ANTIOXIDATIVE AND ANTIHYPERTENSIVE EFFECTS OF THE AQUEOUS LEAF OF *GMELINA ARBOREA* ON RATS FED WITH HIGH SODIUM CHLORIDE DIET

Sylvie Lea Wansi[1*], Fodouop Siaka Fodouop Kayem[1], Paulin Nyadjeu[1], Albert Kamanyi[1]
[1]Department of Animal Biology, Faculty of Science, University of Dschang, PO.BOX 67 Dschang, Cameroon
E mail: wansylvie@yahoo.fr Tel. (237) 77699146

Summary

Reactive oxygen species play an important pathophysiological role in the development of hypertension. Plant extracts have been shown to exert antihypertensive effects by a combination of vasodilator and antioxidant actions. *Gmelina arborea* is a medicinal plant which belongs to the family Verberaceae. It is widely distributed in subtropical regions. In Cameroon, their leaves are commonly used in antihypertensive herbal prescriptions. This study investigated the effects of the aqueous extract of the leaves of *G. arborea* on some oxidative stress parameters, on the blood pressure and on the vascular response of isolated rat aorta, in order to scientifically validate this traditional therapeutic claim and even postulate the possible mechanisms involved in the antihypertensive properties of the plant. The extract (150mg/kg and 300mg/kg) exerted a protective effect by increasing significantly the levels of antioxidants namely, superoxide dismutase, catalase and nitric oxide. The extract also demonstrated free radical-scavenging activity in DPPH assay and the ability to protect β-carotene against peroxidation by neutralizing the linoleate-free radical and other free radicals formed in the system, confirming it in vivo antioxidant effect. The extract exhibited vascular relaxant effect on aortic rings precontracted with phenylephrine 5µM. This relaxation was little inhibited by denudation of the endothelial layer, showing that it might act through the functional endothelium or directly on the smooth muscle cells. On the blood pressure, the extract revealed a dose dependent antihypertensive action. At doses of 30 and 50 mg/kg, the aqueous extract provoked a significant decrease ($p<0.01$) of blood pressure (15.48 and 24.39 % respectively) which was sustained for about 30 min. These results indicated that bioactive compounds such as flavonoids and saponins identified in the extract might be responsible to these pharmacological properties; thereby lending some validation of its traditional therapeutic usage in handling cases of hypertension.

Key words: *Gmelina arborea*, antioxidative, antihypertensive effects.

Introduction

Hypertension is considered as a major risk factor for development and progression of cerebro- and cardiovascular disorders. The pathogenesis of hypertension is complex and multifactorial. Amongst the factors implicated in the pathophysiology of hypertension, factors related to genetics, environment, psychosocial and inflammation have all been advocated to play an important role (1, 2). A balance of endothelium-derived vasodilators, especially NO, and reactive oxygen species (ROS) modulates endothelial function. Therefore, an imbalance of NO and ROS, so-called oxidative stress, is involved in endothelial dysfunction through the inactivation of NO (3). This state of oxidative stress which is a result of impaired endothelial nitric oxide activity has been implicated in the pathogenesis of hypertension (4, 5).

Prevailing economic conditions in many developing populations necessitate the development of alternative low-cost therapy to prevent and to manage various human ailments. Plant extracts present another advantage relative to its low toxicity. *Gmelina arborea* is a verberaceae widely distributed in Central and West Africa. Their leaves are generally used in traditional medicine for treatment of abdominal disorders, rheumatism, headache and cardiovascular diseases especially hypertension. Recently, *G. arborea* leaves showed some in vivo and in vitro cardiovascular effects in rat (6) and spasmolytic effects in rat intestine (7). Previous phytochemical studies reveal the presence of butyric acid, tartric acid and saccharin in both fruits and roots. However, there is no information about the effects of *G. arborea* extract on pharmacological actions in hypertension associated with oxidative stress. In the present study, we evaluated the antihypertensive effects of aqueous extract from the leaves of *Gmelina arborea* on salt hypertensive rats. In addition, we also examined the antioxidant activity and the effects on the vascular responses of isolated rat aorta.

Materials and Methods

Plant material and the plant extract preparation

Gmelina arborea leaves were harvested in Yaoundé, Cameroon in October 2006, and identified in the National herbarium where a voucher specimen has been deposited under acquisition number HNC49461. The harvested fresh leaves were air-dried at room temperature, cut into small pieces and ground into a powder. The powdered leaves (250 g) were soaked in distillate water (1L) at room temperature for 48 hours, with occasional shaking. After filtration, the filtrate was freeze-dried to obtain 12.7 g of the leave water soluble residue, corresponding to an extraction yield of 5.08 %.

Phytochemical screening

The extract was submitted to the Lieberman Buchard, ferric chloride, copo of magnesium and Vanillin-sulphuric acid tests to determine the presence of sterols, phenolic compounds, flavonoids and saponins respectively.

Experimental animals

The experiments were carried out on forty male Wistar rats (weight: 150-180 g; age: 6-8 weeks old), bred and housed in the animal house of the Department of Animal Biology, Faculty of sciences, University of Dschang, Cameroon. The rats were maintained on a 12-h light/12-h dark regime, and given both laboratory rat diet (Granules) with water *ad libitum*.

In vivo antioxidant assay

At the start of the experiment, the rats were weighed and records kept. Twenty five young Wistar rats were divided into five groups of five rats each. The normal rats named Group I received distilled water, the control group (group II) received high sodium chloride solution (9 % NaCl) and the positive group (group III) was treated with vitamin C dissolved into high sodium chloride solution. Groups IV and V were given a combination of high sodium chloride solution and aqueous suspension of *G. arborea* orally at dose levels of 150 and 300 mg/kg body weight. Every test solution was given to each animal by oral gavage (1 ml/kg body weight). Apart from the first group, in all treated rats, drinking water was replaced by 2 % sodium chloride solution *ad libitum*. Experimental rats were fed daily for a total of two months.

Blood sampling and analysis

After any month, all animals were fasted overnight; the rat in each group was weighted. At the end of the second month of treatment, all rats were anaesthetized by the intraperitoneal injection of 0.1 ml/100 g body weight of Thiopental at a dose of 50 mg/kg. Blood samples were collected from the abdominal artery, and allowed to coagulate. The serum was separated before being centrifuge at 3000g for 15 minutes. The resultant supernatant was assayed for total cholesterol (TC), high density lipoprotein (HDL), triglycerides (TG), low density lipoprotein (LDL) and total protein. TC, HDL, TG and LDL were estimated according to the previous describe method (8, 9, and 10) with a commercial available kit Dialab. The serum and the liver total proteins were determined using the Biuret method (11)

Tissue analysis

Immediately after collecting the blood samples, the organs such as liver, heart, kidneys and aorta were removed and weighted immediately on an electronic balance and into many pieces. A part of the kidney, liver and the heart was homogenized in a volume of a phosphate buffer (pH = 7.4) and, then centrifuged at 5000g for 15 minutes to yield a supernatant that was used to determine catalase (CAT). CAT activity was measured spectrophotometrically (12), using hydrogen peroxide as substrate. The pseudo-first order reaction constant (k) of the decrease in H_2O_2 absorption/s at 25 °C was determined and the enzyme specific activity was expressed as k/mg protein. Another part of the heart, kidney and liver was homogenized in a carbonate buffer (pH = 9.5) and, centrifuged at 5000g for 15 minutes to produce a supernatant that was used to determine malondialdéhyde (MDA) level as an index of lipid peroxidation (13), based on the reaction of MDA with thiobarbituric acid (TBA) at 95°C. Superoxide dismutase (SOD) activity in the three organs was determined spectrophotometrically based on its ability to inhibit the autooxidation of epinephrine to adrenochrome at alkaline pH (14). SOD activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit. The rest of the heart and the aorta were homogenized in a carbonate buffer and, then centrifuged at 5000g for 15 minutes and the supernatants obtained were used to determine the nitric oxide level by measuring the accumulation of nitrite. NO production was determined indirectly by assaying the supernatant for accumulated nitrite, the stable end product of NO reacted with molecular oxygen as previously described (15). Briefly, 100 µl of isolated supernatants were allowed to react with Griess reagent (1% sulfanilamide, 0.1% N-1-naphthylethylenediamine dihydrochloride and 2.5% phosphoric acid) at room temperature for 10 min. Nitrite products in supernatants were determined by measuring absorbance at 550 nm versus a $NaNO_2$ standard curve and the results were shown as µM.

In vitro antioxidative activity

The diphenyl-p-picrylhydrazyl (DPPH) radical scavenging activity was measured as described previously (16), with slight modification. Briefly, one millilitre from 0.16 mM methanol solution of DPPH radical was mixed with 0.25 ml of either extract or ascorbic acid at various concentrations. The mixtures were well shaken and kept at room temperature in the dark for 30 min. The absorbance was measured at 515 nm using a double beam Helios Epsilon spectrophotometer. Methanol was used as negative control. The radical scavenging activities of the tested samples, expressed as percentage inhibition of DPPH, were calculated according to the equation: $(\%I) = [(A_0 - As)/A_0] \times 100$

Where A_0 is the absorbance of the control (containing all reagents except the test compound), and As is the absorbance of test compound.

The antioxidant activity of the aqueous extract was also evaluated using β-carotene-linoleic acid model system (17). β-carotene (1.5 mg) in 7.5 ml of chloroform was added to 37.5 µl of linoleic acid, and 300 mg of Tween 80 emulsifier mixture. Chloroform was evaporated at 40°C. Then, 75 ml of distillate water was added and aerated for 15 minutes; time during which the solution was vigorously agitated to for a stable emulsion. 1340 µl of the mixture were transferred into test tubes containing 160 µl of either the extract or ascorbic acid prepare in ethanol at different concentrations as soon as the emulsion was add to each tube, zero time absorbance was measured at 492 nm using a spectrophotometer. The emulsion system was incubated for 105 minutes at 55°C and the absorbencies were measured one more time. The antioxidant activity coefficient (AAC) was given by the equation $AAC = [(A_{A,105} - A_{B,105}) / (A_{B,0} - A_{B,105})] \times 1000$ (a modified version of the formula of Chevolleau et al (18) where $A_{A,105}$ and $A_{B,105}$ are absorbencies of the test and the control at t = 105 minutes, respectively, and $A_{B,0}$ is the absorbance of the control tube at t = 0 minute.

Blood pressure measurement

Fifteen rats received daily and by gastric intubations, high sodium chloride solution (9 % NaCl) for a total of two months; during this time, drinking water was replaced by 2 % sodium chloride solution *ad libitum*. The blood pressure was determined by the invasive method. Briefly, the treated rats were anaesthetized by the intraperitoneal injection of 1ml/100g body weight of urethane at a dose of 1g/kg. The femoral vein was canulated for the administration of plant extract while the carotid artery was also canulated for the blood pressure measurement using a blood pressure transducer model COULD P 23 ID. The preparation was allowed to stabilise for at least 30 minutes before administration of the plant extract. Blood pressure variation was detected and recorded on a Universal Havard polygraph.

Preparation of rat thoracic aorta rings

Thoracic aortas were obtained from both male and female adult Wistar rats. The aorta was immediately excised after cervical dislocation of the rat. While immersed in aerated Krebs solution (composition in M: NaCl, 6.96 g; KCl, 0.35 g; $CaCl_2$, 0.28g; $MgCl_2$, 0.11g; $NaHCO_3$, 1.18 g, KH_2PO_4, 0.16 g and glucose, 2 g; 1l). A section of the thoracic aorta was carefully cleaned of adhering fat and connective tissue and cut into rings approximately 4-5 mm lengths. For endothelium-intact rings, extreme care was taken to avoid injury of endothelium. For endothelium-denuded strips, the endothelium was mechanically removed by gently rubbing the luminal surface three times with distillate water-moistened cotton wool. The tissue was placed in a well-oxygenated (95% O_2, 5% CO_2) bath of Krebs solution 10 ml at 37°C with one end connected to a tissue holder and the other to a force-displacement transducer (Ugo Basile 7003/4/5). The tissue was equilibrated for 60 minutes under a resting tension of 1.0 g. During this time, the Krebs Solution (KS) in the tissue bath was replaced every 15 minutes.

After equilibration, each aortic ring was contracted by treatment with phenylephrine (PE 5 µM). Endothelial integrity and successful removal of the functional endothelium was assessed by the presence or absence, of relaxant response to Carbachol (10 µM) on PE pre-contracted vessels. Carbachol-induced relaxation less or equal to 5% was taken as satisfactory removal of the functional endothelial layer. The aortic ring was then washed for 45 minutes with Krebs solution to remove PE and carbachol before starting the subsequent experiments. To evaluate the *G. arborea* extract (GAE)-induced vasorelaxation, cumulative doses (0.5 to 1.5 mg/ml) of GAE were added to induce relaxation after a maximal contraction of aortic vessel by 5 µM PE.

Chemicals and Solutions

Phenylephrine, Carbachol were obtained from Sigma chemical, ST Louis, MO, USA. Ascorbic acid (vitamin c), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), β-carotene and linoleic acid were purchased from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany) and were dissolved in distilled water. In addition, GAE was dissolved in distilled water.

Statistical analysis

Data are expressed as means ± standard error of the mean (SEM). Significant differences between responses were analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer post-hoc test for multiple comparisons using Graph Pad prism 3.0. For the in vitro antioxidant studies, the EC_{50} values were calculated using Graph Pad prism. In all comparisons, the difference was considered statistically significant at $p \leq 0.05$.

Results

Phytochemical screening

Preliminary phytochemical screening of the extract of *G.arborea* indicated that the aqueous extract contains phenolic compounds, flavonoids and saponins.

Effects of different treatments on body weight

The body weight increase in all treated animals during the first month except the rats receiving vitamin c. During the second month of treatment, the body weight increase in the normal animals was lower significantly ($p \leq 0.05$) in groups receiving only sodium chloride solution. Both plant extract and vitamin c also decrease none significantly animal's body weight compare to the control group (fig 1).

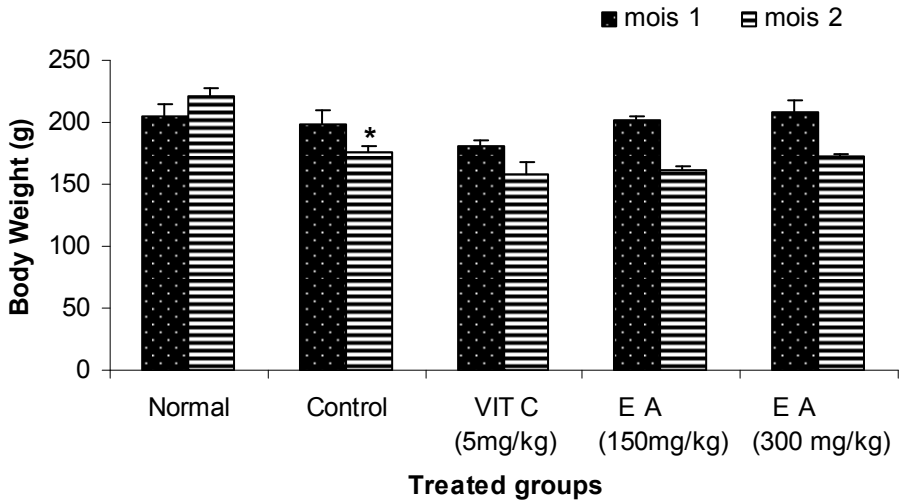

Fig 1: Effects of chronic treatments on the rat body weight. N = 5, *p<0.5 significantly different compared to normal.

Effects of different treatments on some organs weight

Figure 2 shows that high salt intake induced left ventricular hypertrophy significantly ($p \leq 0.05$) of 25.31 ± 2.18 %; this figure also indicates that even though the effect of the extract was not significant, it was able to reduce the left ventricular hypertrophy more than vitamin c.

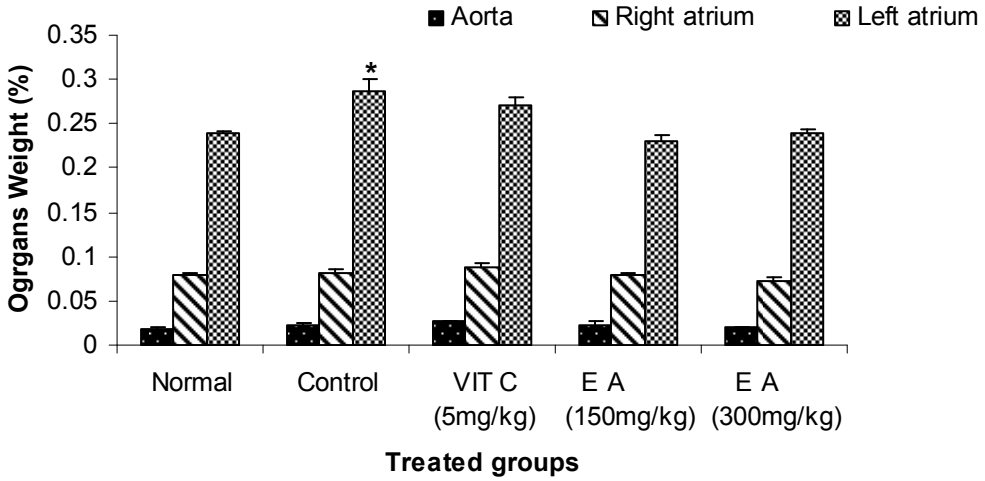

Fig 2: Effects of chronic treatments on organs weight. N = 5, *p<0.5 significantly different compared to normal group.

In figure 3, it is shown that neither the extract nor the vitamin c affected significantly the weight of kidneys compared to groups treated with distilled water and NaCl. However, the weight of the liver was attenuated by the extract.

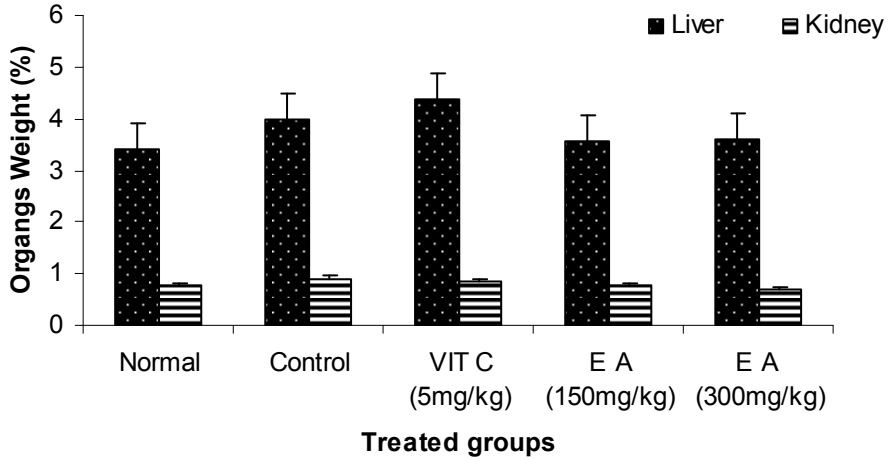

Fig 3: Effects of chronic treatments on liver and kidney weight. N = 5.

Effects of the aqueous extract of *G.arborea* on some biochemical parameters

Effects on plasma and liver proteins

Table 1 shows that high sodium chloride solution elevated significantly ($p \leq 0.05$) both plasma and liver proteins from 27.70 mg/ml and 51.40 mg/g to 38.24 mg/ml and 96.11 mg/g respectively. The extract produced no significant decrease of those biochemical parameters; meanwhile, the vitamin c slows down significantly ($p \leq 0.05$) the liver proteins to 69.95 mg/g compared to the control.

Table 1: effects of chronic treatments on the plasma and the liver proteins

		Total proteins	
Treatments	**Nomber of rat**	**Plasma (mg/ml)**	**Liver (mg/g tissue)**
Normal (distillate water)	**5**	27.70 ± 0.82	51.40 ± 0.95
Control (9% NaCl)	**5**	38.24 ± 1.59[a]	96.11 ± 1.08[a]
Vit C (5mg/kg)	**5**	34.07 ± 1.23	69.95 ± 0.68*
GAE (150mg/kg)	**5**	35.13 ± 1.04	79.08 ± 1.09
GAE (300mg/kg)	**5**	35.32 ± 1.5	82.71 ± 1.25

Results are expressed as mean ± SEM of five rats. [a] $p \leq 0.05$ (compared to normal group)
* $p \leq 0.05$ (compared to control group)

Effects on lipid profile

The plasma cholesterol and triglyceride levels increased significantly ($p \leq 0.05$) following high NaCl loading compared to the normal; however, only the second parameter was significantly reduced in group treated with vitamin c. In animals receiving the plant extract, the dose of 300mg/kg was able to increase significantly ($p \leq 0.05$) the level of HDL cholesterol. The atherogenic index which was elevated in control group was slowed down in all treated groups (table 2).

Table 2: Effects of chronic treatments on the plasma lipids

Parameters	**Normal**	**Control**	**Vit c (5mg/kg)**	**Extrait aqueux**	
				150mg/kg	**300mg/kg**
total Cholesterol (mg/dl)	23.92 ±2.21	27.95±3.12[a]	26.90±4.22	26.56±4.63	27.80±2.55
Triglycerides (mg/dl)	6.88±1.30	13.95±1.04[a]	9.04±0.97*	10.41±2.15	12.08±2.23
HDL Cholesterol (mg/dl)	17.41±0.74	12.42±1.07[a]	15.04±2.54*	12.72±0.94	14.98±0.83*
LDL Cholesterol (mg/dl)	5.13±2.61	12.7 4±1.82[a]	10.05±1.49	11.75±2.54	10.41±1.63
atherogenic Index	0,29	1,00	0,66	0,92	0,69

Results are expressed as mean ± SEM of five rats. [a] $p \leq 0.05$ (compared to normal group)
* $p \leq 0.05$ (compared to control group)

Antioxidant activities of the extract

From the results obtained in vivo, it is shown that SOD activities in the kidney and heart increased significantly ($p \leq 0.05$) in the control group as compared to the normal group of rat while the SOD activity observed an equally significant increase in the experimental groups of rat treated with 150 mg/kg of the plant extract and vitamin C compared to the control group. The extract also produced a significant increase in CAT levels on the heart while the vitamin c elevated the level of CAT in both heart and kidney. The results also indicated that, both extract and vitamin c decreased significantly ($p \leq 0.05$) the level of MDA in the liver compared to the control. The NO level in the heart decreased significantly in the control group while the extract as the vitamin c caused an equally significant increase ($p \leq 0.05$) Table 3.

Table 3: Effects of chronic treatments on some antioxidative biomarkers

	MDA (µM/mg tissue)			SOD (µl/mg tissue)			Catalase (µM/mim/mg tissue)			NO (µM/mg tissue)	
Treatment	**Liver**	**Kidney**	**Heart**	**Liver**	**Kidney**	**Heart**	**Liver**	**Kidney**	**Heart**	**Heart**	**Aorta**
Normal	0.29 ± 0.09	0.70 ± 0.06	0.25 ± 0.04	22.28 ± 1.02	66.77 ± 0.89	63.37 ± 1.38	1.7 ± 0.29	2.46 ± 0.39	2.95 ± 0.57	29.20 ± 3.53	43.1 ± 2.81
Control	0.78 ± 0.13	1.50 ± 0.11[a]	2.60 ± 1.23[a]	24.68 ± 3.14	83.54 ± 1.72[a]	79.45 ± 2.38[a]	1.4 ± 0.41	1.71 ± 0.09	1.88 ± 0.32[a]	14.58 ± 1.39[a]	34.63 ± 0.79
Vit c (5mg/kg)	0.49 ± 0.11*	0.96 ± 0.11	0.46 ± 0.04**	28.07 ± 2.97	97.66 ± 1.47*	93.60 ± 1.24*	1.6 ± 0.09	2.20 ± 0.21*	2.92 ± 0.19*	23.67 ± 2.82*	38.49 ± 1.20
EA (150mg/kg)	0.61 ± 0.22*	1.24 ± 0.19	1.71 ± 1.02	27.05 ± 3.00	90.57 ± 2.07*	81.04 ± 167	1.51 ± 0.31	2.07 ± 0.14	2.34 ± 0.25*	20.13 ± 1.52*	39.19 ± 1.76
EA (300mg/kg)	0.54 ± 0.17*	1.31 ± 0.12	1.64 ± 1.04	27.10 ± 2.97	89.35 ± 1.35*	89.91 ± 2.64	1.56 ± 0.08	1.99 ± 0.23	2.09 ± 0.23*	20.22 ± 3.89*	38.23 ± 1.06

Results are expressed as mean ± SEM of five rats. [a] $p \leq 0.05$ (compared to normal group)
* $p \leq 0.05$, ** $p \leq 0.01$ (compared to control group)

Antioxidant activity of *G. arborea* aqueous extract was also evaluated in vitro by two different test systems namely DPPH and β-carotene/linoleic acid. All of the data is presented in Table 4. As shown in that table, *G. arborea* plant extract showed free radical scavenging activities in a concentration-dependent manner with an IC_{50} value 620.6 µg/ml. In comparison, Vitamin c as positive control was used and IC_{50} value obtained was 0.21 µg/ml.

In β-carotene/linoleic acid model system, the aqueous extract from the leaves of *G.arborea* also exhibited an antioxidant activity. The efficacy (EC_{50}) of the extract in inhibiting the bleaching of β-carotene was notified even though it was inferior (334.4 µg/ml) to that of the positive control (2.16 µg/ml).

Table 4: In vitro antioxidant activity of the aqueous extract of *Gmelina arborea*

Sample	**Concentration (µg/ml)**	**DPPH[a]**	IC_{50} (µg/ml)	**β-carotene/linoleic acidc[b]**	EC_{50} (µg/ml)
Aqueous extract of *Gmelina arborea*	3	21.63	620.6	7713.28	334.4
	10	30.14		9579.94	
	30	33.45		7918.58	
	100	38.33		7908.04	
	300	56.12		5694.23	
	1000	84.19		3110.61	
Vitamin c	1	17.07	0.21	-245.92	2.16
	3	65.01		986.96	
	10	94.61		1252.45	
	30	94.51		203.58	
	100	95.96		996.74	
	300	95.55		1574.92	

[a] radical scavenging activity given as percentage inhibition
[b] antioxidant activity given as antioxidant activity coefficient

Antihypertensive Effects in salt loaded hypertensive rats

Acute intravenous administrations of the aqueous extract from the leaves of *Gmelina arborea* (10-50 mg/kg) into anaesthetised, hypertensive rats produced dose-related reductions in the systemic arterial blood pressure; this antihypertensive effect varied with time. At the dose of 10 mg/kg, the extract did not produce any significant modification in the blood pressure. Intravenous injection of 30 mg/kg of the extract reduced significantly ($p<0.01$) the blood pressure. The systolic blood pressure dropped from 168 mmHg to 142 mmHg, corresponding to a reduction of 15.42 % which persisted for about 7 min; this fall in blood pressure was followed by a progressive increase in blood pressure (163 mmHg) which yet remained below the initial level. The hypertension correction of the extract was more pronounced with the dose of 50 mg/kg; the systolic blood pressure dropped from 164 mmHg to 124 mmHg, representing a significant ($p<0.01$) drop of 24.39 % which persisted for about 30 min (figure 4).

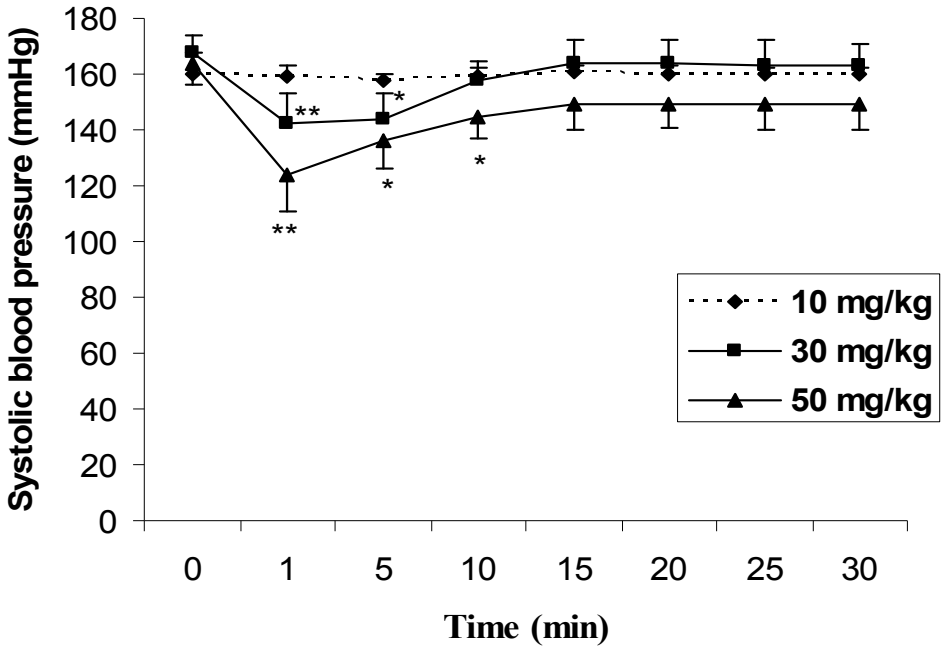

Fig 4: Effects of *G. arborea* aqueous extract on the blood pressure in salt loaded hypertensive rats. Each point represents the mean ± SEM of 5 animals. *$p<0.05$, **$p<0.01$; significantly different from initial value

Vasorelaxants Effects on Aortas from normotensive rats

Cumulative addition of the extract (0.5-1.5 mg/ml) to the bath solution caused concentration-dependent relaxation of aorta rings precontracted with PE (5 µM). The relaxation of aorta ring segment with endothelium intact (+E) increased with increasing doses of *G.arborea* leaf extract, from 17.61 % at a concentration of 0.5 mg/ml to 59.65 % at a concentration of 1.5 mg/ml. (figure 5).

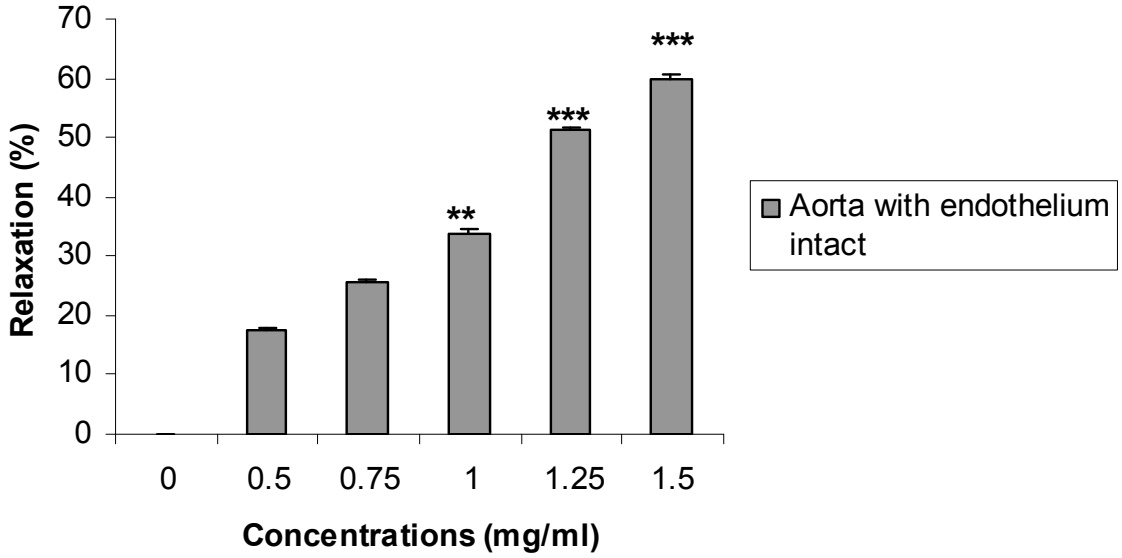

Fig 5: Relaxation effect of cumulative concentrations of *G. arborea* aqueous extract on the aorta precontracted with PE (5μM). Results are expressed as mean ± SEM. N = 5. **p<0.01, ***p<0.001

When aortic rings with or without endothelium (-E) were precontracted with PE (5 μM), the highest dose of the extract (1.5 mg/ml) produced 49.27 % and 42.06 % relaxation, respectively.

Discussion

Gmelina arborea is commonly used by herbalist in treating cardiovascular diseases especially high blood pressure. It has been shown in the present study that administration of high sodium chloride solution for at least two months will induce oxidative stress in rats and create hypertension in normal rats, since it is known that high-salt diet induced or aggravated hypertension in animal models of hypertension and in humans (19). It is known that increase production of free radicals (ROS) can cause oxidative damage to biological macromolecules and finally impair functions of organs (20). The results of this study indicate no significant change in the body weight of the experimental animals. However, in rats receiving the extract, the results indicated a slight decrease of the liver weight which might be due to decrease protein synthesis justifying the slight loose in animals' body weight.

Antioxidant therapy has been well documented to help in the improvement of organ functions. We assessed the effects of *G. arborea* extract by studying its effects on lipid peroxidation. MDA is a direct and stable metabolite of the free radical-mediated lipid peroxidation cascade. Therefore, the extent of lipid peroxidation can be assessed by measuring MDA levels in tissues. SOD plays an important role in the oxygen defence mechanism by intercepting superoxide and reducing it to hydrogen peroxide, which is easily reduced to water, principally by CAT (21). It is the major enzyme for scavenging oxygen free radicals, and its activity can reflect its functional status (22). In the present study, *G. arborea* as well as vitamin C was demonstrated to inhibit MDA production and increase SOD and CAT activities, suggesting that the inhibition of lipid peroxidation may be one of the mechanisms attributable to the protective effects of the extract to sodium chloride-induced oxidative stress.

In this study, increase levels of SOD and CAT were observed in the experimental rats compared to the control group. SOD is an important antioxidant enzyme having an antitoxic effect against superoxide anion on which it induces dismutation to hydrogen peroxide. The scavenging of superoxide anion by SOD protects cells against its deleterious effects (23). CAT is an enzyme which protects the cells from accumulating hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant, in which it works as a peroxidase (24). These results reflect that the extract might act by activating and increasing the synthesis of those enzymes. In the present study, using in vitro experiments, we found that extract has potent free radical-scavenging activity in DPPH assay and also has the ability to protect β-carotene against destruction by neutrilizing the linoleate-free radical and other free radicals formed in the system (25). The preceding results indicated that *G. arborea* could be very helpful to hypertensive patients by its ability to reduce their oxidant status.

The present study shows that the aqueous extract reduces cholesterol and triglycerides levels in rats, an effect attributed to its ability to slow down the lipid peroxidation process and to enhance antioxidant enzyme activity (26). It is known that high cholesterol level leads to endothelial dysfunction (27). In our study, we noted a reduction of circulating cholesterol and triglycerides in the experimental rats. Moreover, the level of LDL was highly elevated in control group compared to the normal group. The role of lipoprotein oxidation in atherosclerosis is well established. A decreased resistance to oxidation of LDL indicates an atherogenic nature of LDL and is associated with an increased risk of atheromatosis, including cardiac and peripheral vascular disease (28, 29). The extract slows down the level of LDL in treated group compared to the control.

Oxidation of LDL is associated with increase left ventricular hypertrophy and reduced endothelium-dependent vasodilation since ROS reduces NO bioactivity through the formation of peroxynitrite, a well known cytotoxic substance susceptible to cause cells injury by nitrosilating proteins (30). The biological availability of NO can reflects the healthy status of the entire body especially that of the heart and the vascular system. On the heart, one of the actions of NO is that, it appears as an important regulator of cardiac remodelling, specifically recognized as an anti-hypertrophic mediator. Our results indicate that the extract why reducing the left ventricular hypertrophy (LVH) induce by the salt loading, was also able to ameliorate the bioavailability of NO in both heart and aorta compared to the control group, indicating that it could act on the one hand, on the heart by facilitating coronary circulation since it is known that one of the negative effect of the LVH is the rarefaction of the coronary circulation (31) and on the other hand on vascular endothelium where it might ensure a protective effect.

In isolated aortic preparation, the aqueous extract from the leaves of *G. arborea* exhibited substantial direct vasodilator effect on phenylephrine-induced contraction of aortic preparations with and without the functional endothelium, indicating it potent vasodilating effects through the endothelium or it direct action on the smooth muscle cells.

In salt loaded hypertensive rats, intravenous injection of the extract induced significant and dose-dependant decrease in systolic blood pressure, indicating that the extract might lower the blood pressure by reducing the total peripheral resistances and/or by decreasing cardiac output via a reduction in the heart rate.

In conclusion, the results from the present study suggest that long-term administration of salt causes hypertension and increase the oxidative status in normotensive Wistar rats. The antihypertensive effect of the extract might be due to a combination of vasodilator and antioxidant actions and even through it remodelling effect on the cardiac myocytes and by ameliorating the coronary circulation. This benefit effect of the plant extract might be attributing to the presence of bioactive compounds such as phenolic components, flavonoids and saponins present in the plant extract.

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