Effect of *Acalypha wilkesiana* Muell Arg Leaf Meal on the Tissue Profile of Enzymes of Energy Metabolism in Salt-Loaded Rats.

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ABSTRACT

The effect of *Acalypha wilkesiana* Muell Arg leaf meal on the lactate dehydrogenase (LDH), pyruvate kinase (PK), and acid and alkaline phosphatase (ACP and ALK) profiles of the tissues of salt-loaded rats was investigated. The kidney and liver LDH activities of the treated rats were significantly ($p<0.05$) higher and lower, respectively, than the test-control and control, while that of the aorta and heart was significantly ($p<0.05$) lower than the control, though not different from the test-control. The kidney and plasma PK activities of the treated rats was significantly ($p<0.05$) higher than the test-control and control, while that of the aorta and heart was significantly ($p<0.05$) lower than the test-control, but not different from the control. The liver ACP activity was significantly ($p<0.05$) lower than the control, but not the test control. The plasma ALP activity of the treated animals was significantly ($p<0.05$) lower than the test-control and control. Our results suggest a likely hepatoprotective role of the plant, and a likely tissue dependent alteration in energy metabolism.

(Keywords: *Acalypha wilkesiana*, acid and alkaline phosphatase, lactate dehydrogenase, pyruvate kinase)

INTRODUCTION

About 30% of modern conventional drugs are derived from plant sources [Murray, 2004]. An herbal medicine is any medicinal product that contains as active ingredient, aerial or underground parts of plants, or other materials or combinations thereof whether in the crude state or as plant preparations [WHO, 1991]. Herbal medicines are the mainstay of about 75–80% of the world population, mainly in developing countries, for primary health care because of better cultural acceptability regarding compatibility with the human body and less side effects [Cunningham, 1993; Kamboj, 2000; Sapna and Ravi 2007]. A resolution of the World Health Organization's 31st Assembly took special interest in herbal remedies, asking for proper identification, sustainable exploitation, scientific development and evaluation of efficacy, safety, appropriate utilization, and standardization of medicinal plants [WHO, 1991].

Acalypha wilkesiana Muell Arg (family Euphorbiaceae) has antimicrobial properties [Akinde, 1986; Alade and Irobi, 1993; Adesina *et al*., 2000; 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 versicolar*, *Impetigo contagiosa*, *Candida intetrigo*, *Tinea versicolor*, *Tinea corporis,* and *Tinea pedis*. The leaves of this plant are eaten as vegetables in the management of hypertension, in Southern Nigeria. Consequent to this, we undertook a study of the effect of the plant leaves on: plasma sodium and potassium levels of normal rabbits [Ikewuchi *et al*., 2008]; blood pressure and aorta contractility [Ikewuchi *et al.*, 2009a], ATPase activity [Ikewuchi and Ikewuchi, 2009], and urinary and plasma chemistry [Ikewuchi *et al*., 2009b], of salt-loaded rats. In the present study,

we looked at the effect of the leaf meal on the lactate dehydrogenase (LDH), pyruvate kinase (PK), and acid and alkaline phosphatase (ACP and ALK) profiles of the tissues of salt-loaded rats.

MATERIALS AND METHODS

Procurement of Experimental Animals and Preparation of the Leaf Meal

Fifteen albino 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 cleaned, oven dried, 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 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 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 group received a diet containing 8% salt, 5% leaf powder and 87% commercial feed. The 8% salt-loading was adopted from Obiefuna *et al*. [1991, 1992] and Ikewuchi *et al*. [2009a]. 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 animals were painlessly sacrificed by decapitation under chloroform anesthesia, and their blood collected into heparin sample bottles. The heart, kidney, liver, and aorta were collected and stored at -10^{o}C, for subsequent biochemical studies. 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^{o}C, for use in the assays. All homogenates were analyzed within a few hours of preparation. The collected blood samples were centrifuged at 4000 rpm for 10 min., after which their plasma was collected and also stored for use.

Enzyme Assay

Lactate dehydrogenase activity was assayed by the method of Bergmeyer [1974]. The enzyme activity was determined by monitoring the rate of oxidation of NADH, which was accomplished by monitoring the change in extinction at 340 nm. The activity of pyruvate kinase was assayed as described by Story and Bialey [1978]. The pyruvate produced by this enzyme was acted upon by lactate dehydrogenase, and the rate oxidation of NADH was used to assign activity to this enzyme. Acid phosphatase activity was determined as reported by Walker and Schutt [1974]. The method of Trietz [1976] was adopted for the assay of alkaline phosphatase activity. The enzyme activity was assayed by measuring the extinction at 404 nm due the liberation of p-nitrophenoxide ion from p-nitrophenylphosphate (PNPP). The protein contents were estimated by the method of Lowry *et al*. [1951]. All reagents used were of analytical grade.

Statistical Analysis of Data

Values are expressed as mean ± SD. Data were analyzed using the student's t test, with the help of SPSS Statistics 17.0 package. $P<0.05$ was assumed to be significant.

RESULTS AND DISCUSSION

The effect of *Acalypha wilkesiana* leaf meal on the lactate dehydrogenase activity of the tissues of salt-loaded rats is given in Table 1. The aorta and heart LDH activity of the treated rats was not significantly different ($p<0.05$) from the test-control, but significantly ($p<0.05$) lower than the control. The kidney LDH activity of the treated rats was significantly ($p<0.05$) higher than the test-control and control; while that of the liver was significantly ($p<0.05$) lower. Lactate dehydrogenase catalyzes the reversible reduction of pyruvate to lactate, using NADH [Mayes and Bender, 2003; Berg *et al*., 2005; Nelson and Cox,

2005; Harris, 2006; Voet *et al*., 2006]. In this study, the leaf meal could not prevent the salt-loading induced reduction in LDH activities in the aorta and heart; but increased and decreased, respectively, the kidney and liver LDH activity. The implication of this is that it will have no effect on anaerobic glycolysis in the aorta and heart of the hypertensive, but will enhance and retard it in the kidney and liver, respectively. It also implies that the leaf meal can reduce the ability of the hypertensive to oxidize lactate in the liver.

Table 2 shows the effect of *Acalypha wilkesiana* on the pyruvate kinase activity of the tissues of salt-loaded rats. The aorta and heart PK activity of the treated animals was significantly ($p<0.05$) lower than the test-control, but not different from the control. That of the kidney and plasma was significantly ($p<0.05$) higher than the test-control and control: while that of the liver was not different from the test-control and control.

Pyruvate kinase converts phosphoenolpyruvate to enolpyruvate, generating 2ATPs in the process [Mayes and Bender, 2003; Berg *et al*., 2005; Nelson and Cox, 2005; Harris, 2006; Voet *et al*., 2006]. In this study, the leaf meal prevented the salt-loading induced increase in aorta, heart and liver PK activity; but increased or potentiated and lowered, respectively, the kidney and plasma PK activity. This implies that the leaf meal will reduce glycolysis in the aorta, heart, liver, and plasma of the hypertensive, while enhancing that of the kidney.

The effect of *Acalypha wilkesiana* leaves on the activity of acid and alkaline phosphatase in the tissues of salt-loaded rats is shown in Table 3. The liver ACP activity in the treated animals was significantly ($p<0.05$) lower than the control, but not the test control. There was no difference in the plasma ACP activity of the three groups. The plasma ALP activity of the treated animals was significantly ($p<0.05$) lower than the test-control and control.

Table 1: The effect of *Acalypha wilkesiana* on the Tissue Profiles of Lactate Dehydrogenase, in Salt-Loaded Rats.

Organ	Activity ($Ux10^{-1}$/mg protein)		
	Normal	Test-control	Treated
Aorta	1.01 ± 0.32^{a}	0.60 ± 0.13^{b}	0.61 ± 0.12^{b}
Heart	3.59 ± 1.56^{a}	1.62 ± 0.33^{b}	1.66 ± 0.48^{b}
Kidney	2.24 ± 0.34^{a}	2.61 ± 0.26^{b}	3.43 ± 0.29^{c}
Liver	3.18 ± 0.55^{a}	3.45 ± 0.42^{a}	2.50 ± 0.14^{b}

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 2: The Effect of *Acalypha wilkesiana* on the Tissue Profiles of Pyruvate Kinase, in Salt-Loaded Rats.

Tissue/Organ	Activity ($Ux10^{-2}$/mg protein)		
	Normal	Test-control	Treated
Aorta	5.63 ± 0.91^{a}	6.58 ± 0.51^{b}	5.08 ± 0.36^{a}
Heart	7.68 ± 2.33^{a}	14.80 ± 4.15^{b}	6.63 ± 1.27^{a}
Kidney	6.96 ± 1.44^{a}	11.61 ± 3.62^{b}	14.90 ± 1.40^{c}
Liver	14.13 ± 4.31^{a}	22.90 ± 7.67^{b}	$17.19\pm3.96^{a,b}$
Plasma	3.20 ± 1.51^{a}	4.62 ± 2.16^{a}	1.37 ± 0.42^{b}

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* on the Tissue Profiles of Acid and Alkaline Phosphatases, in Salt-Loaded Rats.

Tissue/Organ	Activity (U/mg protein)		
	Normal	Test-control	Treated
a. Acid phosphatase ($x10^{-4}$).			
Liver	8.31±0.00[a]	6.76±0.62[b]	6.69±0.23[b]
Plasma	982.00±277.50[a]	1237.00±998.00[a]	796.70±173.30[a]
b. Alkaline phosphatase (10^{-2})			
Plasma	35.40±12.60[a]	69.50±53.40[a]	15.10±8.80[b]

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 leaf meal protected against the salt-loading induced changes in the aorta, heart and plasma ACP activities; but had no effect on those of the kidney and liver. Acid phosphatases hydrolyze biological phosphate esters under slightly acidic conditions (pH 5.0) [Nelson and Cox, 2005; Crook, 2006], while alkaline phosphatases hydrolyze biological phosphate esters under very alkaline conditions (very high pH) [Crook, 2006]. The leaf meal lowered the plasma ALP activity, possibly indicating a possible hepatoprotective effect of the plant. According to Sallie et al. [1991] and Pari and Amali [2005], serum ALP is one of the most sensitive markers employed in the diagnosis of hepatic functionality, as its elevation indicate induction or cholestasis.

In conclusion, our results suggest a likely hepatoprotective role of the plant, and a likely tissue dependent alteration in energy metabolism.

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