Effect of *Acalypha wilkesiana* Muell Arg on the Blood Pressure and Aorta Contractility of Salt-Loaded Rats.

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ABSTRACT

The effect of *Acalypha wilkesiana* leaves on the blood pressure and aortic contractile and relaxation responses of salt-loaded rats was investigated. Three groups of five animals each were used. The control group received a diet consisting 100% of the commercial feed; the test-control received a diet consisting 8% salt and 92% commercial feed, while the test received diet containing 8% salt, 5% leaf powder and 87% commercial feed. The systolic and pulse pressure of the treated animals was significantly ($p<0.05$) lower than those of the test-control and the control, while their diastolic pressure was significantly ($p<0.05$) higher than the test-control and not the control. The treated group had the highest median effective concentration (EC_{50}) for both noradrenalin (NA) and acetylcholine (AcCh).

The maximal contractile response of the aorta from the treated animals was significantly ($p<0.05$) higher than both the test-control and control, while their maximal relaxation response was significantly ($p<0.05$) lower. Our result suggests that the mechanism of action of the leaves may be alteration of the contractile responses of the aortas; and also supports the use of the leaves in the management of hypertension.

(Keywords: *Acalypha wilkesiana*, acetylcholine, aortic contraction, blood pressure, noradrenalin, relaxation)

INTRODUCTION

The pressure necessary to enable the blood to circulate is provided by the pumping action of the heart [cardiac output (CO)] and the tone of the arteries (peripheral resistance, PR). The contraction of the heart propels blood through the arterial tree. However, it is the dynamic regulation of arterial diameter, especially in the smaller branches of the network, which controls blood pressure (BP) and flow in the periphery. Acutely, BP and flow may change under the control of neural and humoral factors (e.g. noradrenalin and acetylcholine) that can rapidly constrict or dilate local arterial segments and/or large arterial beds to meet short-term circulatory demands [Huang *et al*., 2005; Zhang *et al*., 2005; Blaustein *et al.*, 2006]. Blood flow in small arteries is governed by Poiseuille's law [Berne and Levy, 2001; Blaustein *et al*., 2006], which states that the resistance to flow (R) is inversely proportional to the fourth power of the internal radius, r (i.e., $R \alpha 1/r^4$). Small increases in myogenic tone (decreases in r) should, therefore, have a profound effect on R (or total peripheral R). There is a direct relationship between myogenic tone in isolated arteries and BP in intact animals [Blaustein *et al*., 2006].

Acalypha wilkesiana Muell Arg belongs to the family Euphorbiaceae (spurge family). Its other names include *A. amentacea* and *A. tricolor*, while its common names are copperleaf, Joseph's coat, fire dragon, match-me-if-you-can [http://www.floridata.com/ref/A/acal_wil.cfm].

It has antimicrobial properties [Ogundaini, 2005; Akinyemi *et al.*, 2006; Oladunmoye, 2006]. According to Ogundaini [2005], the expressed juice or boiled decoction is used for the treatment of gastrointestinal disorders and fungal skin infections such as *Pityriasis versicolar*, *Impetigo contagiosa*, *Candida intetrigo*, *Tinea versicolor*, *Tinea corporis* and *Tinea pedis*. In Southern Nigeria, the leaves of this plant are eaten as vegetables in the management of hypertension. Therefore, in order to provide an explanation for the possible mechanism of its anti-hypertensive action, the present study was designed to investigate the effect of the leaves on the blood pressure parameters, the NA-induced contraction and the AcCh-induced relaxation of NA pre-contracted aortas of salt-loaded rats.

MATERIALS AND METHODS

Collection of Animals, Feed and Reagents: Six weeks old Sprague Dawley (SD) rats were collected from the animal house of the Pathology Department of Lagos University Teaching Hospital (LUTH), Lagos, Nigeria. The feed used is Guinea grower's marsh from Bendel Feed and Flour Mill Limited, Ewu, Nigeria. All the reagents used were of analytical grade.

Collection and Preparation of the Leaves: The leaves were collected from within Hall 1 of the Ugbowo campus of the University of Benin, Benin City, Nigeria. After due identification at the Department of Plant Science and Biotechnology, Faculty of Life sciences, University of Benin, Benin City, Nigeria, they were rid of dirt, oven dried and ground into powder and used for compounding the test diet.

Experimental Design and Composition of Diet: The rats were randomly sorted into three groups of five animals each, so that the average weight difference was ±1.3g. The animals were individually housed in plastic metabolic cages. After a three-day acclimatization period, the treatment commenced and lasted for 6 weeks. The control group received a diet consisting 100% of the commercial feed; the test-control received a diet consisting 8% salt and 92% commercial feed, while the test received diet containing 8% salt, 5% leaf powder and 87% commercial feed. The 8% salt-loading was adopted from Obiefuna *et al*. [1991]. The animals were allowed food and water *ad libitum*.

Blood Pressure Measurement: At the end of the treatment period, the rats were anaesthetized by intra-peritoneal injection of 5mg/kg body weight of 25% Urethane saline solution. The blood pressure was measured as reported by Obiefuna *et al*. (1992). While under anesthesia, the trachea was exposed by blunt dissection and cannulated. The left common carotid artery was similarly exposed and cannulated. 50 units of heparin was injected into the cannulated artery to prevent clotting. The arterial cannula was connected to a Stathan P23 pressure transducer which was connected to a Grass Polygragh Model 7D. The blood pressure (mmHg) was obtained by calibrating the pressure transducer with mercury manometer

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Measurement of Aortic Contraction and Relaxation: Studies on aortic contraction and relaxation was carried out as reported by Kamanyi *et al*. (1991) and Obiefuna *et al*. (1991, 1992). The aorta of the animals was immediately removed, cleansed of connective tissues and cut into 2mm ring segments which were suspended in 20mL jacketed tissue baths containing physiological salt solution (PSS). The bath temperature was maintained at $37^{O}C$ and the solution bubbled with 5% CO_2 (pH 7.35-7.40). The rings were mounted on fine stainless steel rods and connected to Grass FT-03 force transducers. Changes in isometric force were recorded on a Grass Model 79D under an initial tension of 1.5g. The rings were equilibrated in PSS for 90 minutes, under a resting tension of 1.5g prior to the commencement of experiment, after which contractile response test to noradrenalin (NA) was conducted by cumulative addition of serial concentrations of the agent, higher concentration being added only when the effect of the previous one has reached a plateau. A relaxation response to acetylcholine (AcCh) was assessed by cumulative addition of the relaxing agent to 10^{-7} $molL^{-1}$ NA-pre-contracted aortic rings.

Data are presented as mean ± SD. Data were analyzed using the student's t test. The half-maximally effective concentration (EC_{50}), i.e. the concentration producing 50% response, was determined from the logit plot.

RESULTS

Table 1 shows the effect of *Acalypha wilkesiana* leaves on the blood pressure parameters of salt-loaded rats. The systolic and pulse pressure of the treated animals was significantly ($p<0.05$) lower than those of the test-control and the control, while their diastolic pressure was significantly ($p<0.05$) higher than the test-control and not the control.

The EC_{50} values and maximal contractile and relaxation responses are shown in Table 2. The treated group has the highest EC_{50} for both NA and AcCh. The maximal contractile response of the aorta from the treated animals was significantly ($p<0.05$) higher than both the test-control and control, while their maximal relaxation response was significantly ($p<0.05$) lower.

Figures 1-2 show, respectively, the NA-induced contractile and the AcCh-induced relaxation responses of the aortas from the three groups.

DISCUSSION

The present result confirmed earlier report by Lüscher *et al.* [1987], that 8% salt-loading produced no significant difference in the systolic pressure of Dahl salt resistant (DR) rats, while negating the report by Obiefuna *et al.* [1992], that 8% salt-loading significantly increased the blood pressure of SD rats. Acetylcholine is a classical example of an endothelium-dependent vasorelaxant, and so its ability to cause relaxation of pre-contracted vascular tissues has been generally employed to assess the integrity of the endothelium in vascular disease [Obiefuna *et al.*, 1991b]. Lüscher *et al*. [1987] reported a depressed AcCh-induced relaxation in Dahl salt-sensitive rats, Obiefuna *et al*. [1991b] and Adegunloye and Sofola [1997] reported unaltered response of aortic rings from Sprague-Dawley salt-loaded rats; however, in the present study, we observed a significantly improved response.

Our results confirmed earlier reports of increased responsiveness to norepinephrine in the aortic rings of salt-loaded Dahl salt-sensitive rats [Lüscher *et al*., 1987], and SD rats [Obiefuna *et al*., 1991a; Adegunloye and Sofola, 1997]. The leaves reduced (though not significantly), the relaxation response of aortic rings of the salt-loaded rats, while significantly increasing their contractile responses to noradrenalin.

Table 1: The Effect of *Acalypha wilkesiana* on the Blood Pressure Parameters of Salt-Loaded Rats.

Parameter	Pressure (mmHg)		
	Control	Test-control	Treated
Systolic Pressure (SP)	$84.00 \pm 10.85^{a,b}$	84.00 ± 6.00^{a}	74.67 ± 3.18^{b}
Diastolic Pressure (DP)	45.75 ± 3.88^{a}	32.50 ± 2.50^{b}	42.67 ± 4.06^{a}
Pulse Pressure (PP)	46.00 ± 0.60^{a}	51.50 ± 3.50^{b}	32.00 ± 1.00^{c}
Values are means ± SD, n=5 per group. Values in the same row with the different superscripts are significantly different at p<0.05.			

Table 2: The EC_{50} Values and Maximal Responses of the Aortas.

Parameter	Control	Test-control	Treated
EC50 (M)			
♦ For NA	4.01×10^{-8}	4.82×10^{-7}	8.84×10^{-8}
♦ For AcCh	1.00×10^{-7}	3.16×10^{-8}	3.16×10^{-7}
Maximal contraction (%)	78.10 ± 1.00^{a}	85.00 ± 0.02^{b}	100.00 ± 0.03^{c}
Maximal relaxation (%)	73.00 ± 2.50^{a}	91.5 ± 6.0^{b}	81.0 ± 3.0^{c}
Values are means ± SD, n=5 per group. Values in the same row with the different superscript are significantly different at p<0.05.			

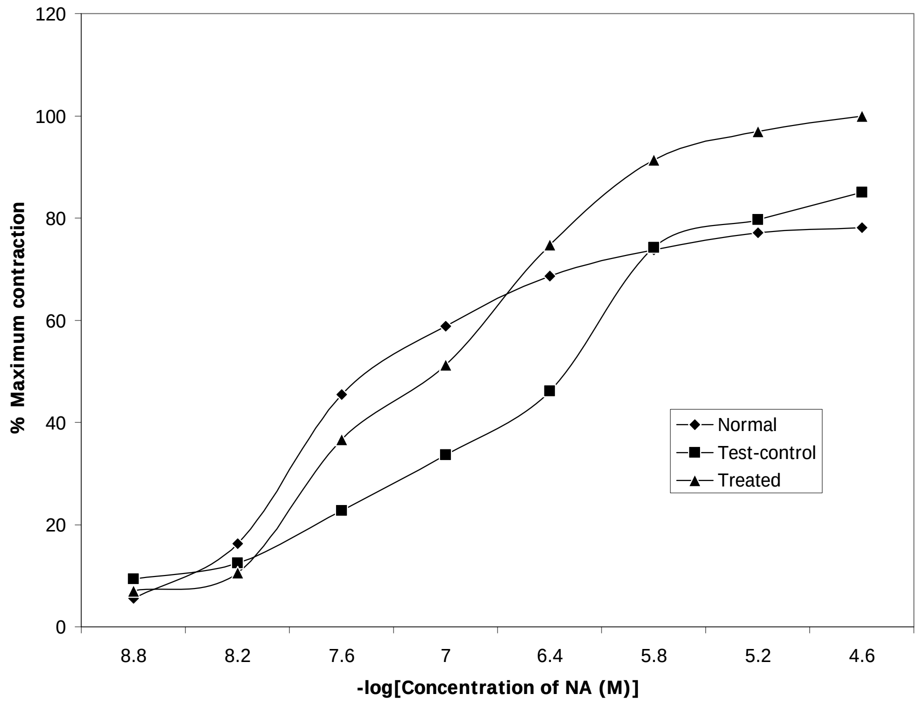

Figure 1: Effect of *Acalypha wilkesiana* Leaves on Noradrenalin (NA) Induced Contractile response of the Aortic rings of Salt-Loaded Rats.

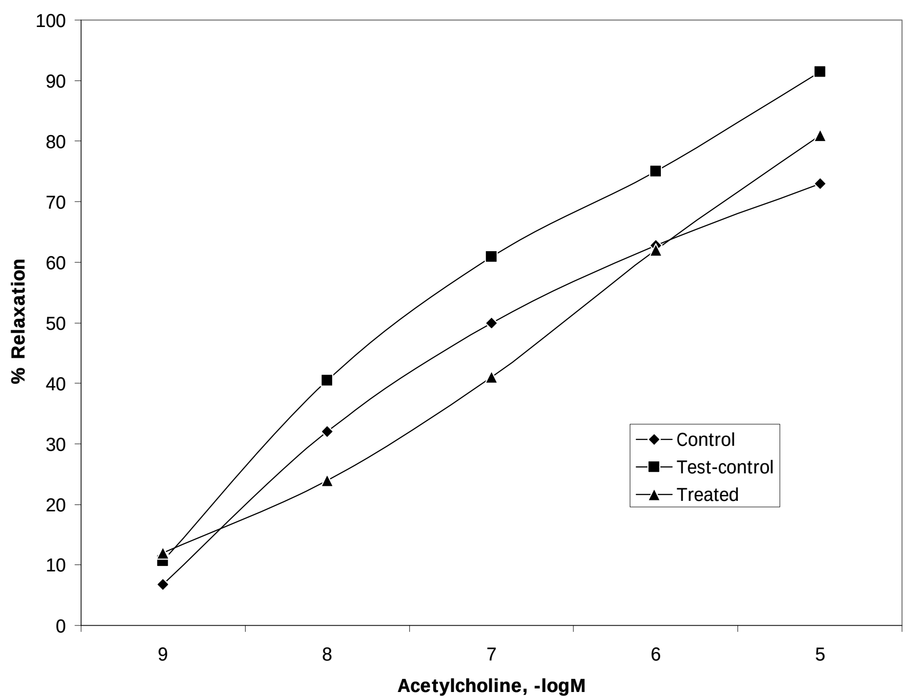

Figure 2: Effect of *Acalypha wilkesiana* Leaves on the Relaxation Response to Acetylcholine (AcCh) by Noradrenalin Pre-Contracted aortic Rings of Salt-Loaded Rats.

According to Khoyi *et al*. [1991], NA acts via increases in Na-Ca exchange in rabbit abdominal aorta. Thus, the leaves may have enhanced sensitivity to NA, by enhancing its effect on Na-Ca exchange. Endothelium-dependent relaxations in response to acetylcholine may be controlled by the simultaneous release of endothelium derived constricting factors and altered release of endothelium derived relaxing factors [Lüscher *et al*., 1987; Lüscher and Vanhoutte, 1986]. Thus, the decreased relaxation response produced by the leaves (Figure 2), may be via a decreased release of the relaxing factors, and an increased release of the constricting factors, or a direct inhibition of the relaxation process. Lüscher *et al*. [1987] observed a significantly high maximal response to NA in the aortic rings of salt-loaded DR rats. They further posited that the effect of dietary salt on contractile response may be mediated by endothelium factors. Again, the improved contraction produced by the leaves (Figure 1), may be mediated via increased release of constricting factors or a direct stimulation of the contraction process.

Finally, our result indicate that *Acalypha wilkesiana* leaves affected the systolic and pulse pressure, increased the contraction while reducing the relaxation response of aortas of salt-loaded rats. Thus, the mechanism of action of the leaves may be alteration of the contractile responses of the aortas. All of these support the use of the plant in the management of hypertension.

REFERENCES

1. Adegunloye, B.J. and O.A. Sofola. 1997. "Effect of Dietary Salt Loading and High-Calcium Diet on Vascular Smooth Muscle Responses and Endothelium Function in Rats". *Clin Exptl Pharmacol Physiol*. 24(11): 814 – 818. Doi:10.1111/j.1440-1681.1997.tb02696.x http://www3.interscience.wiley.com/doiinfo.html

2. Akinyemi, K.O., O.K. Oluwa, and E.O. Omomigbehin. 2006. "Antimicrobial Activity of Crude Extracts of Three Medicinal Plants used in South-Western Nigerian Folk Medicine on some Food Borne Bacterial Pathogens". *Afr J Trad CAM*, 3(4):13-22. http://www.bioline.org.br/abstract?id=tc06048&lang=en, http://www.africanethnomedicines.net/ojs2/index.php/ajtcam

3. Berne, R.M. and M.N. Levy. 2001. *Cardiovascular Physiology (8th edition)*. Mosby: St. Louis, MO. 115–153.

4. Blaustein, M.P., J. Zhang, L. Chen, and B.P. Hamilton. 2006. "How Does Salt Retention Raise Blood Pressure?". *Am J Physiol Regul Integr Comp Physiol*. 290(3): R514-R523. doi:10.1152/ajpregu.00819.2005. http://ajpregu.physiology.org/cgi/reprint/290/3/R514

5. Huang, B.S., H. Wang, and F.H. Leenen. 2005. "Increases in CSF [Na+] Precede the Increases in Blood Pressure in Dahl S Rats and SHR on a High-Salt Diet". *Am J Physiol Heart Circ Physiol*. 288(2): H517–H524. http://ajpregu.physiology.org/cgi/reprint/288/2/H517

6. Kamanyi, A., M. Bopelet, C.P. Aloamaka, P.C.M. Obiefuna, and A.B. Ebeigbe. 1991. *J Ethnopharmacol*. 34: 283-286.

7. Khoyi, M.A., R.A. Bjur, and D.P. Westfall. 1991. "Norepinephrine Increases Na-Ca Exchange in Rabbit Abdominal Aorta". *Am J Physiol Cell Physiol*. 261(4): C685-C690. PMID:1928328 http://ajpcell.physiology.org/cgi/reprint/261/4/C685

8. Lüscher, T.F., L. Raij, and P.M. Vanhoutte. 1987. "Endothelium-Dependent Vascular Responses in Normotensive and Hypertensive Dahl Rats". *Hypertension*. 9(2):157-163. http://hyper.ahajournals.org/cgi/reprint/9/2/157

9. Lüscher, T.F. and P.M. Vanhoutte. 1986. "Endothelium-Dependent Contractions to Acetylcholine in the Aorta of the Spontaneously Hypertensive Rat". *Hypertension*. 8:344-348.

10. Obiefuna, P.C.M., A.B. Ebeigbe, O.A. Sofola, and C.P. Aloamaka. 1991a. "Altered Responses of Aortic Smooth Muscle from Sprague-Dawley Rats with Salt-Induced Hypertension". *Clin Exptl Pharmacol Physiol*. 18(12): 813 – 818. Doi: 10.1111/j.1440-1681.1991.tb01400.x

11. Obiefuna, P.C.M., O.A. Sofola, and A.B. Ebeigbe. 1991b. "Dietary Salt-Loading Attenuates Endothelium Dependent Relaxation in Response to Histamine but not to Acetylcholine in Rat Aortic Rings". *Exptl Physiol*. 76(1): 135-138. http://ep.physoc.org/cgi/reprint/76/1/135.pdf

12. Obiefuna, P.C.M., O.A. Sofola, and A.B. Ebeigbe. 1992. "Contractile Response of Normotensive Rat Aorta to Serum from Salt-Loaded Spague-Dawley Rats". *Nig J Physiol Sci*. 8(1-2):54-57.

13. Ogundaini, A.O. 2005. "From Greens Into Medicine: Taking a Lead From Nature". An Inaugural Lecture Delivered at Oduduwa Hall, Obafemi Awolowo University, Ile-Ife, Nigeria. Inaugural Lecture Series 176. OAU Press Limited, Ile-Ife, Nigeria, pp: 12-15. http://www.oauife.edu.ng/faculties/pharmacy/aogund.pdf

14. Oladunmoye, M.K. 2006. "Comparative Evaluation of Antimicrobial Activities and Phytochemical Screening of Two Varieties of Acalypha wilkesiana". *Trends Appl Sci Res*. 1: 538-541. http://www.cababstractsplus.org/abstracts/Abstract.aspx?AcNo=20073213450

15. Zhang, J., M.Y. Lee, M. Cavalli, L. Chen, R. Berra-Romani, C.W. Balke, G. Bianchi, P. Ferrari, J.M. Hamlyn, T. Iwamoto, J.B. Lingrel, D.R. Matteson, W.G. Wier, and M.P. Blaustein. 2005. "Sodium Pump $\alpha 2$ Subunits Control Myogenic Tone and Blood Pressure in Mice". *J Physiol*. 569(1): 243–256. http://ajpregu.physiology.org/cgi/reprint/569/1/243

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