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Hydroethanolic Extract of *Eribroma oblongum* (Malvaceae) Stem Bark Prevents Hypertension, Oxidative Stress and Dyslipidemia in L-NAME Induced Hypertension in Wistar Rats

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Abstract: *Introduction: Eribroma oblongum* (Malvaceae) stem bark is used in Cameroonian ethnomedicine to treat various metabolic illnesses including cardiovascular diseases. The present study was designed to investigate the mechanisms of hypotensive effects of hydroethanolic extract of *Eribroma oblongum* and its cardioprotective effects after a subchronic treatment. *Study Design:* This was a prospective cross-sectional analytical study *Methodology:* Preventive effects were studied after oral administration of hydroethanolic extract of *Eribroma oblongum* (HEEO) (100 mg/kg body weight/day) or captopril (25 mg/kg body weight/day) simultaneously with L-NAME (40 mg/kg body weight/ day) in rats for 28 days. Body weight and food intake were measured after 5 days. At the end of treatment, blood pressure and heart rate were recorded. Aorta, heart, liver and kidneys were weighted. Reduced nitrite, superoxide dismutase, catalase, gluthatione, Malondialdehyde and lipid profile were measured. *Results:* The extract prevented L-NAME hypertension and improved food intake and body weight of rats. HEEO improved lipid profile and antioxidant status. L-NAME caused a significant ($P<0.001$) increase in the levels of serum transaminase relative to the normal. L-NAME treated rats had markedly decreased catalase (CAT), superoxide dismutase (SOD), nitrite (NO_2^-) and reduced glutathione (GSH) levels in the tissue. Also, L-NAME caused a significant ($P<0.001$) induction of lipid peroxidation (MDA) in the animals tissue relative to the normal range. Administration of HEEO with L- NAME caused significant ($P<0.001$) inhibition of MDA relative to LNHR and augmented tissue antioxidant indices. *Conclusion:* The hydroethanolic stem bark extract of *Eribroma oblongum* showed a promising potential cardioprotective effect in L-NAME induced hypertension. This effect could be due at least in part by improving endothelial function, lipid profile and oxidative status. These findings justify the traditional use of HEEO as treatment in cardiovascular disorders and serve as a promising traditional pharmacopoeia for the development of new chemical entity in the phytomedicine development platform.

Keywords: *Eribroma oblongum*, L-NAME, Blood Pressure, NO, Antihypertensive and Antioxidant Activities, Wistar Rat

1. Introduction

Previous study showed that the hydroethanolic extract of *Eribroma oblongum* possesses antihypertensive and antiatherogenic properties in a model that atherogenic diet induced hypertension in Wistar rats. Essential hypertension accounts for approximately 90% to 95% of patients diagnosed with hypertension [1] and it is mainly caused by endothelial dysfunction. Endothelial dysfunction itself results from NO deficiency. In fact, it has been found that vascular endothelium of hypertensive patients produces less nitric oxide, a key regulator of cardiovascular system and metabolic homeostasis [2]. The inhibition of NO production by L-arginine analogue like Nw Nitro-L- arginine methyl-ester (L-NAME) therefore results in arterial hypertension, dyslipidemia and histological damages. L-NAME-induced hypertension is thus a suitable model to study the cardiovascular effects of new active substances. *Eribroma oblongum* stem bark is widely used in Cameroon for treatment various metabolic illness such as hypertension. Cardiovascular disease is the leading cause of death and a major cause of disability not only in the United States but also worldwide [3]. Also known as the "silent killer", hypertension is estimated to cause 4.5% of current global disease burden and is as prevalent in many developing countries, as in the developed world [4].There are controllable factors such as poor diet, obesity, excessive consumption of alcohol or salt, stress and physical inactivity, and non-controllable factors such as age and heredity. Treatments of hypertension are usually diuretics, beta blockers, alpha blockers, calcium channel blockers, angiotensin II receptors inhibitors, inhibitors of angiotensin-converting enzyme and mimetic nitric oxide. High blood pressure is particularly dangerous because it usually remains silent, which greatly increases the risk of complications. Patients balk the necessity of treatment in the absence of obvious symptoms, and most importantly, because of the high cost of drugs in modern medicine. Therefore in developing countries such as Cameroon, people resort to traditional medicine by phytotherapy to solve their health problems. They are encouraged by the affordability, efficiency and availability of this medicine [5]. However, scientific investigations are necessary to confirm these therapeutic claims and to regulate the use of these herbal drugs by populations. *Eribroma oblongum* (Malvaceae) is a tree of about 15 m high found in Central Africa. It is widely used in African traditional medicine to treat various metabolic illnesses including cardiovascular diseases. In Cameroon, decoction of *E. oblongum* dried stem bark is used by traditional healers of Centre Region, for the management of cardiovascular diseases, especially hypertension in combination with other plants [6]. Phytochemical investigations have shown the presence phytochemical analysis revealed the presence of reduced sugar, flavonoides, tannins, phenols and saponins. Terpenoides, alkaloids, lipids, steroid cardiac glycosides, anthraquinones, and triterpenes were absent [6]. The present investigation was undertaken to examine the antihypertensive activities of the stem bark hydroethanolic extract of *E. oblongum* in L-NAME-induced hypertensive rats (LNHR). For that purpose, we analyzed the effect of subchronic administration of the extract on blood pressure and heart rate, on oxidative stress and lipid profile, transaminase and creatinine induced by Nw-Nitro-L-arginine Methyl ester hydrochloride (L-NAME) by oral administration.

2. Material and Methods

2.1. Experimental Design

This study was performed on male Wistar rats from the Laboratory of Animal Physiology in the Department of Physiology, Faculty of Sciences, University of Yaoundé I-Cameroon. Male albino Wistar rats of 10-12 weeks of age, weighting 200-250 g were selected for these experiences. The animals were kept in a room maintained under environmentally controlled condition of temperature 23-25°C and 12 h light/dark cycle, with free access to tap water *ad libitum* and standard commercial diet. L-NAME induced hypertensive rats (LNHR) were used. To obtain LNHR, L-NAME was orally administrated to normotensive rats (NTR) for 28 days. Body weight and food intake were measured every 5 days until the end of experimentation. Then, rats with a systolic blood pressure higher than 140 mmHg were considered as hypertensive. The experimental protocol and the maintenance of the experimental animals was done in accordance with the standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986 [7].

2.2. Plant Material

Fresh stem bark of *Eribroma oblongum* were collected at Eseka, Centre province of Cameroon, in August 2013. The plant material was identified at the National Herbarium (HNC) of Yaoundé-Cameroon where a voucher specimen N°27489SRFCam has been deposited [6].

2.3. Drying and Preparation of the Extract of HEEO

Before drying could start, the plants were washed to remove sand and dust particles. The stem bark was separated from the rest of the plant and dried separately under shade in an airy condition. The stem bark was air dried at room temperature and ground into a powder. The extraction was done by using cold maceration process. The grounded plant material (2 kg) was soaked in 5 L of water-ethanol mixture

(50:50) for 48 hours at room temperature. After two days of occasional shaking, the whole material was filtered using a funnel and filter paper and then kept in a flask. Removal of the solvent from the obtained extract under reduced pressure using a rotary evaporator. The crude extract was then air-dried to obtain a solid mass with a yield of 25%. The extracts were then kept separately in small bottles and put in the fridge for later use [6].

2.4. Animal Studies

2.4.1. Animal Regulatory Issues

Healthy male albino Wistar rats (body weight 150–250 g) are preferred for the experiment according to the ICH guidelines. The experimental protocol and the maintenance of the experimental animals was done in accordance with the regulations of the OEDD guide since in Cameroon the ethics committee focuses only on clinical studies. The animal experiment protocols was carried out in accordance with the guidelines of the ICH on preclinical pharmaceutical testing in mouse..Male albino Wistar rats of 12 weeks old weighting 180-250 g were used. The animals were maintained on a 12 h light/dark cycle, with free access to water and standard laboratory diet. Normotensive rats (NTR) were used to evaluate hypotensive effect in the ethyl acetate extract and compounds on arterial blood pressure, heart rate and its mechanisms of action.

2.4.2. Animal Selection

Male albino Wistar rats of 12 weeks old weighting 190-250 g were used. The animals were maintained on a 12 h light/dark cycle, with free access to water and standard Laboratory diet. Normotensive rats (NTR) were used to evaluate effects of the plant extract on arterial blood pressure, heart rate and its mechanisms of action. To determine the antihypertensive activity mechanism of *Eribroma oblongum*, atherogenic diet induced- hypertensive rats (ADHR) were used. To obtain ADHR, NTR were fed by

atherogenic diet for 45 days. Body weight and food intake were of study. The feeding for 45 days was done to get a steady trend that can be validated and also to be able to assess the effect of possible organ toxicity which was an aspect of interest of the study to us. Then, rats with a systolic blood pressure higher than 140 mmHg were considered as hypertensive.

2.4.3. Subchronic Effects of Hydroethanolic Extract of Eribroma Oblongum in L-NAME Induced Hypertension

Normotensive rats were randomly divided into four groups. The first group made of 5 rats served as control and received distilled water; the second group made of 5 rats received only L-NAME (40 mg/kg body weight/day); the third group constituted of 5 rats received concomitantly L-NAME (40 mg/kg body weight/day) and captopril (25 mg/kg body weight/day) and the last group made of 5 rats received concomitantly L-NAME (40 mg/kg body weight/day) and plant extract (100 mg/kg body weight/day). All these products were dissolved in water and given daily per os to the animals for a period of 28 days. During this period, food consumption and body weight were noted. At the end of treatment, the rats were fasted for 12 h before the rats were anesthetized using an intraperitoneal injection of urethane (1.5 g/kg body weight/day). The trachea was exposed and cannulated to facilitate spontaneous respiration. A polyethylene catheter was inserted into the rat carotid artery. This catheter was linked to the transducer connected to the recorder hemodynamic signal was transferred to an acquisition system (Biopac Student Lab MP type 35) coupled with a computer. Another catheter was inserted into the femoral vein and a bolus injection of 10% heparin (0.1 mL/100 g body weight) was immediately administered. The animals were allowed to stabilize for at least 30 minutes [8; 9]. The blood was collected from carotid arterial of the animals into dried tubes. Serum was prepared by centrifugation at 1000 g for 15 minutes. The clear supernatant was used for the estimation of serum lipid profile, hepatic and renal functions.

2.5. Preparation of Tissues

Aorta, heart, liver and kidneys were dissected out, washed in ice-cold 9% NaCl solution, dried and weighed. All these tissues and the remaining parts of liver homogenized. The Mc Even solution (mM NaCl, 147; $CaCl_2$, 2.6; CO_3HNa 11.6; D-glucose, 11; KCl, 5.6; NaH_2PO_4, 0.66; $MgCl_2$, 0.24) was used for aorta (10% m/v), heart (20% m/v) and Tris-HCl 50mM buffer solution was used for liver and kidneys (20% m/v). The tissues were centrifuged at 15,000 g for 20 minutes to obtain post-mitochondrial supernatant fraction. All procedures were carried out at temperature of – 4°C [6].

2.6. Biochemical Analysis

2.6.1. Oxidative Stress Markers

Tissue levels of Superoxide dismutase (SOD) activity was assayed according the method described by Misra and Fridovich (1972) [10], catalase (CAT) by sinha (1972) [11], reduced glutathione (GSH) by Ellman (1959) [12]; nitrites ($N0_2^-$) assay was performed using the methods of Ikeda *et al.*, 2003 [13] and malondylaldehyde (MDA) was assayed according to Wilbur *et al*., 1949 [14].

2.6.2. Lipid profile Assays

Blood samples were collected in dried tubes and the serum was obtained by centrifugation at 1000 g during 15 minutes at -4°C for serum activities. The lipoproteins were measured using the enzymatic colorimetric method. Samples were assayed for total cholesterol HDL-cholesterol and triglycerides by using the kits (Fortress diagnostics). The low-density lipoprotein (LDL) was calculated using the formula by Friedewald *et al*., 1972 [15]. All these parameters were determined by measurement of the optical density of the reaction products at the corresponding wavelengths with spectrophotometer (Genesys 20, Thermo Fisher Scientific, Waltham, MA, USA).

2.6.3. Determination the Atherogenic Index And the Protection Percentage

$$Atherogenic\ index\ 1 = \frac{Total - Cholesterol}{HDL - Cholesterol}$$

or

$$\text{Atherogenic index 2} = \frac{\text{LDL} - \text{Cholesterol}}{\text{HDL} - \text{Cholesterol}}$$

$$* \text{ Protection (\%)} = \frac{\text{Atherogenic index of control} - \text{Atherogenic index group treated}}{\text{Atherogenic index of control}}$$

2.7. Statistical Analysis

The results were expressed as means ± SEM and analyzed with Graph Pad Instat Software. The comparisons within the experimental groups were made using one way analysis of variance (ANOVA) followed by Dunnett as post hoc test. P values less than 0.05 were considered significant

3. Results

3.1. Subchronic effects of E. Oblongum

3.1.1. Body Weight

As shown in table 1, the body weight increased in all groups during the treatment, however this increase was low in test groups compared to normal group. Rats treated with L-NAME were showed the lowest increasing of body weight which was significant (P<0.01) compared to normal group. Thus the percentages of growth were 66.32, 52.65 and 57.71% respectively for L-NAME, captopril, extract at the doses of 100 mg/kg body weight/day. In rats treated with HEEO and captopril, the increase of body weight was significantly (P<0.01) reduced from the fifteenth day compared to normal group.

Table 1. *Effects of E. oblongum on body weight of L-NAME hypertensive rats after 4 weeks of treatment.*

Body weight variations %						
Group	**day-5**	**day-10**	**day-15**	**day-20**	**day-25**	**day-28**
Normal	7.9±0.23	9.06±1.28	12.5±2.8	16.8±3	18.44±3.2	19.7±5.54
L-NAME	3.3±0.6**	5.19±0.38**	7.06±2.16**	9.1±1.9**	9.5±0.53**	9.8±1.2**
L-NAME + captopril	5.7±0.78$^{\beta}$	6.42±0.53**$^{\beta}$	8.6±0.72**$^{\alpha}$	10.4±0.86**$^{\gamma}$	11.66±0.93**$^{\beta}$	12.04±1.77**$^{\beta}$
L-NAME+ HEEO	6.3±3.91$^{\beta}$	7.95±6.64*$^{\beta}$	9.8±10.81**$^{\alpha}$	12.6±14.8**$^{\beta}$	13.42±17.8**$^{\beta}$	14.9±17.45**$^{\beta}$

Data represent the mean ± SEM of each group. HEEO: hydroethanolic stem bark extract of *E. oblongum*. *P< 0.05, **P<0.01, significantly different compared to normal group. $^{\alpha}$P<0.05, significantly different compared to L-NAME (Nw Nitro-L- arginine methyl-ester) group. $^{\beta}$P<0.01, significantly different compared to L-NAME group.

3.1.2. Food Consumption

The effects of chronic administration of HEEO on food are showed in table 2. Treatment of rats during 28 days with L-NAME has induced a significant (P < 0.01) drop in food consumption compared to normal group. Treatment with captopril and plant extract blunted the decrease in food intake observed in hypertensive group. This effect was significantly marked with plant extract at the dose of 100 mg/kg during the treatment. So, the percentage of decrease of food intake at the fourth week.

Table 2. *Effects of E. oblongum on food intake of L-NAME hypertensive rats after 4 weeks of treatment.*

Food intake (g / 100g of body weight)						
Group	**day-5**	**day-10**	**day-15**	**day-20**	**day-25**	**day-28**
Normal	6.16±0.47	9.06±0.45	12.79±0.88	14.16±0.55	15.44±0.91	16.59±0.26
L-NAME	3.07±0.23**	5.19±0.65**	7.14±0.51**	8.58±0.53**	9.22±0.18**	9.62±0.47**
L-NAME + captopril	5.56±0.6	6.42±0.8**	8.03±0.66*	9.03±0.76*	10.66±0.34**	11.47±0.22**
L-NAME+ HEEO	5.41±0.33	7.95±0.12$^{\alpha}$	9.42±0.18*	10.43±0.49*	12.42±0.49*$^{\alpha}$	13.73±0.42*$^{\alpha}$

Data represent the mean ± SEM of each group; g/100 g of body weight: quantity taken in g per animal per week. HEEO: hydroethanolic stem bark extract of *E. oblongum*. *P<0.05, **P<0.01, significantly different compared to normal group. $^{\alpha}$P < 0.05, $^{\beta}$P<0.01significantly different compared to L-NAME group (Nw Nitro-L- arginine methyl-ester).

3.2. Antihypertensive Effects of E. Oblongum

Subchronic oral administration of L-NAME (40mg/kg body weight /day) for 28 days resulted in a significant increase in mean systolic blood pressure (MSBP) by 46.94% and heart rate (HR) by 8.76% in L-NAME group as compared to normotensive rats (normal group). The hydroethanolic stem bark extract of *E. oblongum* at 100 mg/kg body weight /day significantly blunted the increase in MSBP in LNHR but also reduced the MSBP level at a value below to that of normotensive rat (NTR). Comparable results were obtained with captotril (25 mg/kg body weight/day). Treatment with *E. oblongum* significantly blunted the increase in heart rate in LNHR at the dose, as well as captopril 25 mg/kg body weight /day (fig 1).

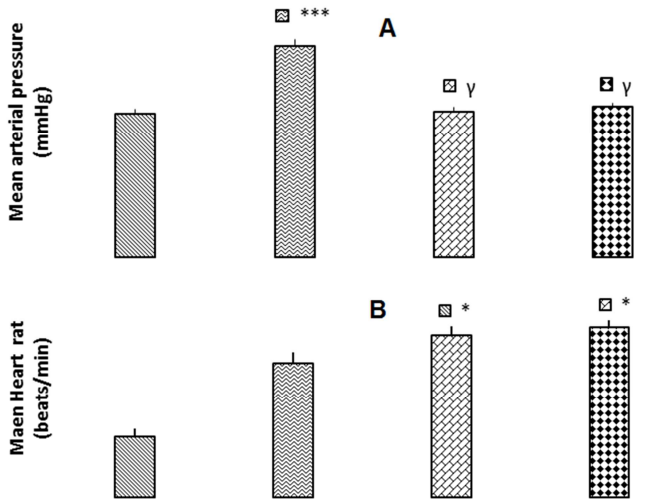

Figure 1. *Effects of Eribroma oblongum on mean arterial pressure (A) and heart rate (B) of L-NAME hypertensive rats after 28 days of treatment.*

Each bar represents the mean ± SEM of group *P<0.05; **P< 0.01, significantly different compared to normal group; $^{\gamma}$P<0.001 significantly different compared to L-NAME group (Nw Nitro-L- arginine methyl-ester) group. L-NAME plus Captopril (25 mg/kg body weight/day); L-NAME plus *Eribroma oblongum* (100 mg/kg body weight/days) extract.

3.3. Subchronic Effects of the Hydroethanolic Extract of Eribroma Oblongum on Lipid Profile

Total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides levels were significantly increased respectively by 34.43% (P<0.001), 41.11% (P<0.001), by 19.24% (P<0.05) and by 64.50% (P<0.001) in LNHR group as compared to normal group. HEEO significantly blunted the increase by 20.35% (P<0.01) and by 38.85% (P<0.001) respectively the level of total cholesterol or HDL-cholesterol as compared to normal group.

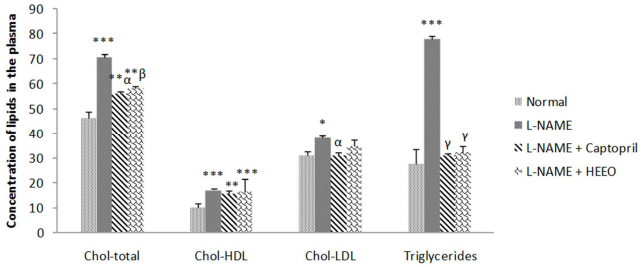

Figure 2. *Effects of the hydroethanolic extract of Eribroma oblongum on lipid profil in L-NAME induced-hypertensive rats.*

Data represents the mean ± S.E.M. of each group; n=5 number of animals in each group; *P<0.05,**P<0.01 and ***P<0.001 significantly different compared to the normal group. $^{\alpha}$P<0.05, $^{\beta}$P<0.01 and $^{\gamma}$P<0.001 significantly different compared to L-NAME (N_W-Nitro-L-Arginine Methyl Ester) group. TC: total Cholesterol; LDL-C: Low Density Lipoproteins Cholesterol; HDL-C: High Density Lipoproteins-Cholesterol.

3.4. Effect of Hydroethanolic Extract of the Bark Stem of E. Oblongum on Cardiovascular Risk

After 4 weeks of gavage with a solution of L-NAME 40 mg/kg body weight /day, in normotensive rats, we found that the hydroethanolic stem bark extract of *E. oblongum* at a dose of 100 mg/kg body weight/day administered concomitantly decreased the risk of developing atherosclerosis of 23.27% or 31.16% (table 3).

Table 3. *Effects of the hydroethanolic extract of Eribroma oblongum on atherogenic Indexe in L-NAME induced-hypertensive rats.*

Group	Normal	L-NAME 40 mg/kg Pc	L-NAME 40 mg/kg Pc +	
			Captopril 25 mg/kg Pc	HEEO. 100 mg/kg Pc
Atherogenic Indexe 1 TC/HDL-C	4.64	4.16	3.58	3.56
*Protection(%) 1	-	-	22.64	23.27
Atherogenic Indexe 2 LDL-C/HDL-C	3.08	2.25	1.98	2.12
*Protection(%) 2	-	-	55.55	31.16

HEEO: hydroethanolic stem bark extract of *Eribroma oblongum*; L-NAME: N_W-Nitro-L-Arginine Methyl Ester; TC: total Cholesterol; LDL-C: Low Density Lipoproteins Cholesterol; HDL-C: High Density Lipoproteins-Cholesterol.

3.5. Effects of Hydroethanolic Extract of E. oblongum on Liver and Kidney Function

Levels of ALT and AST increased significantly from 40.44% (P <0.001) and 44.71% (P <0.001) compared to the normal group. The hydroethanolic stem bark extract of *E. oblongum* caused a significant increase in ALT levels 26.92% (P <0.01) compared to the control group and 18.50% (P <0.001) compared to the group treated with L-NAME and a significant decline in AST 28.95% (P <0.01) compared to group rats rendered hypertensive by L-NAME (table 4).

Table 4. *Effects hydroethanolic extract of Eribroma oblongum on liver and kidney functions.*

Group	Normal	L-NAME 40mg/kg Pc	L-NAME 40mg/kg Pc +	
			Captopril 25mg/kg Pc	HEEO. 100 mg/kg Pc
ALAT (U/L)	27.77±1.02	46.63±10***	29.36±2.16$^{\beta}$	38±3.93**$^{\alpha}$
ASAT (U/L)	43.27±4.70	78.27±4.13***	50.72±2.22**	55.61±3.6$^{\beta}$
ASAT/ALAT	1.55	1.67	1.72	1.46
Creatinine (mg/dL)	1.47±0.28	1.10±0.26	1.81±0.28	1.31±0.14

Data represent the mean ± SEM of each group. n=5: number of animals in each group; **P<0.01 and ***P<0.001 significantly different compared to the normal group. $^{\alpha}$P<0.05 and $^{\beta}$P<0.01 significantly different compared to L-NAME (N_W-Nitro-L-Arginine Methyl Ester) group. ALAT: Alanine animotransferase; ASAT: Aspartate animotransferase; HEEO: Hydroethanolic extract of *Eribroma oblongum*.

3.6. Effect of E. oblongum on Oxidative Stress Biomarkers

The concentration of nitrite (NO_2^-) decrease significantly in heart by 49.83% (P<0.05), liver by 64.43% (P<0.001) and kidney by 68.30% (P<0.05) in LNHR as compared to the normal group. The HEEO prevented the deleterious effects of LNHR in the tissues. It decreased significantly by 61.45% (P<0.01) and 43.46% (P<0.05) the concentration of nitrite in aorta and liver respectively compared to the normal group Table 4 shows that reduced glutathione (GSH) levels were significantly decreased in aorta by 57.27% (P<0.05), heart by 65.51% (P<0.01) and liver by 80.64% (P<0.001) of LNHR compared to normal group. Likewise, the extract (100 mg/kg body weight/day) like captopril significantly prevented the decrease in GSH levels. Group of LNHR exhibited a significant decrease in aorta by 56.06% (P<0.05), liver by 74.19% (P<0.001) and kidney by 26.24% (P<0.01) GSH activity as compared to normal group (table 4) whereas GSH significantly increased in the liver by 18.94% (P<0.001) as compared to LNHR group. Conversely, L-NAME-induced hypertension was associated with an increase in aortic SOD activity, the values (U/mg proteins) rising from 25.10±2.05 in normal group to 42.94% (P<0.01), in LNHR group. Treatment with the plant extract prevented the increase in SOD activity in the aorta, heart, liver and kidneys from LNHR group. Captopril treatment also reduced the increase in aorta SOD activity and significantly blunted the decrease in SOD activity in the heart, liver and kidney (table 5).

The catalase activity was decreased significantly in aorta by 85.94% (P<0.01), heart by 70.53% (P<0.01), liver by 70.56% (P<0.001) and kidney by 74.21% (P<0.001) as compared to normal group. The HEEO significantly prevented the deleterious effects of LNHR group in the tissues. It increased catalase activity in kidney by 44.34% (P<0.01) as compared to the normal group and 18.13% (P<0.05) or 66.97% (P<0.001) respectively in heart and liver as compared to LNHR group (table 5).

MDA levels were significantly P<0.001 higher in aorta by 42.02%, liver by 41.56%, and kidney by 43.11% tissues of L-NAME treated rats as compared to normal group. Treatment with *E. oblongum* (100 mg/kg/day) for 28 days significantly P<0.001 prevented the rise in tissue MDA levels in aorta by 55.05%, liver by 55.60% and kidney by 44.57% as compared to LNHR group. The same effect was observed with captopril (table 5).

Table 5. *Effects of hydroethanolic extract of Eribroma oblongum on oxidative stress markers.*

		Normal	L-NAME 40 mg/kg Pc	L-NAME 40 mg/kg Pc + Captotril 25 mg/kg Pc	L-NAME 40 mg/kg Pc + HEEO 100 mg/kg Pc
NO_2^- (µM/mg of protein)	Aorta	20.94±2.10	10.14±0.04	27.57±3.92$^{\beta}$	26.31±3.65$^{\beta}$
	Heart	30±5.90	15.05±0.32*	20.79±0.61	17.77±1.18
	Liver	35.82±6.09	12.74±0.37***	22.08±2.04*	20.25±1.03*
	Kidneys	50.19±12.4	15.91±0.53*	37.17±4.4	26.57±2.03
Gluthatione (µmol/L)	Aorta	3.3±0.63	1.41±0.17*	4.14±0.45$^{\beta}$	1.45±0.05*
	Heart	2.61±0.07	0.90±0.04**	3.20±0.43$^{\gamma}$	3.22±0.4$^{\gamma}$
	Liver	2.48±0.13	0.48±0.05***	0.82±0.04***$^{\alpha}$	0.64±0.06***
	Kidneys	1.41±0.11	1.03±0.1	1.81±0.15$^{\gamma}$	0.77±0.06**
SOD (U/mg of protein)	Aorta	25.10±2.05	14.32±0.77**	34.52±2.24**$^{\gamma}$	15.89±1.49**
	Heart	50.54±6.48	25.16±1.09*	35.66±1.07	38.15±8.79
	Liver	43.68±1.15	25.69±2.71***	34.93±1.87	33.82±3.75
	Kidneys	36.96±5.16	21.45±0.74**	31.62±0.8	33.53±2.02$^{\alpha}$
Catalase (U/mg of protein)	Aorta	50.03±12.8	7.03±1.33**	22.92±1.09	31.06±5.28
	Heart	40.08±7.41	11.81±0.43**	37.53±4.1$^{\beta}$	32.81±1.22$^{\alpha}$
	Liver	72.13±3.91	21.23±3.91***	52.39±8.84$^{\beta}$	64.28±2.53$^{\gamma}$
	Kidneys	65.82±6.93	16.97±3.66***	49.96±5.29$^{\beta}$	36.63±2.42**
Malondialdehyde (µmol/mg of protein)	Aorta	44.9±0.68	77.45±0.53***	65.76±7.81*	34.81±3.02$^{\gamma}$
	Heart	22.60±1.76	38.69±9.27	28.31±2.37	24.36±0.69
	Liver	15.08±0.6	35.34±2.74***	29.94±3.93**	15.69±1.01$^{\gamma}$
	Kidneys	20.65±0.77	36.30±2.2***	29.31±0.77**$^{\alpha}$	20.12±1.56$^{\gamma}$

Data represent the mean ± SEM of group; n=5: number of animals in each group. $N0_2^-$: nitrite; SOD: super oxide dismutase; HEEO: hydroethonolic stem bark extract of *Eribroma oblongum*.*P<0.05, **P<0.01 significantly different compared to normal group and, $^{\alpha}$P<0.05, $^{\beta}$P<0.01,$^{\gamma}$P<0.001; significantly different compared to L-NAME (N_W-Nitro-L-Arginine Methyl Ester) group

4. Discussion

This study aimed to evaluate the antihypertensive effect of hydroethanolic stem bark extract of *E. oblongum* on L-NAME-induced hypertensive rats. the administration of the solution with L-NAME 40 mg/kg bw/day is a non selective inhibitor of NO synthase [16, 17], during 28 days leads to the increase significantly P<0.001 of mean systolic arterial blood pressure (MSBP) by 46.94% in L-NAME group as compared to normal group. HEEO 100 mg/kg bw/day prevented significantly P<0.001 hypertension by 28.39% in rats simultaneously treated with L-NAME, in comparison to L-NAME group (fig.1 A). The blocking oxide synthase nitrite by L-NAME resulted dyslipidemia, which play an important

role in the pathogenesis of hypertension [18]. Hypertension and hyperlipidemia are two cardiovascular risk factors. Thus to reduce the cardiovascular risk, it is important to qualitative and quantitative change one or more parameters of plasma lipids [19]. The observed dyslipidemia was characterized by hypertriglyceridemia, hypercholesterolemia, elevated HDL-cholesterol and LDL-cholesterol. Dyslipidemia observed in the group of hypertensive rats made was prevented by the ethanolic extract and captopril, which improved the lipid profile by increasing the production of nitrite oxide. These results clearly showed that the hydroethanolic extract of *E. oblongum* has hypotriglycéridémiantes and cholesterol-lowering properties on atherogenic risk. The pharmacological activity of this extract could be explained by the presence of phytochemical constituents such as flavonoids and saponins in the plant. These bioactive phytomolecules are known to possess vasorelaxant activity, antihypertensive and antihyperlipidémiantes [20]. Chronic administration of L-NAME rats is a source of hepatotoxicity, characterized by, among other malfunction in serum transaminase levels. The transaminases (ALT, AST) are well known enzymes as good indicators of liver function and as possible toxicity markers [21]. ALT and AST are important enzymes produced by the liver and serum levels of these enzymes are widely used as biomarkers of liver health [22]. Regarding the activity of transaminases, significant increase or decrease in transaminase activity in the group of rats made hypertensive with L-NAME may reflect liver damage. Co-administration of L-NAME and the extract allowed to significantly increase serum transaminases compared to rats made hypertensive group, suggesting that the hydroethanolic extract *E. oblongum* protect the liver toxicity due to L-NAME. L-NAME is an L-arginine analog which blocks nitric oxide production in endothelial cells resulting in elevation of blood pressure [23;24]. HEEO and captopril significantly prevented development of hypertension in rats. Captopril, an angiotensin converting enzyme (ACE) inhibitor blocks the production of angiotensin II and reduces blood pressure [25]. HEEO could prevent hypertension by NO/GMPC releasing. However, the possible hypotensive effect of the extract through ACE system could be explored. Indeed, the nitrite oxide (NO) plays a critical role in the maintenance of basal vascular tone. Factors other than the inhibition of NO may be involved in the development of hypertension induced by L-NAME, such as the activation of vasoconstrictor systems like the renin-angiotensin system, endothelin or the sympathetic nervous system [26; 27]. The mechanisms involved in this beneficial effect may involve inhibiting the sympathetic system as suggested by the results of intravenous study. However, other mechanisms may also be involved, including the reduction of oxidative stress. During installation of hyperlipidemia, cell membranes and extracellular matrix may change their lipid composition and thus be more capable of generating free radicals. Oxidative stress alters lipids creating a disturbance of self-generation cycle of free radicals and protein modification [28]. In addition, patients with hypertension, the decrease in antioxidant defenses is well documented [29]. Our results showed that in this model of hypertension several oxidative stress markers are altered. Reduced glutathione (GSH) is an endogenous antioxidant most abandant in eukaryotic cells that interacts with activated oxygen species, thereby preventing the oxidation of organic substrates (proteins, DNA, fatty acids). GSH is also a scavenger of superoxide radicals and protects the thiol groups of proteins against oxidation [30]. Our results showed a general decrease in the level of glutathione reduced in aorta, heart, liver and kidneys of rats made hypertensive by L-NAME. The determination of the specific activity of superoxide dismutase (SOD), an enzyme that catalyzes the dismutation of the superoxide anion (O_2^-) into water and hydrogen peroxide [31] shows a decrease of activity SOD in the aorta, heart and kidney of hypertensive rats. The increase in SOD activity has been reported in the aorta of rats [32] and would be secondary to the increased production of O_2^-. Our results suggest that in this model the aorta, heart, liver and kidneys would be affected by oxidative stress. The hydroethanolic stem bark extract of *E. oblongum* reduced the nitrite levels around the normal value at the aorta. These results suggest that the extract has a protective role against the endothelial dysfunction induced by L-NAME. Malondialdehyde (MDA) is used as an index of lipid peroxidation resulting from the reaction of active oxygen species with the membrane fatty acids [33]. In our study, it was observed in hypertensive rats made a significant increase in MDA levels especially in the liver. The hydroethanolic stem bark extract of *E. oblongum* has had a beneficial effect on tissue parameters of oxidative stress; namely a reduction rate of SOD, catalase and MDA and nitrites increase the rate. These results suggest that the extract of *E. oblongum* would be capable of preventing lipid peroxidation induced by L-NAME and increase vasodilation of vessels.

5. Conclusion

In conclusion, present study showed that the oral administration of hydroethanolic stem bark extract of *Eribroma oblongum* possesses antioxidant activity, protective effects on the vessels by improving the NO bioavailability. This helps in prevention of hypertension induced by L-NAME. Overall data justified the empirical uses of this plant in the treatment of arterial hypertension associated with NO deficiency.

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