

# Effect of *Acalypha wilkesiana* Muell Arg on the ATPase Activities of Salt-Loaded Rats.

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## ABSTRACT

The effect of *Acalypha wilkesiana* leaves on the ATPase activities of the tissues of salt-loaded rats was investigated. The control group received a diet consisting 100% commercial feed; the test-control received a diet consisting 8% salt and 92% commercial feed, while the test group received diet containing 8% salt, 5% leaf powder and 87% commercial feed. The Na<sup>+</sup>,K<sup>+</sup> ATPase activity in the aorta, heart, kidney and RBC of the treated animals was significantly higher than the test-control, while that in liver was significantly lower. There was no significant difference in the Ca<sup>2+</sup>-ATPase activity observed in the aorta, heart, liver and RBC of the treated animals and the test control, while that in the kidney of the treated animals was significantly higher than the test-control. The Mg<sup>2+</sup>-ATPase activity in heart, liver and RBC of the treated animals was significantly higher than the test-control, that of the kidney was significantly lower, while that of the aorta was not significantly different. This alteration of tissue ATPase activities of salt-loaded rats may well be the basis of the diuretic as well as the hypotensive/antihypertensive effect of *Acalypha wilkesiana* leaves.

(Keywords: *Acalypha wilkesiana*, ATPases, hypertension, salt loading)

## INTRODUCTION

The mechanism induced by dietary salt in essential hypertension and the spontaneously hypertensive rats (SHR) is, in part, due to an increase in the plasma's capacity to inhibit Na<sup>+</sup>, K<sup>+</sup>-ATPase (NKA), which raises the blood pressure by inhibiting the sodium-calcium

exchange pump in vascular smooth muscle [Meneton *et al.*, 2005].

Vasdev *et al.* [1988] reported higher total, ouabain-sensitive and ouabain-insensitive NKA activities in the aortas of Dahl Salt Sensitive (DS) rats as compared to Dahl salt resistant (DR) rats on similar salt diet; with both DS and DR rats on high salt diet having higher NKA activity than on low salt diet. They found no evidence for a decrease in vascular sodium pump activity accompanying hypertension. According to Zhang *et al.* [1996, 1998] NKA catalytic activity in the basolateral membranes decreases in arterial hypertension and the dynamic regulation of proximal tubule sodium transport by fluctuations in blood pressure may be mediated by changes in sodium transporter characteristics at both the apical and basolateral membranes. Increased expression and maximal activity of the renal Na-K pump is the most important mechanism regulating constitutive tubular sodium reabsorption in the kidney [Ferrari *et al.*, 1999], and is mediated primarily by apical entry via sodium/hydrogen exchangers (NHE) and extrusion via basolateral sodium pumps (Na-K-ATPase) [Zhang *et al.*, 1998].

Hypertension is also accompanied by decreases in erythrocyte membrane NKA, Ca<sup>2+</sup>-ATPase and Mg<sup>2+</sup>-ATPase activities [Touyz and Milne, 1995]. Inhibiting cardiac Na pump activity decreases the driving force for the Na<sup>+</sup>,Ca<sup>2+</sup> exchanger (NCE) transport that increases cellular Ca stores and contractility [Magyar *et al.*, 1995]. Studies using NCE inhibitors and genetically engineered mice indicated that vascular NCE exchanger type 1 (NCE1) is involved in the development of salt-dependent hypertension [Iwamoto and Kita, 2006].

*Acalypha wilkesiana* Muell Arg (family Euphorbiaceae), is also called *A. amentacea* and *A. tricolor*. Its common names are copperleaf, Joseph's coat, fire dragon, match-me-if-you-can [[http://www.floridata.com/ref/A/acal\\_wil.cfm](http://www.floridata.com/ref/A/acal_wil.cfm)]. It has antimicrobial properties [Akinde, 1986; Ogundaini, 2005; Akinyemi *et al.*, 2006; Oladunmoye, 2006]. According to Akinde [1986] and Ogundaini [2005], the expressed juice or boiled decoction is used for the treatment of gastrointestinal disorders and fungal skin infections such as *Pityriasis versicolor*, *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. Earlier, Oladunmoye (2006) reported that the mechanism of antimicrobial activity of *A. wilkesiana* is the release of sodium and potassium ions, thus implying that the leaf extract may affect sodium and potassium pumps or Na<sup>+</sup>,K<sup>+</sup> ATPases. Consequent upon this, the present study was designed to monitor the effect of the leaves on the ATPase activities of the tissues of salt-loaded rats.

## MATERIALS AND METHODS

**Collection of Animals and Preparation of the Leaves:** Sprague-Dawley rats were collected from the animal house of the Pathology Department of Lagos University Teaching Hospital (LUTH), Lagos, Nigeria. 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  $\pm 1.3g$ . The animals were individually housed in plastic metabolic cages. After a one-week acclimatization period, the treatment commenced and lasted for 6 weeks. The control group received a diet consisting 100% of the commercial feed (Guinea grower's marsh from Bendel Feed and Flour Mill Limited, Ewu, Nigeria); 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.* [1991b]. The animals were allowed food and water *ad libitum*.

**Collection of Tissues and Preparation of Tissue Homogenates:** 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. While under anesthesia blood was collected from each rat via heart puncture and transferred into heparin sample bottles after which they were painlessly sacrificed, and their heart, kidney, liver, and aorta collected and stored at 4°C. Known masses of each of the tissues were separately homogenized in 10mL of ice-cold distilled water, and the resultant tissue homogenates were stored in the refrigerator at 4°C, for use in the assays. All homogenates were analyzed within a few hours of preparation.

The collected blood samples were centrifuged at 3000g, to isolate the cells which were used in preparing ghost cells. The ghost cells were prepared based on the method of Hamlyn and Duffy [1978]. Red cells were washed with 0.15M NaCl, pH 7.4. Membrane was prepared by hypotonic hemolysis in 5mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.7. The mixture was centrifuged at 7000 revs for 30min. The supernatant was removed and the ghost washed once in 10mM Tris-HCl (pH 7.5). The ghost was later suspended in 3mL distilled water for 12h at 4°C.

**Enzyme Assay:** The activities of the ATPases were determined as reported by Bonting [1970] as modified by Takeo [1980]. ATPase activity was measured by the amount of inorganic phosphate liberated following incubation with disodium ATP. The inorganic phosphate liberated was estimated by the method of Fiske and Subbarow [1925] while the protein was estimated by the method of Lowry *et al.* [1951].

**Statistical Analysis of Data:** Values are expressed as mean  $\pm$  SD. Data were analyzed using the student's t test.

## RESULTS

The effect of *Acalypha wilkesiana* leaves on NKA activity of the tissues of salt-loaded rats is given in Table 1.

**Table 1:** The Effect of *Acalypha wilkesiana* Leaves on Na<sup>+</sup>/K<sup>+</sup> ATPase Activity of Salt-Loaded Rats.

Tissue	Activity (Ux10 <sup>-3</sup> /mg protein)		
	Normal	Test-control	Treated
Aorta	105.662±16.186 <sup>a</sup>	35.217±9.840 <sup>b</sup>	59.953±4.822 <sup>c</sup>
Heart	58.744±11.917 <sup>a</sup>	29.361±3.260 <sup>b</sup>	43.701±6.902 <sup>c</sup>
Kidney	30.806±10.583 <sup>a</sup>	40.142±8.803 <sup>a</sup>	58.787±9.806 <sup>b</sup>
Liver	49.598±1.668 <sup>a</sup>	53.671±1.830 <sup>b</sup>	39.458±6.143 <sup>c</sup>
RBC	64.434±5.118 <sup>a</sup>	31.584±3.355 <sup>b</sup>	45.499±4.418 <sup>c</sup>

Values are expressed as mean ± SD, n=5 per group. Values in the same row with the different superscripts are significantly different at p<0.05.

The NKA activity in the aorta, heart and RBC of the treated animals was significantly higher than the test-control, but lower than the control. That of their kidney was significantly higher than the test-control and control, while that of their liver was significantly lower than the test-control and control.

Table 2 shows the effect of *Acalypha wilkesiana* leaves on the Ca<sup>2+</sup>-ATPase (CA) activity of the tissues of salt-loaded rats. The increased CA activity observed in the aorta, heart and liver of the treated animals were not significantly higher than those of the test control: although compared to the control, that of the aorta was significantly lower, that of the heart was significantly higher, while that of the liver was not significantly lower. The kidney CA activity of the treated animals was significantly higher than that of the test-control, but not significantly different from the control. There was no significant difference in the RBC CA activity of the three groups.

The effect of *Acalypha wilkesiana* leaves on Mg<sup>2+</sup> ATPase (MA) activity of the tissues of salt-loaded rats is given in Table 3. The MA activity in the aorta of the treated animals was not significantly higher than the test-control, but significantly lower than the control. That found in their heart was significantly higher than the test-control, but not significantly lower than the control. Their kidney MA activity was significantly lower than the test-control and control, while the liver MA activity was significantly higher than the test-control and control. The RBC MA activity was significantly higher than that of the test-control, but lower than that of the control.

## DISCUSSION

We assumed that the rats became hypertensive following 6 weeks salt-loading, on the basis of earlier reports by Nwaigwe and Sofola [1989] and

Obiefuna *et al.* [1991b], of a significantly increased blood pressure in rats fed 8% salt for 6 weeks.

According to Magyar *et al.* [1995], Köksoy [2002] and Meneton *et al.* [2005], inhibition of vascular smooth muscle Na pump activity decreases the driving force for the NCE transport leading to decreased cytosolic Ca and subsequent reduction in contractility, and increase in blood pressure. Thus, the significantly higher NKA activity found in the test, portends an increased intracellular Ca concentration, and by extension, increased contraction of aortic and cardiac muscles, with accompanying lowering of blood pressure. The low aortic NKA activity observed in the test control negates earlier report of higher NKA activity in rats on high salt diet than those on low salt diet [Vasdev *et al.*, 1988], but confirmed the report by Obiefuna *et al.* [1991a] of impairment of NKA activity in hypertension induced by salt-loading. Our result corroborated earlier reports that erythrocyte NKA activity is diminished in hypertension [Touyz and Milne, 1995; Osiecki, *et al.*, 2008], while revealing that *Acalypha wilkesiana* leaves significantly protected against the salt-loading induced lowering of NKA activity: since the RBC NKA activity of the test was significantly higher than that of the test control.

Cells contain two types of CA pumps: the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR) CA, which functions to resequester Ca<sup>2+</sup>, and the plasma membrane (PM) CA whose role is to extrude Ca<sup>2+</sup> from the cell, both of which helps to maintain steady Ca<sup>2+</sup> balance in the cytosol [Blaustein and Lederer, 1999; Iwamoto, 2006]. We found no significant difference in RBC CA activities of the three groups. This result negates earlier reports of reduction in erythrocyte CA activity in hypertension [Touyz and Milne, 1995].

**Table 2:** The Effect of *Acalypha wilkesiana* Leaves on Ca<sup>2+</sup> ATPase Activity of Salt-Loaded Rats.

Tissue	Activity (Ux10 <sup>-3</sup> /mg protein)		
	Normal	Test-control	Treated
Aorta	36.053±5.918 <sup>a</sup>	13.606±1.626 <sup>b</sup>	16.797±4.470 <sup>b</sup>
Heart	11.695±1.704 <sup>a</sup>	15.389±0.962 <sup>b</sup>	21.632±8.552 <sup>b</sup>
Kidney	27.154±9.139 <sup>a</sup>	12.185±0.130 <sup>b</sup>	24.232±5.613 <sup>a</sup>
Liver	16.478±2.534 <sup>a</sup>	11.644±1.796 <sup>b</sup>	13.979±1.966 <sup>a</sup>
RBC	12.608±2.608 <sup>a</sup>	13.068±3.575 <sup>a</sup>	11.036±2.684 <sup>a</sup>

Values are expressed as mean ± SD, n=5 per group. Values in the same row with the different superscripts are significantly different at p<0.05.

**Table 3:** The Effect of *Acalypha wilkesiana* Leaves on Mg<sup>2+</sup> ATPase Activity of Salt-Loaded Rats.

Tissue	Activity (Ux10 <sup>-3</sup> /mg protein)		
	Normal	Test-control	Treated
Aorta	45.244±9.681 <sup>a</sup>	19.194±6.426 <sup>b</sup>	26.672±8.694 <sup>b</sup>
Heart	25.569±6.429 <sup>a</sup>	5.602±2.049 <sup>b</sup>	20.942±5.315 <sup>a</sup>
Kidney	16.286±5.454 <sup>a</sup>	12.231±4.406 <sup>a</sup>	5.797±1.376 <sup>b</sup>
Liver	9.267±2.067 <sup>a</sup>	7.671±2.264 <sup>a</sup>	17.175±4.309 <sup>b</sup>
RBC	17.598±3.620 <sup>a</sup>	7.766±1.765 <sup>b</sup>	11.698±2.897 <sup>c</sup>

Values are expressed as mean ± SD, n=5 per group. Values in the same row with the different superscripts are significantly different at p<0.05.

Recall that the treatment produced increased NKA which is expected to elicit high cytosolic Ca, yet there was no proportionate increase in the PMCA activity of the erythrocytes. This may have been as a result of adaptive changes by the cells, involving the NCE [Blaustein and Lederer, 1999; Köksoy, 2002; Meneton *et al.*, 2005]. The high kidney CA activity recorded for the treated group may have been precipitated by high cytosolic Ca concentration that may itself have resulted from the increased NKA activity [Meneton *et al.*, 2005].

The proximal tubule cells contain CA pump in their basolateral membranes, which mediates the extrusion of Ca<sup>2+</sup> from the proximal tubules cells across the basolateral membrane [Blaustein and Lederer, 1999]. Similarly, the high CA activity recorded here for the heart and aorta, may have been precipitated by the high NKA activity.

Our result also confirmed earlier report of reduced erythrocyte membrane MA activity [Touyz and Milne, 1995], while indicating that *Acalypha wilkesiana* leaves significantly protected the

animals against salt-loading induced reduction in erythrocyte membrane MA activity.

Finally, this study revealed that *Acalypha wilkesiana* leaves altered the tissue ATPase activities of salt-loaded SD rats. This may well be the basis of its diuretic activity, as well as reduction of blood pressure.

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