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EVALUATION OF THE ANTIHYPERTENSIVE ACTIVITY OF R-(+)-PULEGONE IN L-NAME INDUCED HYPERTENSIVE WISTAR RATS

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Keywords:

Monoterpenes, L-NAME hypertension, Lipid profile, Endogenous biomarkers, Nitric oxide, Rats

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ABSTRACT: Objectives: The present study was aimed to assess the anti-hypertensive effect of PLG in L-NAME-induced hypertensive rats. **Methods:** Wistar rats of 200-240g were given L-NAME (40 mg/kg body weight/day/p.o.) in drinking water daily for 4 weeks to induce hypertension. Rats were randomly divided into six groups: control, L-NAME control, Verapamil (20 mg/kg, p.o.), and PLG (5, 10, and 20 mg/kg, p.o.). PLG and standard drug Verapamil (20 mg/kg) was also administered daily for 4 weeks. At the end of the 4th week, hemodynamic and biochemical parameters were evaluated. **Result:** PLG (10 and 20 mg/kg) significantly ($p < 0.01$ and $p < 0.001$) and dose-dependently decreased the elevated (SBP), (DBP) and (MABP). PLG also prevented the increase in heart weight and body weight. Undesirable changes such as increased (MDA) levels and decreased concentration of enzymatic antioxidants (SOD) and (GSH) in the heart tissue were rectified after the administration of PLG (10 and 20 mg/kg). Oral administration of PLG (10 and 20 mg/kg) also restored altered levels of hepatic and renal markers, lipid profile values, and histological abnormalities. Verapamil (20mg/kg) showed a maximum antihypertensive effect. Meanwhile, we found that NO concentration in plasma and aorta was significantly increased in the PLG (20 mg/kg) treated group. **Conclusion:** It is suggested that PLG possessed an antihypertensive effect in L-NAME-induced hypertensive Wistar rats. The results thus proposed that the blood pressure-lowering effect of PLG may be due to its antioxidant nature and prevention to decrease the bioavailability of NO.

INTRODUCTION: Hypertension is a significant public-health challenge because of its high frequency and concomitant risks of cardiovascular disease throughout the world. In the USA, 45% of adults suffer from hypertension [1]. According to the study, rates of awareness, treatment, and control of hypertension can be increased up to 2030 [2]. In preventing or treating cardiovascular diseases, monoterpenes have significant therapeutic potential [3].

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Pulegone is a monoterpene ketone found mainly in the oils of the family Labiatae. In Labiatae oils, it mainly exists in R-(+)-form [4]. R-(+)-Pulegone was reported to show anti-inflammatory & anti-allergic properties, wound healing properties, and ambulatory effects [5-7]. R-(+)-Pulegone was also reported to produce vasorelaxation using isolated guinea pig ileum; negative inotropic effect by the blockage of Ca^{+2} channels using isolated guinea pig left atria and using isolated myocyte by patch-clamp technique [8-10].

Conventional antihypertensive drugs cause certain adverse effects [11, 12]. It is essential to develop alternative options for synthetic drug therapy to treat hypertension. Herbal medicines are safer, economical, and more effective in chronic diseases than conventional drugs [13, 14].

The emerging scenario of widespread usage and therapeutic potential of phytomedicines and phytoconstituents prompted us to investigate a phytoconstituent as a potential therapy to treat hypertension. The monoterpenes are secondary metabolites of plants. Monoterpenes possesses various pharmacological properties including antifungal, antibacterial, antioxidant, anticancer, anti-spasmodic, anti-inflammatory, analgesic, antipyretic, hypotensive and vasorelaxant. In the prevention or treatment of cardiovascular diseases, monoterpenes have significant therapeutic potential [3]. Few monoterpenes such as thymol and carvacrol using isolated rat aorta produced vasorelaxant effect [15]. Monoterpene like eucalyptol in normotensive rats 16 has been reported to produce antihypertensive activity. Pulegone is structurally and functionally related to other monoterpenes [17].

Monoterpenes possess the therapeutic potential of preventing or treating cardiovascular diseases [3]. Pulegone epoxide has shown vasorelaxant activity by relaxation of mesenteric artery rings in a pharmacological assay [17]. *Ziziphora clinopodioides Lam.* is widely used in the treatment of hypertension in China. It exerted concentration-dependent inhibition of spontaneous contraction of the ileum, and the effect was 23 times as potent as the essential oil of *Calamintha nepata* in inhibiting contractions.

R-(+)-Pulegone (PLG) was recently reported to have vasorelaxant, anti-inflammatory and antioxidant properties. The objective of the current study is to assess the antihypertensive effect of PLG in L-NAME-induced hypertensive rats. In the present study, Nω-Nitro-L-arginine methyl ester (L-NAME) treated hypertensive rats were used. Acute and chronic L-NAME treatment leads to alterations in blood pressure and vascular reactivity due to decreased nitric oxide (NO) bioavailability. The sympathetic tone plays an important role in initiating and maintaining hypertension, and vasoconstriction in response to L-NAME was mediated by the sympathetic drive [18]. In the present study, L-NAME treated hypertensive rats were used. Nitric oxide (NO) is released from vascular endothelial cells in a Ca^{+2} -dependent manner. It leads to relaxation of blood vessels through stimulation of soluble guanylyl cyclase and consequent accumulation of guanosine 3':5'-cyclic monophosphate (cGMP) in smooth muscle cells [19]. However, this mechanism plays an important role in the regulation of blood flow and the control of blood pressure in men [20]. L-arginine analogs are widely used inhibitors of nitric oxide synthase (NOS) activity both in vitro and *in vivo*[21]. The antihypertensive activity of R-(+)-Pulegone in chronic disease conditions has not been well explained in animals. The present study aimed to reveal the antihypertensive activity of R-(+)-Pulegone (PLG) in L-NAME-induced hypertensive rats.

MATERIALS AND METHODS:

Experimental Animals and Protocol Approval: Wistar rats of either sex with body weight ranging from 200-240 gm were purchased from Batlekar (National Institute of Bioscience), Pune, and were maintained in an air-conditioned room (25±1ºC) with 12 h light/12 h dark cycle. The animals had access to food pellets (Neutrivet Life, Pune) and water *ad libitum*. The protocol was approved by the Institutional Animal Ethics Committee of Sinhgad Institute of Pharmacy, Narhe, Pune, Maharashtra, India (IAEC approval no. SIOP/IAEC/2018/02/02) constituted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (1139/PO/a/07/CPCSEA).

Drugs and Chemicals: N_{ω}-Nitro-L-arginine methyl ester (L-NAME) was purchased from Sigma-Aldrich Corporation, USA. R-(+)-Pulegone was purchased from TCI Chemicals Pvt. Ltd., India. Verapamil tablets were purchased from Abbott Healthcare Pvt. Ltd., Gujarat, India.

Preparation of Drug Solution and Selection of PLG Dose: PLG was diluted with soyabean oil as a vehicle. Verapamil tablets were dissolved in distilled water. This study is carried out using three doses of PLG *i.e.* 5, 10 & 20 mg/kg/p.o. and one dose of verapamil (20 mg/kg/p.o.).

Experimental Design and Protocol for L-NAME Induced Hypertension: Hypertension was induced by giving L-NAME in drinking water at a 0.4 mg/ml concentration to account for a daily intake of 40 mg/kg throughout the experimental period (4 weeks) [22]. The rats were randomly divided into the following 6 groups each containing 6 rats.

Group I: Normal; Group-II: L-NAME control (L-NAME 40 mg/kg/day); Group-III: L-NAME (40 mg/kg/day) + Verapamil (20 mg/kg *p.o.*); Group-IV: L-NAME (40 mg/kg/day) + PLG (5 mg/kg *p.o.*);Group-V: L-NAME (40 mg/kg/day) + PLG (10 mg/kg *p.o.*);Group-VI: L-NAME (40 mg/kg/day) + PLG (20 mg/kg *p.o.*).

Test drug PLG was dissolved in vehicle (soyabean oil) and administered to the (Group-IV, V, and VI) rats p.o. using an oral feeding needle once in a day (every morning) for four consecutive weeks. Standard drug verapamil tablets were also dissolved in the vehicle (distilled water) and administered to the rats (Group-III) *p.o.* once in a day (every morning) for four consecutive weeks.

After the administration of the last dose, at the end of the study, the blood was collected from each rat by a retro-orbital puncture to measure various biochemical parameters. The collected blood was separated in a centrifuge tube to separate serum and blood plasma.

The blood collected without anticoagulant was centrifuged at 3000 rpm for serum collection for 15 minutes. The serum samples were stored at -80°C until being analyzed.

Assessment of Hemodynamic Changes: The rats were anesthetized with the administration of urethane (1.25 g/kg) by the *i.p.* route. The rat trachea was cannulated to assist respiration. By invasive technique, systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MABP) was measured.

A polyethylene cannula (PE 50) filled with heparinized saline (100 IU/mL) was inserted into the right carotid artery of the rat. Then the cannula was connected to a transducer, and the signal was amplified by using the PowerLab 8-channel data acquisition system (AD Instruments Pvt. Ltd., with Lab Chart 7.3 Pro software, Australia).

Estimation of Biological Serum Markers: Cardiac damage markers [creatine kinase (CK-MB), lactate dehydrogenase (LDH)] were estimated using kits from coral Clinical Systems Pvt. Ltd., Goa, India. Hepatic markers [aspartate aminotransferase (AST), alanine aminotransferase (ALT)] and renal markers [blood urea nitrogen (BUN), creatinine] were estimated using commercially available measurement kits (BioLab Diagnostics Pvt. Ltd., Mumbai, India) and lipid profile [triglycerides (TG) and high-density lipoprotein (HDL)], total protein, renal marker [uric acid] were estimated using commercially available measurement kits (Transasia Bio-Medicals Ltd., Himachal Pradesh, India).

Estimation of Endogenous Antioxidant Enzyme: The rats were humanely euthanized after recording hemodynamic changes. The heart of each animal was excised, weighed, sliced into pieces, and processed for further antioxidant estimation.

The portions of the heart tissues were individually homogenized in 10% ice-cold Tris-hydrochloride buffer (10 mmol/L, pH 7.4) using a tissue homogenizer (Remi motors Ltd, Mumbai-53, India) and centrifuged at 7500 rpm for 15 min at 0ºC.

The clear supernatant was collected after the centrifugation and used for biochemical and molecular estimations. The level of glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) in the heart tissues were measured by the method previously described elsewhere [23-25].

Determination of NO Concertation: No concentration in plasma and aortic tissue was determined as nitrite using Griess reaction. Rapid simple spectrophotometric method is used to perform the assay [22, 26].

Histopathology of Heart: The excised heart samples were collected, cleaned, and immediately fixed in a neutral buffered 10% formalin solution. They were processed and embedded in paraffin routinely.

Then the specimens were cut into sections of 5 µm thickness by microtome and stained with hematoxylin and eosin (H&E) for microscopic examination. Myocardial degeneration, collagen deposition, and fibrosis of the heart sections were evaluated.

Statistical Analysis: The data were expressed as mean ± standard error mean (SEM) in the present study. Analysis of the data was performed using GraphPad Prism 5.0 software (GraphPad Software,

Inc., La Jolla, CA). The heart rate and body weight data were analyzed by two-way variance (ANOVA) analysis and Bonferroni's test. However, hemodynamic, antioxidant and biochemical parameters were analyzed using a one-way analysis of variance (ANOVA) and Dunnett's test was applied for *posthoc* analysis. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS:

Effect of PLG on Hemodynamic Parameters: Fig. 1, 2 shows the effect of PLG on hemodynamic parameters at three concentrations (5, 10, and 20 mg/kg) in I to VI groups after 4 weeks. The L-NAME control rats showed a significant ($p < 0.001$ each) increase in SBP, DBP and MABP after 4 weeks. Verapamil (20 mg/kg) and PLG (20 mg/kg) treatment respectively showed a significant ($p < 0.001$) decrease in the SBP, DBP and MABP.

PLG (10 mg/kg) treatment also showed significant ($p < 0.01$each) decrease in SBP, DBP and MABP compared to L-NAME control animals. However, PLG in low doses (5 mg/kg) did not significantly affect hemodynamic parameters.

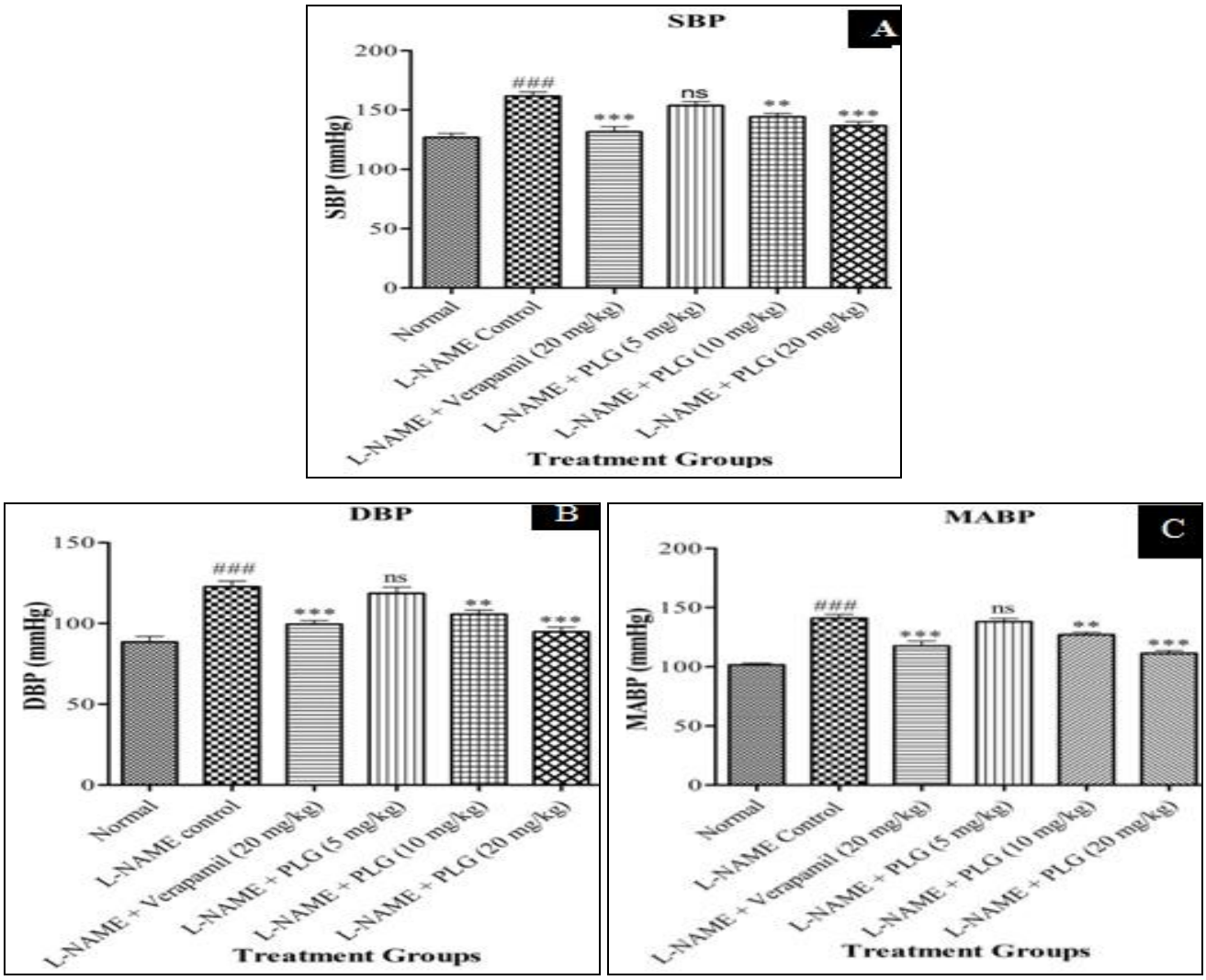

FIG. 1: EFFECT OF PLG (5, 10 AND 20 mg/kg) AND VERAPAMIL (20 MG/KG) ON (A) SBP, (B) DBP, (C) MABP IN L-NAME INDUCED HYPERTENSIVE RATS. Values are expressed as mean ± SEM for n=6 rats. Data were analyzed by one-way ANOVA followed by Dunnett's test. ns = non-significant, ###p<0.001as compared with normal group at same time point whereas **p <0.01, ***p<0.001 as compared with L-NAME control group at same time point.

Effect of PLG on Heart Weight and Body Weight: L-NAME control rats significantly ($p < 0.001$) decreased body weight and increased heart weight. The treatments with verapamil (20mg/kg) and PLG (10, 20 mg/kg) showed significant results by bringing back those values to near normal levels **Table 1.**

TABLE 1: EFFECT OF PLG AND VERAPAMIL ON ENDOGENOUS ANTIOXIDANT ENZYMES IN L-NAME HYPERTENSIVE RATS

Parameter		Treatment					
		Normal	L-NAME Control	L-NAME + Verapamil (20 mg/kg)	L-NAME + PLG (5 mg/kg)	L-NAME + PLG (10 mg/kg)	L-NAME + PLG (20 mg/kg)
Organ wt.	**Heart (g)**	0.85 ± 0.059	1.14 ± 0.034$^{###}$	0.93 ± 0.056**	1.06 ± 0.034^{ns}	0.95 ± 0.024*	0.94 ± 0.029**
Body weight (g)	0th week	200.8 ± 2.48	212.3 ± 3.28^{ns}	214.3 ± 3.38^{ns}	212.5 ± 3.66^{ns}	215.7 ± 1.47^{ns}	217.8 ± 3.98^{ns}
	1st week	208.0 ± 2.45	205.6 ± 2.77^{ns}	215.7 ± 3.31^{ns}	213.7 ± 3.77^{ns}	213.0 ± 1.63^{ns}	215.0 ± 3.40^{ns}
	2nd week	216.2 ± 2.88	200.0 ± 3.31$^{##}$	213.3 ± 5.22*	210.7 ± 3.65^{ns}	211.3 ± 1.99^{ns}	214.7 ± 2.68*
	3rd week	223.0 ± 2.77	194.9 ± 3.25$^{###}$	211.3 ± 5.11**	205.3 ± 2.68^{ns}	208.7 ± 2.19*	212.0 ± 2.63**
	4th week	230.8 ± 2.86	188.0 ± 3.05$^{###}$	214.7 ± 4.6***	201.5 ± 2.77*	204.7 ± 2.77**	213.3 ± 2.95***

Values are expressed as mean ± SEM for n=6 rats. Data regarding organ weight were analyzed by one-way ANOVA followed by Dunnett's test. ns = non-significant, $^{###}p$<0.001 as compared with control group at same time point whereas *p <0.05, **p <0.01 as compared with L-NAME control group at same time point. Data regarding body weight were analyzed by Two-way ANOVA followed by *post hoc* Bonferroni tests. ns = non-significant, $^{##}p$<0.001, $^{###}p$<0.001 as compared with control group at same time point whereas *p <0.05, **p <0.01, ***p<0.001 as compared with L-NAME control group at same time point.

Effect of PLG on Lipid Peroxides, Enzymatic and Non-Enzymatic Antioxidant Enzymes: The MDA level in the heart tissues of L-NAME control rats was elevated significantly (p <0.001). Oral administration of PLG (10 and 20 mg/kg) and verapamil (20 mg/kg) significantly (p <0.01) decreased the levels of MDA in the heart tissues as compared to L-NAME control. However, PLG (5 mg/kg) treated group did not show any significant restoration **Table 2.**

The activities of SOD, in the heart tissue of L-NAME control rats, were decreased significantly (p <0.001) after four weeks. Treatment with Verapamil (20 mg/kg) and PLG (20 mg/kg) significantly (p <0.001) restored the activities of SODin the heart tissue. PLG (10 mg/kg) also showed significant (p <0.01) restoration of the SOD **Table 2.**

The level of reduced glutathione (GSH) in the heart tissue was decreased significantly (p <0.001) in L-NAME-induced control rats. Verapamil (20 mg/kg) and PLG (20 mg/kg) treatment significantly improved values toward the normal with p <0.01 and p <0.001 respectively. PLG (10 mg/kg) group rats showed p <0.05 restoration of GSH activity in the heart tissue, whereas the PLG (5 mg/kg) treated group did not show any significant change in activity **Table 2.**

TABLE 2: EFFECT OF PLG AND VERAPAMIL ON ENDOGENOUS ANTIOXIDANT ENZYMES IN L-NAME HYPERTENSIVE RATS

Parameter	Treatment					
	Normal	L-NAME Control	L-NAME + Verapamil (20 mg/kg)	L-NAME + PLG (5 mg/kg)	L-NAME + PLG (10 mg/kg)	L-NAME + PLG (20 mg/kg)
SOD (Unit /mg protein)	5.19 ±0.16	3.49 ± 0.14$^{###}$	4.77 ± 0.24***	3.61 ± 0.09^{ns}	4.29 ± 0.11**	4.68± 0.17***
GSH (µg/mg protein)	8.55 ±0.11	4.14 ± 0.36$^{###}$	5.50 ± 0.41**	4.50 ± 0.20^{ns}	5.34 ± 0.22*	6.10± 0.14***
MDA (nmol /mg protein)	4.87 ±0.20	9.20 ± 0.15$^{###}$	7.81 ± 0.25**	8.31± 0.31^{ns}	7.76 ± 0.22***	6.67 ± 0.27***

Values are expressed as mean ± SEM for n=6 rats. Data were analyzed by one-way ANOVA followed by Dunnett's test. ns = non-significant, $^{###}p$<0.001 as compared with control group at same time point whereas *p <0.05, **p <0.01, ***p<0.001 as compared with L-NAME control group at same time point.

Effect of PLG on Serum Cardiac, Hepatic, and Renal Markers: The activities of CK-MB, LDH, AST, ALT, total protein, BUN, uric acid, and creatinine were significant (p <0.001 each) increased in L-NAME control rats. The treatments with verapamil (20mg/kg) and PLG (20mg/kg) decreased these values toward near normal. Verapamil (20mg/kg) showed the highest activity

by a significant (p <0.001) reduction in hepatic and renal markers. The treatment with PLG (10 and 20mg/kg) showed a significant reduction in serum levels of AST, ALT, total protein, blood urea nitrogen, uric acid, creatinine, CK-MB and LDH. On the other hand, PLG showed a non-significant reduction in urea levels. But PLG (5 mg/kg) did not show any significant decrease in serum cardiac, hepatic, and renal parameters **Table 3.**

TABLE 3: EFFECT OF PLG ON SERUM CARDIAC, HEPATIC, AND RENAL MARKERS IN L-NAME HYPERTENSIVE RATS

Parameter	Treatment					
	Normal	L-NAME Control	L-NAME + Verapamil (20 mg/kg)	L-NAME + PLG (5 mg/kg)	L-NAME + PLG (10 mg/kg)	L-NAME + PLG (20 mg/kg)
Aspartate aminotransferase (AST) (IU/L)	91.49± 3.79	166.8± 4.03$^{###}$	105.0± 3.65***	151.0 ± 4.51^{ns}	139.0± 5.80***	111.5± 4.07***
Alanine aminotransferase (ALT) (IU/L)	40.78 ± 1.49	74.0± 1.63$^{###}$	58.4± 2.58***	67.38 ± 2.30^{ns}	61.02± 2.46***	48.79± 2.39***
Total protein (mg/dl)	6.55 ± 0.19	8.78 ± 0.29$^{###}$	7.46 ± 0.28**	8.57 ± 0.21^{ns}	7.78 ± 0.14*	7.62 ± 0.28**
Blood urea nitrogen (BUN) (mg/dl)	18.85 ± 0.48	39.50 ± 2.18$^{###}$	25.31± 1.24***	37.20± 2.53^{ns}	33.03± 2.25^{ns}	28.02 ± 2.14**
Uric acid (mg/dl)	2.14 ± 0.16	5.04± 0.15$^{###}$	4.04± 0.20***	4.42± 0.20^{ns}	3.82± 0.18***	2.72± 0.09***
Creatinine (mg/dl)	0.75 ± 0.04	1.83 ± 0.04$^{###}$	0.97± 0.08***	1.59± 0.13^{ns}	1.28± 0.07***	1.07± 0.11***
Creatinine kinase (CK-MB) (IU/L)	356.5 ± 11.3	570.7 ± 9.0$^{###}$	484.5± 19.9**	535.0± 20.8 ^{ns}	498.8± 9.34**	471.3± 13.7***
Lactate dehydrogenase (LDH) (IU/L)	665.8± 14.05	833.2± 26.3$^{###}$	714.8± 20.5**	784.2± 25.9^{ns}	734.8± 18.9*	710.2± 24.5**

Values are expressed as mean ± SEM for n=6 rats. Data were analyzed by one-way ANOVA followed by Dunnett's test. ns = non-significant, #p < 0.05, ##p < 0.01, ###p < 0.001 as compared with control group at same time point whereas *p <0.05, **p <0.01, ***p<0.001 as compared with L-NAME control group at same time point.

Effect of PLG on Lipid Profile: There was a significant (p <0.001each) increase in levels of TG, and a significant (p <0.001) decrease in the level of HDL in L-NAME control rats. After the treatments with verapamil (20 mg/kg) and PLG (5, 10, and 20mg/kg), there was a decrease in serum TG levels and an increase in HDL levels as compared to L-NAME control rats.

TABLE 4: EFFECT OF PLG ON LIPID PROFILE IN L-NAME HYPERTENSIVE RATS

Parameter	Treatment					
	Normal	L-NAME Control	L-NAME + Verapamil (20 mg/kg)	L-NAME + PLG (5 mg/kg)	L-NAME + PLG (10 mg/kg)	L-NAME + PLG (20 mg/kg)
Triglyceride (TG) (mg/dl)	56.00± 2.68	151.3± 2.79$^{###}$	77.00± 2.24***	135.7 ± 3.18*	112.3± 5.77***	98.00± 2.81***
High density lipoprotein (HDL) (mg/dl)	32.65 ± 1.57	17.81± 0.43$^{###}$	26.07 ± 1.87***	22.16 ± 1.29^{ns}	24.05± 0.69**	26.56± 1.01***

Values are expressed as mean ± SEM for n=6 rats. Data were analyzed by one-way ANOVA followed by Dunnett's test. ns = non-significant, #p < 0.05, ##p < 0.01, ###p<0.001 as compared with control group at same time point whereas *p <0.05, **p <0.01, ***p<0.001 as compared with L-NAME control group at same time point.

Verapamil (20mg/kg) and PLG (20 mg/kg) showed significant (p <0.001) reduction in TG levels and also showed significant (p <0.001) increase in serum HDL level. PLG (10mg/kg) showed similar activity (*i.e.p* <0.001) as compared to a higher dose (20 mg/kg) of PLG in the case of TG levels but showed relatively less activity (p <0.01) in the case of HDL. The lower dose of PLG (5 mg/kg) also showed (p <0.05) a decrease in TG levels but

showed a non-significant increase in HDL levels **Table 4.**

Effect of PLG on Nitrite/Nitrate Production: L-NAME hypertensive rats showed a significant (P< 0.001) decrease in nitric oxide metabolite (nitrite /nitrate) in plasma and aortic tissue. The rat treated with Verapamil (20 mg/kg) and PLG (10 mg/kg and 20 mg/kg) showed significant (P< 0.001, *p* <0.05 and P< 0.01, respectively) increase in plasma NO and aortic tissue NO concentration. However, PLG (5 mg/kg) did not significantly affect **Table 5.**

TABLE 5: EFFECT OF PLG ON NITRITE/NITRATE IN L-NAME HYPERTENSIVE RATS

Parameter	Treatment					
	Normal	L-NAME Control	L-NAME + Verapamil (20 mg/kg)	L-NAME + PLG (5 mg/kg)	L-NAME + PLG (10 mg/kg)	L-NAME + PLG (20 mg/kg)
Plasma nitrite and nitrate concertation (µ mol/l)	86.20 ± 2.81	60.14± 2.57$^{###}$	79.32± 2.16***	62.53 ± 1.76^{ns}	69.82± 2.26*	73.35± 2.62**
Aorta nitrite and nitrate concertation (µ mol/l)	109.4 ± 3.02	63.06± 3.060$^{###}$	100.6 ± 2.11***	63.22 ± 1.813^{ns}	74.64± 4.53*	79.55± 2.25**

Values are expressed as mean ± SEM for n=6 rats. Data were analyzed by one-way ANOVA followed by Dunnett's test. ns = non-significant, #p< 0.05,##p< 0.01,###p<0.001 as compared with control group at same time point whereas *p <0.05, **p <0.01, ***p<0.001 as compared with L-NAME control group at same time point.

Effect of PLG (5, 10 and 20 mg/kg) on Histopathology of Heart in L-NAME Induced Hypertensive Rats: The normal rat has shown normal histopathological manifestation without any myocardial damage at the microscopic level in the heart. L-NAME control group rats were found to show severe myocardial degeneration, hypertrophy, and fibrosis. Administration of verapamil (20 mg/kg) and PLG (10 and 20 mg/kg) reduced this myocardial damage, collagen deposition and fibrosis. However, PLG (5 mg/kg) did not show any significant protection against L-NAME-induced hypertension **Fig. 2.**

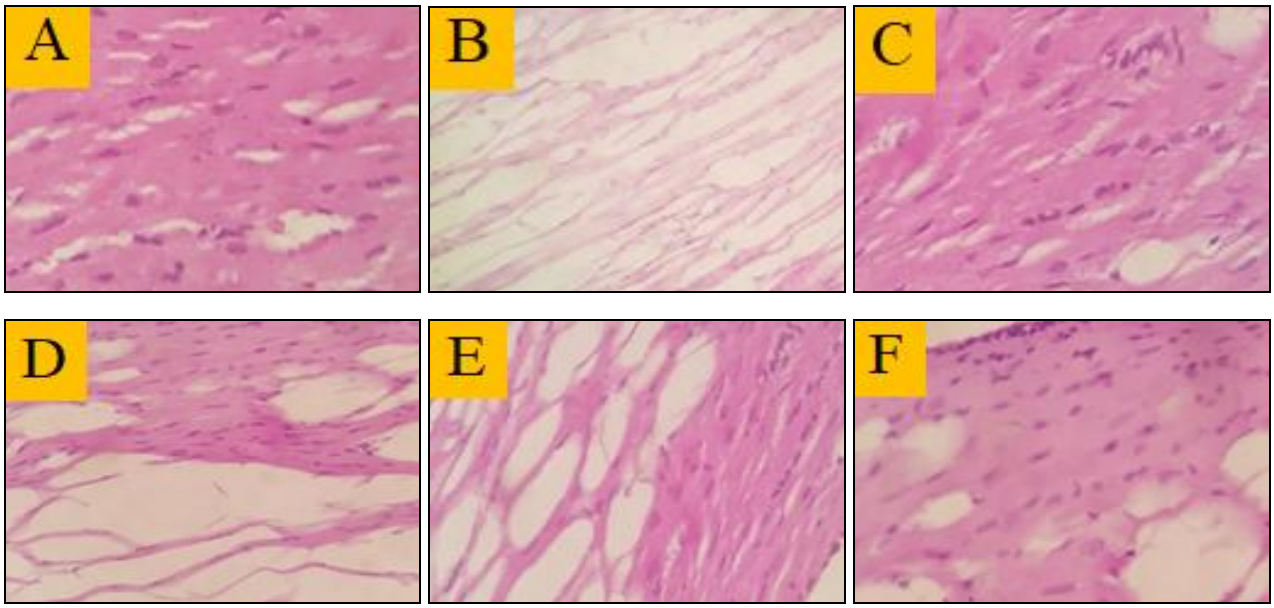

FIG. 2: EFFECT OF PLG (5, 10 AND 20 mg/kg) AND VERAPAMIL ON HISTOPATHOLOGY OF HEART FROM (A) NORMAL, (B) L-NAME CONTROL, (C) VERAPAMIL (20 mg/kg), (D) PLG (5 mg/kg), (E) PLG (10 mg/kg) AND (F) PLG (20 mg/kg)

DISCUSSION: In the present study, L-NAME is used to induce hypertension in Wistar rats, and it is a well-known animal model to evaluate antihypertensive drugs. The L-NAME model of experimental hypertension to induce high blood pressure has become a widely accepted method for testing antihypertensive agents. In this model, the sympathetic nervous system also contributes to developing high blood pressure [27]. The present discussion is based upon the results obtained with

the optimum dose of PLG, *i.e.*, 5, 10 and 20 mg/kg as well as verapamil 20 mg/kg once daily. It is well known that L-NAME is a nitric oxide synthase (NOS) inhibitor. The oral administration of the same cause chronic inhibition of NO biosynthesis by hypertension, cardiac remodeling, and vasoconstriction will be produced in rats [28]. In the present investigation, L-NAME control (hypertensive) rats showed a significant increase in SBP, DBP and MABP. PLG (10 and 20 mg/kg) and verapamil (20 mg/kg) produced the maximum antihypertensive effect. Antihypertensive activity of PLG may be due to its L- type calcium channel blockage and negative in tropism as proposed in a previous study using guinea pig atria [9]. Antihypertensive effect of PLG may also be due to smooth muscle relaxation, *i.e.*, vasorelaxation as proposed in one study by using guinea pig ileum [8]. The vasorelaxant property had also been studied using rat thoracic aorta [29].

Chronic L-NAME hypertension is associated with marked body weight loss in laboratory animals [22, 44]. The present investigation found that L-NAME hypertensive group rats showed significant loss of body weight in rats. Treatment with PLG and verapamil significantly improved the weight loss which may be due to its ability to oppose hypertension and destruction of structural proteins. Results produced by PLG are consistent with previous reports [22, 30]. In the L-NAME hypertensive group, weight of the heart was significantly increased, which was in line with the previous studies [27, 31]. The treatment with the verapamil (20 mg/kg) and PLG (10 and 20 mg/kg) prevented hypertrophy of the heart and significantly reduced the heart weight.

This is maybe due to the antihypertensive activity of PLG. An increase in blood pressure and oxidative stress are associated with each other [32]. In the present study, serum MDA levels were significantly increased in the heart tissue of the L-NAME control group compared to the normal group. This may be due to the increase in blood pressure which became responsible for the production of oxidative stress. PLG significantly protected the heart tissue from hypertension-induced oxidative damage, as evidenced by the decreased MDA level in L-NAME hypertensive rats, which may be due to its ability to decrease ROS and antioxidant nature [33]. Results of the present study demonstrated that the L-NAME control group showed a significantly decreased level of SOD and GSH in the heart tissue. PLG significantly improved the activity of enzymatic antioxidants in L-NAME induced hypertensive rats according to previous reports that proved PLG has free radicle scavenging activity [34, 35]. CK-MB and LDH are known to be the standard cardiac markers for identifying cardiac damage. The measurement of CK-MB in serum is known to be the gold standard clinical laboratory test [23]. An increase in blood pressure may be responsible for myocardial damage to leak CK-MB and LDH in blood and detected in serum in the L-NAME control rats [36, 37]. This increase in levels of serum cardiac markers indicates cardiac damage. Decreased serum levels of these enzymes in PLG (10 and 20 mg/kg) and verapamil (20 mg/kg) treated groups showed the protective effect of PLG in L-NAME induced hypertensive rats.

Oxidative stress in hypertension leads to liver damage or hepatotoxicity [38]. In the present investigation, there was an increase in the serum level of this enzyme in the L-NAME control hypertensive group confirming liver damage. After the administration of PLG and verapamil, there was a significant decrease in serum levels of AST, ALT and total protein, which clearly showed the prevention of oxidative damage in the L-NAME hypertensive rats [39]. Levels of serum renal marker enzymes in the L-NAME hypertension animal model were increased in the present study, consistent with previous study [40]. PLG significantly reduced serum urea, creatinine, and uric acid levels, suggesting its protective effect in hypertension, which may be due to the prevention of oxidative damage [41, 42].

In the present investigation, serum HDL level was decreased, and serum TG level was increased in L-NAME control rats. PLG (10 and 20 mg/kg) and verapamil (20 mg/kg) increased HDL whereas decreased TG serum levels. It has been reported that in hypertension, levels of TG increase, and the level of HDL decreases [43]. In the present study, the restoration of lipid profile values may be due to the antihypertensive activity of PLG and verapamil. It is reported that L-NAME hypertension is responsible for releasing excessive reactive oxygen

species that react with NO and reduce its concentration [45]. Oxidation of NO produces various compounds like peroxynitrite.

These compounds are thus responsible for oxidative stress and increased blood pressure [46, 47]. Significant elevation of plasma and aortic tissue nitrite/nitrate levels in this study revealed the ability of PLG to prevent the loss of NO, explaining its antihypertensive effect. In the present investigation, histopathological examination revealed that L-NAME-induced hypertension was responsible for the heart tissue damage in the L-NAME control group. L-NAME control group rats showed severe myocardial degeneration, hypertrophy, and fibrosis. PLG (10 and 20 mg/kg) and verapamil (20 mg/kg) significantly reduced heart tissue damage. This showed the cardio-protective activity of PLG (10 and 20 mg/kg) in the L-NAME-induced hypertensive rats.

CONCLUSION: The present investigation was designed to evaluate the protective activity of PLG in L-NAME-induced hypertensive Wistar rats. The results indicated that PLG at higher doses (10 and 20 mg/kg) possesses therapeutic properties in restoring hypertension-induced insults. It significantly restored elevated blood pressure, biochemical changes, altered lipid profile, and oxidative injury associated with L-NAME hypertension. It also restored decreased NO bioavailability in L-NAME hypertension. It was concluded that PLG possessed an antihypertensive effect in L-NAME-induced hypertension in rats. The results thus suggested that the blood pressure-lowering effect of PLG may be due to its antioxidant nature, which fights against reactive oxygen species. It was also recommended that PLG reduce blood pressure by vasorelaxation activity, increased NO level and possible calcium channel blocking activity. A histopathological study of heart tissue further revealed the protective nature of PLG.

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