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Effect of Ethanolic Extract of Seeds of *Linum usitatissimum* (Linn.) on Hemodynamic Changes and Left Ventricular Function in Renal Artery Occluded Renovascular Hypertension in Rats

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Abstract: Background: Renal Artery Occlusion (RAO) induced hypertension is a major health problem associated with structural and functional modifications of the renal and cardiac vasculature. The present study was designed to investigate the antihypertensive activity of ethanolic extract of seeds of *Linum usitatissimum* (EELU) in RAO induced hypertensive rats. **Materials and methods:** Male Wistar rats (180-200 g) were divided in four groups Sham, RAO, EELU 200 mg kg^{-1} and EELU 400 mg kg^{-1}. Treatment group were pretreated with EELU (200 and 400 mg kg^{-1}) for 4 weeks. After that on last day of the experiment, left renal artery was occluded with renal bulldog clamp for 4 h. The right carotid artery was cannulated, renal clamp was removed and immediately subjected to hemodynamic recording and assessment of left ventricular function. **Results:** RAO group significantly increased hemodynamic parameters at 15, 30 and 45 min of clamp removal. EELU (400 mg kg^{-1}) treated group significantly decreased hemodynamic parameters at 15 min after clamp removal which remained for 60 min. EELU (400 mg kg^{-1}) treated group showed significant improvement in left ventricular function at 15, 30 and 45 min of clamp removal. The flow cytometric analysis showed significant decrease in Reactive Oxygen Species (ROS) production by renal cells in EELU (400 mg kg^{-1}) treated group as compared with RAO group which indicated antioxidant activity of EELU. **Conclusion:** It is concluded from the present investigation that the antihypertensive activity of EELU may be resulted through the action on rennin angiotensin system and inhibition of ROS.

Key words: Renal artery occlusion, antioxidants, hypertension, rennin angiotensin system and reactive oxygen species

INTRODUCTION

Renal hypertension induced acute renal failure is a major health problem in clinical nephrology. It is associated with structural and functional modifications of the renal and cardiac system. Rennin Angiotensin Aldosterone System (RAAS) is a hormone system that regulates cardiac and renal function. It plays important role in hypertension associated vascular remodeling and is consider one of the most important etiological candidates in hypertension (Atlas, 2007). It is proved that Angiotensin Converting Enzyme (ACE) plays an important role in the regulation of peripheral blood pressure and cardiovascular function. Renal ischemia reperfusion injury triggers the release of rennin which further responsible for secondary elevation in blood pressure. Local ACE in renal cells promotes conversion of inactive decapeptide angiotensin I to potent vasoconstricting octapeptide angiotensin II, causing severe vasoconstriction and aldosterone release, which plays an important role in long-term stabilization of hypertension (Vogel and Vogel, 1997).

In ischemia reperfusion induced renal damage sodium and water excretion capacity of kidney is affected hence, volume plays an additive role in the hypertension. Blocking the effect of ROS and angiotensin II may offers a possibility to achieve protection in ischemic injury of the cardiovascular and renal systems (Ozcan *et al*., 2007). Current treatment of hypertension and heart failure includes use of ACE inhibitors like captopril, enalapril and ramipril. However, use of synthetic ACE inhibitors is limited due to their adverse side effects. Recently attention has been focused towards herbal and mineral formulations which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases (Moline *et al*., 2000). Dietary

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antioxidants may also be protective against hypertension. Phytoestrogens have been evaluated for their effects on blood pressure. The hypotensive effect of soy has been reported in rats (Teede *et al*., 2001; Washburn *et al*., 1999). Recently prasad (2004) observed fall in blood pressure after intravenous administration of SDG in rats.

Flaxseed (Linum usitatissimum Linn.) is commonly known as linseed. It belongs to family linaceae. SDG is the main lignan isolated from flaxseed. Prasad (2004) has reported the antihypertensive potential of flaxseed and postulated that the hypotensive effects may be mediated through stimulation of guanylate cyclase activity. Zanwar *et al*. (2010) showed *in vitro* antioxidant potential of ethanolic extract of *Linum usitatissimum* (EELU) and reported that EELU has more DPPH radical scavenging activity, reducing power, hydroxyl radical scavenging and hydrogen peroxide radical scavenging activity. A phenolic content present in EELU may be responsible for its antioxidant activity. Recently we have reported renoprotective effects of ethanolic extract of seeds of *Linum usitatissimum* through conservation of antioxidant enzymes in ischemia reperfusion induced renal injury in rats (Ghule *et al*., 2011). The objective of the present investigation was to determine the antihypertensive effect of EELU in renal artery occlusion induced acute hypertension in rats.

MATERIALS AND METHODS

Collection and authentication of plant: Authenticated seeds of *Linum usitatissimum* (variety NL-97) were obtained from Dr. P. B. Ghorpade, Principal, Scientist and Linseed breeder, Punjabrao Deshmukh Krushi Vidyapeeth, College of Agriculture, Nagpur, India, Maharashtra State, India and voucher specimen was deposited at the institute.

Drugs and chemicals: Urethane (Hi media), H_2DCFDA (Sigma Chemicals, St. Louis, MO, USA) were obtained from respective vendors. All chemicals used were of analytical grade.

Preparation of ethanolic extract of *Linum usitatissimum:* The authenticated seeds of *Linum usitatissimum* (variety NL-97) were procured and processed for extraction of oil at our Omega-3-oil unit, Sangamner, Maharashtra, India. The double cold pressed flaxseed cake/meal obtained from this oil unit was defatted by n-hexane in soxhlet apparatus to remove residual oil. The defatted cake was then hydrolyzed with 1 M aqueous sodium hydroxide for 1 h at room temperature with intermittent shaking, followed by extraction with 50% ethanol. Then filtrate was acidified to pH 3 using 1 M hydrochloric acid. The filtrate was dried on tray dryer at 50°C. The yield of dry powder was 14.81% w/w. Weighed powder quantity of EELU was dissolved in distilled water to prepare the different doses for pharmacological studies. The analysis of EELU samples by high performance thin layer chromatography (HPTLC) has been reported earlier by Zanwar *et al*. (2011). The SDG lignan content in EELU was 40 mg g^{-1}.

Experimental animals and research protocol approval: Male Wistar rats (200-250 g) were purchased from National Toxicology Centre, Pune, India. Rats were housed in an air conditioned room having temperature 22±2°C, relative humidity 45 to 55% and 12 h light: 12 h dark cycle. The animals had free access to standard food pellets (Chakan Oil Mills, Pune, India) and water was available *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India.

Renal artery occluded renovascular hypertension in rats: Rats were divided into four groups containing twelve animals each. Animals in sham group (Group I, Sham) were pretreated with saline for 4 week and underwent the exposure of the left renal artery but did not receive occlusion. The animals from RAO group (Group II, RAO) were pretreated with saline for 4 weeks and on last day of the experiment renal artery were occluded for 4 h with renal clamp. EELU 200 mg kg^{-1} (Group III, RAO+EELU 200) and EELU 400 mg kg^{-1} (Group IV, RAO+EELU 400) were administered orally for 4 weeks. After 4 weeks of treatment period, animals were anaesthetized by intraperitoneal injection of urethane (1.25 g kg^{-1}). A small incision was made on the left side of peritoneal cavity to expose left kidney of the animal. The left renal artery was occluded for the period of 4 h by using rat bulldog clamp. EELU 200 and 400 mg kg^{-1} treated (Group III and IV) animals were surgically treated the same way as the RAO group. The jugular vein was cannulated for the administration of test drug. The carotid artery was cannulated and connected to the blood pressure transducer of powerlab assembly to measure the hemodynamic changes. After stabilization of blood pressure, the rat bulldog clamp was removed. Then 1/10th of the administered dose of the EELU, i.e., 20 and 40 mg kg^{-1} was injected to group III and IV respectively through jugular vein and hemodynamic changes and left ventricular function were measured at different time intervals (0, 5, 15, 30, 45, 60 and 90 min) out of twelve

animals in each group six animals were subjected to hemodynamic measurement and remaining six animals were used for the assessment of left ventricular function. (Vogel and Vogel, 1997; Sakat *et al*., 2009).

Invasive measurement of hemodynamic changes: The rats were anesthetized with urethane (1.25 g kg^{-1}, i.p.) and subjected to the measurement of hemodynamic changes by means of a polyethylene cannula (PE 50) filled with heparinised saline (100 IU mL^{-1}) and inserted into the right carotid artery. The cannula was connected to a transducer and the signals were amplified with the help of bioamplifier. Left ventricular systolic pressure was measured by means of a Millar mikro-tip transducer catheter (Model SRP-320, Millar instrument, INC 320-7051, Houston, Texas) inserted into the left ventricle via the right carotid artery and connected to a bioamplifier. Heart rate, Maximum first derivative of ventricular pressure with respect to time (dp/dt max), minimum first derivative of ventricular pressure with respect to time (dp/dt min) and left ventricular end diastolic pressure (LVEDP) signals were obtained from primary signals (left ventricular systolic pressure and blood pressure) by means of an acquisition data system (AD Instruments Pty Ltd with LABCHART 6 software, Unit 13, 18-22 Lexington Drive, Bella Vista NSW 2153, Australia).

Preparation of single cell suspension from kidney: Single cell suspension from kidney tissue of renovascular hypertensive rats and normotensive rats were prepared by the trypsinization method as described earlier by us Ghule *et al*. (2011). Briefly kidney samples were extensively perfused in situ in Phosphate Buffered Saline (PBS) to get rid of blood and irrigated in a buffer containing Hepes (10 mM), KCl (3 mM), NaCl (130 mM), NaH_2PO_4, H2O (1 mM) and glucose (10 mM, pH 7.4). The kidney tissue was homogenized and suspended in Hanks balanced salt solution. It was then treated with trypsine for 10 min and trypsine inhibitor for 5 min. Cell suspension was obtained by passing the trypsinized renal tissue through a nylon mesh. Cells were washed twice with cold PBS and then resuspended in 1X binding buffer at a concentration of 1 to 2×10^6 cells mL^{-1}.

Flow cytometric estimation of ROS production in renal cell suspension by H_2DCFDA probe: ROS production was quantified by the H_2DCFDA method according to Lawler *et al*. (2009) based on the ROS-dependent oxidation of DCFH-DA to DCF according to the method described elsewhere (Kobayashi *et al*., 2008). An aliquot of 100 μL of renal cell suspension was transferred to falcon tube (Becton and Dickinson India Pvt. Ltd., Gurgaon, India) and incubated with 10 μL of 10 Mmol H_2DCFDA for 15 min in a dark condition. The H_2O_2, OH^- and $ONOO^-$ produced during the cellular oxidative response oxidized the nonfluorescent intracellular DCFH into highly fluorescent dichloroflurescein (DCF). DCF fluorescence was assayed at 530 nm after excitation of cells at 488 nm. Acquisition and analysis of the processed sample was performed on flow cytometer by using CELL Quest software (Becton and Dickinson, San Diego CA, USA) (Urbanits *et al*., 2002).

Statistical analysis: All the data were expressed as Mean± SEM. Statistical analysis was carried out by one-way ANOVA and Two-way ANOVA followed by post hoc Bonferroni tests performed using GraphPad InStat version 5.00 for Windows Vista™BASIC, Graphpad Software, San Diego California USA. P value was considered significant when <0.05.

RESULTS

Hemodynamic changes in RAO induced hypertensive rats: Administration of vehicle in sham treated animals did not change the heart rate up to 45 min post administration but showed a non significant decrease at 60 and 90 min which was considered as normal. In RAO treated animals administration of vehicle significantly reduced the heart rate after 5 min ($p<0.001$), 15 min ($p<0.001$) and 30 min ($p<0.001$). The observations thus indicated that temporary occlusion of renal artery followed by reperfusion reduces the heart rate. The bradycardia caused due to renal artery occlusion was considered as a parameter to assess the cardiovascular action of EELU.

Administration of EELU 400 mg kg^{-1} in RAO animals showed non significant reduction in heart rate at 15 ($p<0.001$) min post administration. The heart rate increased during subsequent 5 and 15 min and showed significant increase at 30 min ($p<0.001$) and 45 min ($p<0.01$) compared to RAO group. The result thus indicated that bradycardia resulting due to occlusion and reperfusion of renal artery was reversed by EELU 400 mg kg^{-1}. A rebound increase in the heart rate was evident after EELU administration. Administration of challenge dose (20 and 40 mg kg^{-1}) of EELU resulted in significant increase in heart rate at 45 and 60 min but a clear dose response effect could not be established (Fig. 1a).

SBP, DBP and MABP were increased significantly in RAO group after 5 min ($p<0.001$ each), 15 min ($p<0.001$ each), 30 min ($p<0.001$ each), 45 min ($p<0.001$ each), 60 min ($p<0.001$ each) and 90 min ($p<0.001$ each) of renal clamp

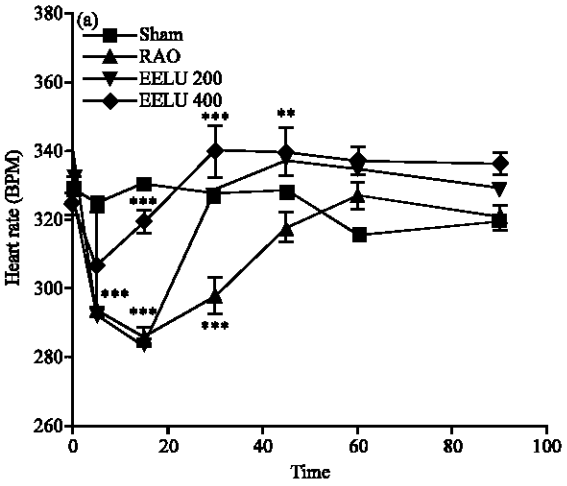

Fig. 1a: Effect of EELU treatment on heart rate in RAO induced hypertension. Heart rate was measured at 0, 5, 15, 30, 45, 60 and 90 min after clamp removal. Rats were pretreated with either vehicle or EELU 400 and 200 mg kg^{-1} as indicated in method section. Results are represented as Mean±SEM (n = 6). Data was analyzed by Two-way ANOVA followed by post hoc Bonferroni test. Compared with RAO at same time point, **p<0.01, ***<0.001

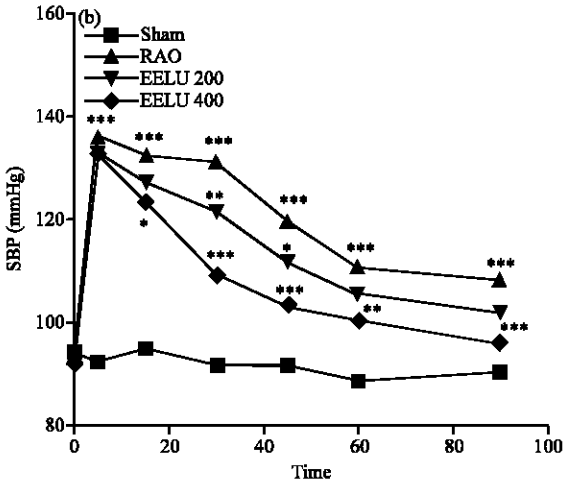

Fig. 1b: Effect of EELU treatment on SBP in RAO induced hypertension. SBP was measured at 0, 5, 15, 30, 45, 60 and 90 min after clamp removal. Rats were pretreated with either vehicle or EELU 400 and 200 mg kg^{-1} as indicated in method section. Results are represented as Mean±SEM (n = 6). Data was analyzed by Two-way ANOVA followed by post hoc Bonferroni test. Compared with RAO at same time point, *p<0.05, **p<0.01, ***<0.001

removal when compared with sham group. The result thus indicated that temporary occlusion (4 h) of renal artery followed by reperfusion showed significant increase in hemodynamic parameters such as SBP, DBP and MABP which confirmed that renal artery occlusion results in hypertension. Administration of EELU (200 and 400 mg kg^{-1}) in RAO induced hypertensive rat showed significant reduction in SBP after 15 min (nonsignificant and p<0.05, respectively), 30 min (p<0.001 and p<0.01, respectively), 45 min (p<0.05 and p<0.001, respectively), 60 min (nonsignificant and p<0.05, respectively) and 90 min (nonsignificant and p<0.001, respectively) (Fig. 1b). Significant reduction in MABP at 30 min (p<0.01) and 60 min (p<0.001) in EELU 400 mg kg^{-1} group was observed (Fig. 1d). Significant reduction in DBP in EELU (400 mg kg^{-1}) treated group was observed at 30 min (p<0.001) after post administration and remain significantly decreased until 90 min (p<0.001) (Fig. 1c).

Left ventricular function in RAO induced hypertensive rats: Occlusion of renal artery for 4 h in RAO treated animals significantly increased LVEDP (4.16±0.37 to 5.48±0.16 with p<0.05 at 15 min; 4.24±0.22 to 6.14±0.10 with p<0.001 at 30 min and 4.61±0.17 to 6.11±0.46, with p<0.01 at 45 min), contractility index (12.19±0.92 to 9.33±0.38 with p<0.05 at 5 min and 11.14±0.62 to 8.11±0.48 with p<0.05 at 15 min) and pressure time index (4.61±0.07 to 7.80±0.72 with p<0.05 at 5 min; 5.24±0.41 to 13.93±1.56 with p<0.001 at 15 min; 4.61±0.08 to 10.42±1.20 with p<0.001 at 30 min and 4.56±0.09 to 8.77±1.07 with p<0.001 at 45 min). The results thus indicated that temporary occlusion and reperfusion of renal artery produces the left ventricular dysfunction. Administration of EELU (400 mg kg^{-1}) in renal artery occluded animals showed significant reduction in LVEDP at 30 min (6.14±0.10 to 4.70±0.49 with p<0.01) and 60 min (6.24±0.53 to 4.97±0.48 with p<0.05) when compared with RAO group. Administration of EELU (200 mg kg^{-1}) in RAO group showed significant increase in contractility index (9.33±0.38 to 12.11±0.26 with p<0.05 at 5 min). EELU treatment (400 mg kg^{-1}) in RAO group also showed significant improvement in pressure time index (7.80±0.72 to 13.95±0.72 with p<0.001 at 5 min and 13.93±1.56 to 8.90±0.83 at 15 min each). The result thus indicated that hypertensive effect and left ventricular dysfunction resulting due to occlusion and reperfusion of renal artery was reversed by EELU 400 mg kg^{-1}. The maximal rate of left ventricular pressure rise (dP/dt max), maximal rate of left ventricular pressure fall (dP/dt min) and time course of relaxation which is also called as exponential tau were non significantly changed in RAO and EELU treated groups after clamp removal and thereafter during study period (Table 1).

ROS production in renal cells by H_2DCFDA probe using flow cytometry: Intracellular ROS was measured with 2, 7-dichlorofluoresce in diacetate (H_2DCFDA) by triple-color analysis using CELL Quest software on flow cytometry. Figure 2a showed representative histogram of fluorescence intensity from experimental group. Animals

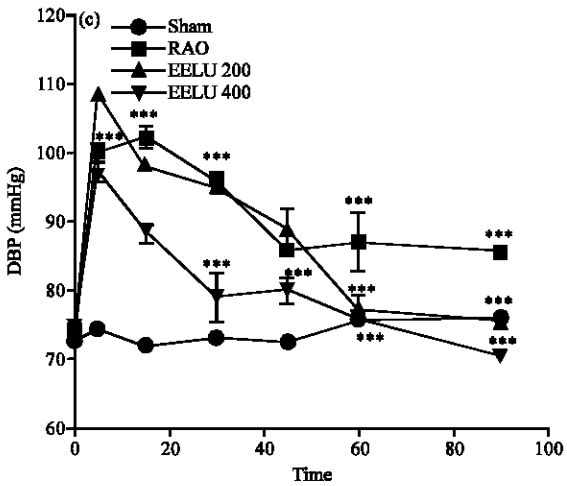

Fig. 1c: Effect of EELU treatment on DBP in RAO induced hypertension. DBP was measured at 0, 5, 15, 30, 45, 60 and 90 min after clamp removal. Rats were pretreated with either vehicle or EELU 400 and 200 mg kg^{-1} as indicated in method section. Results are represented as Mean±SEM (n = 6). Data was analyzed by Two-way ANOVA followed by post hoc Bonferroni test. Compared with RAO at same time point, ***<0.001

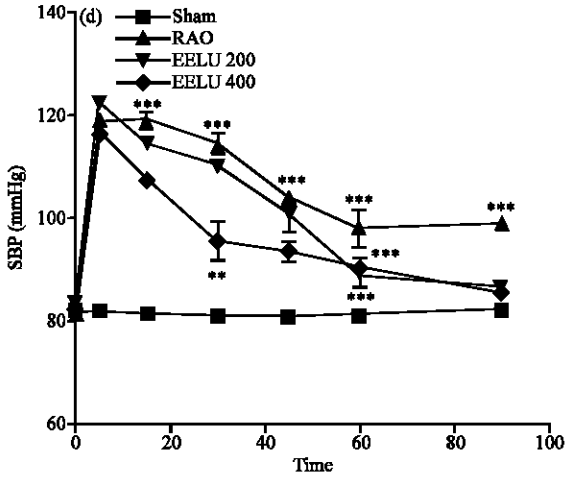

Fig. 1d: Effect of EELU treatment on MABP in RAO induced hypertension. MABP was measured at 0, 5, 15, 30, 45, 60 and 90 min after clamp removal. Rats were pretreated with either vehicle or EELU 400 and 200 mg kg^{-1} as indicated in method section. Results are represented as Mean±SEM (n = 6). Data was analyzed by Two-way ANOVA followed by post hoc Bonferroni test. Compared with RAO at same time point, **p<0.01, ***<0.001

Table 1: Effects of EELU on left ventricular function in RAO induced hypertensive rats

Parameter	No.	Treatment (mg kg^{-1})	Pre administration	Post (5 min) administration	15 min	30 min	45 min	60 min	90 min
LVEDP (mmHg)	I	Sham	4.68±0.14	4.51±0.19	4.16±0.37	4.24±0.22	4.61±0.17	5.15±0.39	4.14±0.18
	II	RAO	4.22±0.27	5.06±0.35	5.48±0.16*	6.14±0.10***	6.11±0.46**	6.24±0.53	4.83±0.27
	III	EELU 200	4.02±0.31	4.74±0.16	5.10±0.28	5.40±0.25	5.18±0.30	5.34±0.27	5.18±0.35
	IV	EELU 400	4.04±0.22	4.75±0.24	5.16±0.54	4.70±0.49**	5.09±0.49	4.97±0.48*	5.08±0.58
Max dp/dt (mmHg/s)	I	Sham	943.9±75.97	951.8±135.28	953.1±48.07	830.5±11.62	867.1±26.14	546.8±10.87	640.6±34.48
	II	RAO	901.3±17.23	1015.6±87.40	937.5±7.13	979.8±15.47	797.5±13.73	824.0±79.12**	854.7±73.98*
	III	EELU 200	890.3±25.68	1065.4±43.27	996.0±10.08	885.1±31.60	826.1±72.58	1007.1±68.19	968.8±39.25
	IV	EELU 400	923.9±50.42	994.4±29.91	966.7±15.74	885.4±67.59	869.1±14.91	911.4±17.94	937.5±23.65
Min dp/dt (mmHg/s)	I	Sham	-503.3±72.9	-586.0±50.4	-528.5±38.1	-411.8±6.1	-423.2±21.0	-444.1±74.23	-311.1±14.8
	II	RAO	-592.5±26.45	-545.4±44.06	-485.6±59.48	-325.5±9.65	-302.7±6.67	-237.6±6.00	-227.8±6.51
	III	EELU 200	-530.4±35.17	-477.0±73.40	-455.2±60.58	-338.5±14.84	-397.7±62.63	-360.2±26.51	-373.9±42.5
	IV	EELU 400	-517.7±49.40	-495.4±51.04	-442.5±60.5	-366.5±34.7	-393.3±55.5	-410.0±64.89	-369.7±47.4
Contractility Index	I	Sham	13.26±0.64	12.19±0.92	11.14±0.62	9.88±0.81	8.36±0.95	6.72±0.15	7.74±0.38
	II	RAO	15.02±1.36	9.33±0.38*	8.11±0.48*	9.18±0.16	7.32±0.15	7.07±0.69	8.06±0.82
	III	EELU 200	14.10±1.06	12.11±0.26*	9.66±0.05	9.13±0.51	6.20±0.33	7.73±0.79	6.48±0.38
	IV	EELU 400	13.64±0.91	9.64±0.59	9.01±0.85	9.79±0.82	7.42±0.45	7.84±0.42	7.30±0.31
exponential Tau (ms)	I	Sham	20.82±0.81	22.34±0.83	22.07±0.88	23.21±0.51	23.93±0.80	20.69±1.08	23.31±0.69
	II	RAO	20.26±0.64	29.68±3.07	27.89±0.87	25.90±0.82	23.50±1.01	22.09±0.89	21.48±1.67
	III	EELU 200	20.97±0.72	26.00±1.16	25.82±1.27	21.76±1.41	22.29±2.28	18.40±0.83	20.92±1.17
	IV	EELU 400	20.86±0.68	23.37±1.22	22.92±0.90	21.41±0.85	21.26±1.12	20.59±1.02	19.55±0.56
Pressure Time Index	I	Sham	5.20±0.43	4.61±0.07	5.24±0.41	4.61±0.08	4.56±0.09	5.27±0.50	4.59±0.05
	II	RAO	4.73±0.10	7.80±0.72*	13.93±1.56***	10.42±1.20***	8.77±1.07***	7.01±0.58	6.22±0.59
	III	EELU 200	4.59±0.06	15.10±1.19***	11.59±0.19	12.58±0.97	9.21±0.92	7.25±1.18	8.28±0.97
	IV	EELU 400	5.46±0.55	13.95±0.72***	8.90±0.83***	8.26±0.49	8.60±0.87	8.29±0.87	6.01±0.63

Results are represented as Mean±SEM, (n = 6). Left ventricular function measured at 5, 15, 30, 45, 60 and 90 min after clamp removal in RAO induced hypertensive rat. Data was analyzed by Two-way ANOVA followed by post hoc Bonferroni tests. Compared with RAO at same time point, *p<0.05, **p<0.01, ***<0.001. (LVEDP: Left ventricular end diastolic pressure, Max dp/dt: Maximum first derivative of ventricular pressure with respect to time, Min dp/dt: Minimum first derivative of ventricular pressure with respect to time)

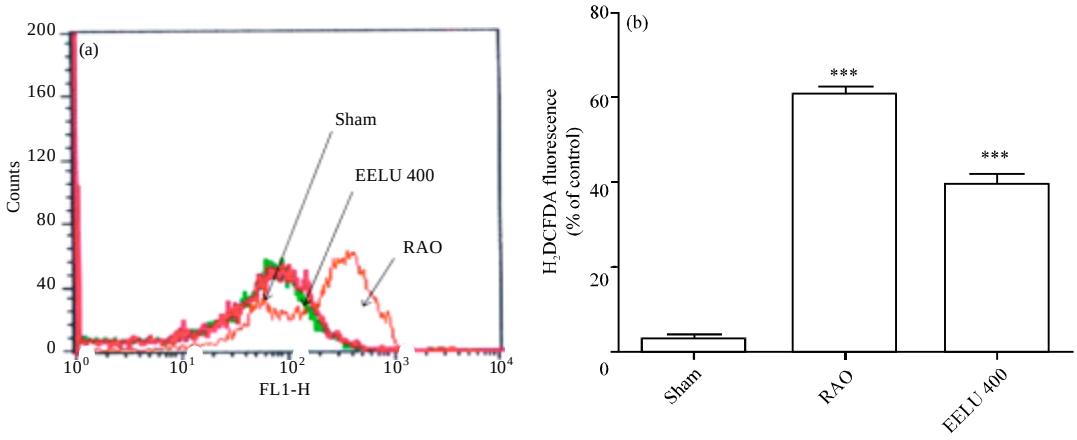

Fig. 2(a-b): Effects of EELU on Intracellular ROS generation by flow cytometry in RAO induced hypertensive rats. Effect of EELU (400 mg kg^{-1}) on Intracellular ROS production detected in renal tissue of renal artery occlusion induced renovascular hypertensive rat by flow cytometry using H_2DCFDA probe. H_2DCFDA fluorescence intensity was measured using FL-1. (a) Representative histogram of fluorescence from experimental groups. (b) Mean fluorescence obtained from the histogram statistics, each bar represents Mean±SEM (n = 4) Compared with RAO ***p<0.001

from RAO group significantly ($p<0.001$) increased intracellular level of ROS in the form of H_2DCFDA fluorescence intensity. Treatment of EELU (400 mg kg^{-1}) significantly decreased H_2DCFDA fluorescence intensity ($p<0.001$) as compared with RAO group which indicated the antioxidant activity of EELU (Fig. 2b).

DISCUSSION

As the cardiac function is concerned, several evidences proved that many antihypertensive agents can modulate the cardiac systolic or diastolic function, such as ACE blockers (Aunapuu *et al*., 2005), β blocker (Brodde *et al*., 1995) and central antihypertensive agents (Stefanadis *et al*., 2001) via cardiac autonomic nerve control. Present study revealed the significant antihypertensive activity of EELU 400 mg kg^{-1} in renal artery occluded hypertensive rats. It is reported that ischemia of the kidney causes elevation of blood pressure by activation of the rennin angiotensin system (Michels *et al*., 2009). In rats acute renal hypertension is induced by clamping the left renal artery for 4 h. After reopening of the vessel, accumulated rennin is released into circulation. The protease rennin catalyzes the first and rate-limiting step in the formation of angiotensin II leading to acute hypertension (Vogel and Vogel, 1997). This concept has been used in the present investigation to evaluate antihypertensive activities of EELU in RAO induced renovascular hypertension.

In present investigation occlusion of renal artery for the period of 4 h results in ischemia mediated renal damage. This ischemia reperfusion results in enhanced oxidative stress and possibly the activation of the RAAS. It is well known that RAAS is activated once the renal artery is temporarily clamped (Atlas, 2007). Similar duration of the renal ischemia has been used by other investigators (Sakat *et al*., 2009). Significant increase in SBP, DBP and MABP were observed at 15, 30, 45 and 60 min after clamp removal, after which the blood pressures tended to recover up to 90 min. The result showed that intravenous injection of EELU 400 mg kg^{-1} significantly decreased hemodynamic parameters such as SBP, DBP and MABP at 30, 45 and 60 min after clamp removal. According to (Rickard *et al*., 1996; Prasad, 2000) SDG is metabolized into secoisolariciresinol and to the mammalian lignans enterolactone and enterodiol. It has been considered that extended hypotensive effect could be due to its metabolites.

In present investigation EELU (400 mg kg^{-1}) treatment significantly restored LVEDP and ventricular pressure time index. These are direct indicators of cardiac systolic function and myocardial oxygen consumption (Zhang *et al*., 2009). When other left ventricular functions were discussed, no pronounced effects of EELU were observed in dp/dt max, dp/dt min and exponential tau in RAO group after clamp removal and throughout study period. Antihypertensive activity of SDG isolated from flaxseed has been studied by Prasad (2004) and reported

SDG as a long acting hypotensive agent. Hypotensive activity of SDG is considered because of stimulation of guanylate cyclase activity and its mechanism of action is similar to that of nitric oxide.

It is reported that kidney is one organ which is extremely sensitive to changes in oxygen tensions within its complex architecture making it very prone to hypoxic injury when the renal artery is temporarily occluded (Michels *et al*., 2009). Renal cells were found to be more sensitive in oxidative damage induced by occlusion of renal artery and useful tool as marker reflecting the systemic symptoms of oxidative stress (Rodriguez-Lopez *et al*., 2006; Gulec *et al*., 2006). The present investigation showed increased ROS generation in renal cells of renal artery occluded rat, which may be because of the increased utilization of these antioxidant enzymes to counteract the ROS generated by renal ischemia. Treatment of EELU 400 mg kg^{-1} in renal artery occluded rat showed significant decrease in ROS generation.

CONCLUSION

It is concluded that EELU (400 mg kg^{-1}) treatment exhibited antihypertensive effects in renal artery occlusion induced hypertension in rat. The antihypertensive activity of EELU may be resulted through the action on rennin angiotensin system and inhibition of ROS in renal cell.

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