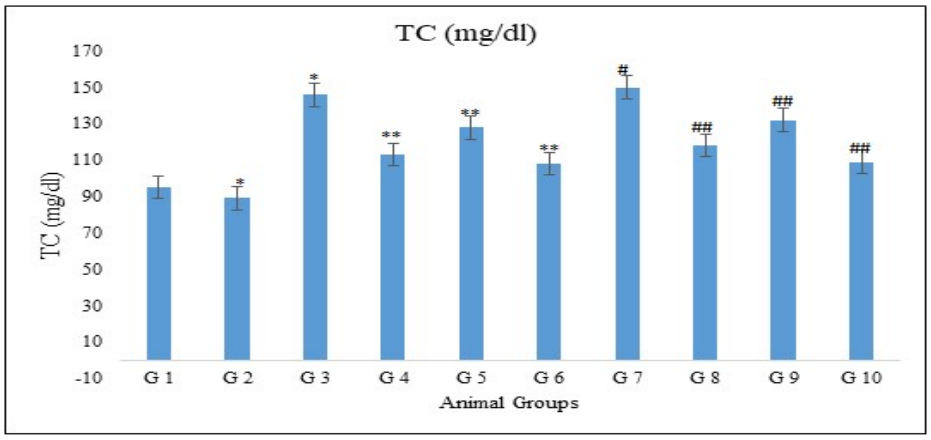

Figure 10: TC (mg/dL).
Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

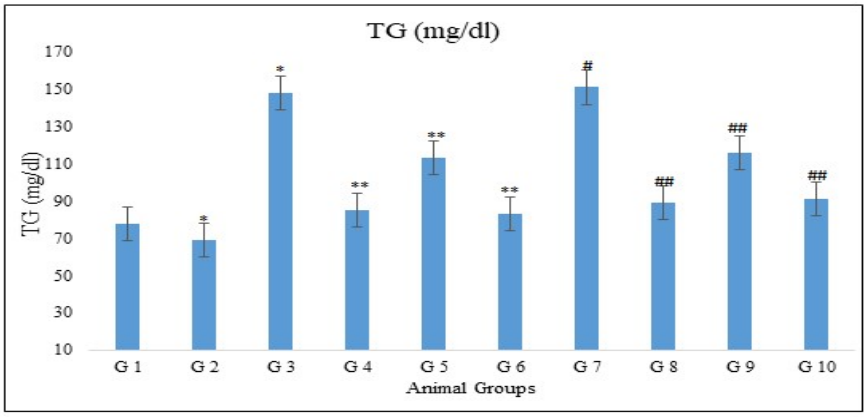

Figure11: TG (mg/dL).
Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

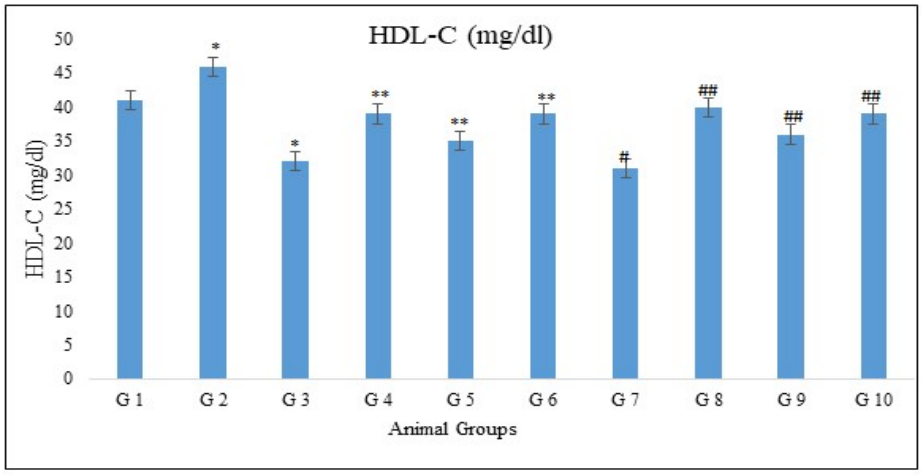

Figure12: HDL-C (mg/dL).
Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

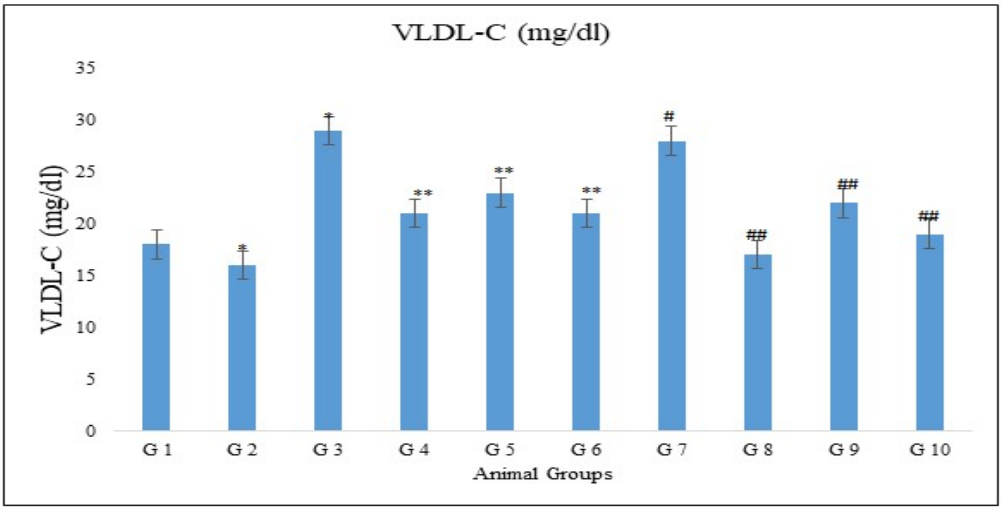

Figure13: VLDL-C (mg/dL).
Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

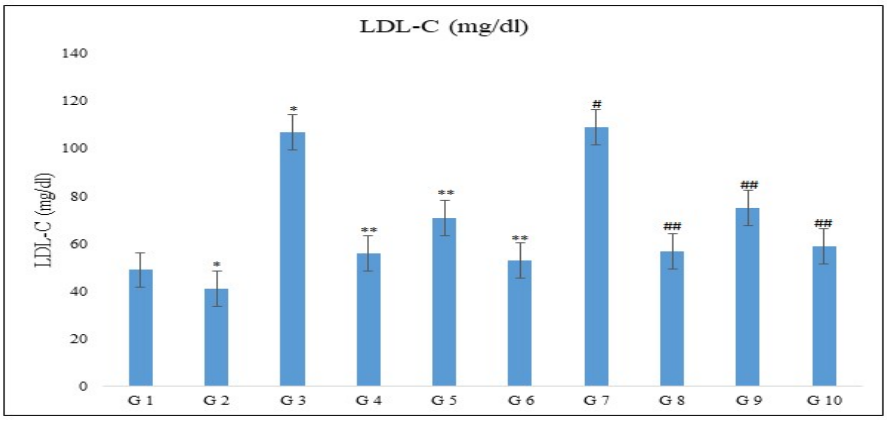

Figure14: LDL-C (mg/dL).
Values are expressed as mean±SEM.*P<0.05 *vs.* Con rats (G1), **P<0.05 *vs.* Negative Con rats (G3), #P<0.05 *vs.* Con rats (G1), ##P<0.05 *vs.* Negative Con rats (G7).

Histopathology of Aorta – Aorta harvested from the sacrificed rat will further be treated the 10% solution of formalin and will be examined for the alteration in endothelium lining

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