

ORIGINAL ARTICLE

The Effect of Ethanol Leaf Extract of *Cnidosculus Aconitifolius* on Cardiorenal Functions in Hypertensive and Normotensive Male Wistar Rats

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ABSTRACT

Background: *Cnidosculus aconitifolius* is widely used by traditional healers in treating plethora of ailments either alone, in combination with other therapeutic agents or as additive to food/drinks. This study investigated the effect of different doses of *C. aconitifolius* on cardiorenal function in both normotensive and hypertensive male Wistar rats.

Methods: Forty-two male Wistar rats (120-150 g) were categorized into two normotensive and hypertensive groups. Normotensive was group 1 receiving food and water; while normotensive groups 2 and 3 received food and water together with 200 mg and 800 mg of *C. aconitifolius* for 4 weeks. The hypertensive group 1 received only high salt diet (HSD) and water and hypertensive groups 2 and 3 received HSD and water together with 200 mg and 800 mg of *C. aconitifolius*, respectively for 4 weeks after confirmation of hypertension. The animals were sacrifice by cervical dislocation and blood sample was collected, and serum was decanted for assays; while heart and kidney were harvested for histological investigation.

Results: There were significant reductions in systolic, diastolic and mean arterial blood pressure in hypertensive animals; while was not different in normotensive animals. Urine creatinine and fractional excretion decreased; while the histomorphology of the heart in hypertensive animals showed restorative and regenerative effects of *C. aconitifolius*.

Conclusion: This study suggests that *C. aconitifolius* potentiates cardiorenal functions by affecting the functional markers, as well as histology.

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Introduction

The kidney is one of the important organs in the body responsible for the removal of metabolic waste, body

fluid homeostasis and the control of erythropoiesis. Particularly, excessive dietary salt consumption over an extended period of time has been associated

with hypertension and cardiovascular diseases. Evidences from a wide variety of studies showed consistent direct relationship between salt intake, blood pressure and deteriorating renal functions (1). Salt consumption has increased tremendously due to increased consumption of carbonated drinks (soft drinks). However, population-based interventions indicate that when salt intake is reduced, blood pressure in the community falls. On the other hand, blood pressure in the body is regulated by the requirement of the kidneys to excrete or conserve enough sodium to maintain normal sodium content and blood volume. Therefore, excess sodium is generally excreted. Any increase in blood volume caused by excess salt increases arterial pressure, glomerular filtration rate and renal perfusion leading to increased water and sodium excretion (2, 3).

However, there is an upper limit to the amounts, which may be excreted and therefore excess salt can lead to increased water retention and if retention exceeds water excretion; this may in turn lead to tissue damage and hypertension (1, 3). Medicinal plants have been employed by traditional medical healers for several years in treating plethora of ailments either singly, in combination with other therapeutic agents or as addictive to food or drinks (4, 5). However, scientific evaluation of these claims is needed to provide evidence of their safety and efficacy (4, 6). *Cnidioscolus aconitifolius*, a perennial shrub that grows fast to about 3 m (10 feet) in height and 2 m (6.5 feet) in width, has large dark-green attractive leaves with no distinct taste, but with low moisture content when compared to spinach or lettuce (7, 8). Due to its plethora, several names have been ascribed to it by different people, culture or region, such as 'Efo Jerusalem' or 'Efo Iyana-upaja' in South-western Nigeria, 'Hospital Too Far' in Niger Delta areas of Nigeria and 'tree spinach' in United Kingdom (9-12).

The origin of *C. aconitifolius* can be traced to Southeast Mexico in the Maya region of Guatemala, Belize, which serves not only as the main source of leafy vegetables for the people, but also used widely for the treatment of several disease conditions (8, 13). Hepatoprotective, nephroprotective and gastroprotective effects, as well as anti-oxidant properties have been studied by Oyagbemi and Odetola (14), Ajiboye *et al.* (15), and Olivia *et al.* (16), respectively. However, the present study was designed to investigate the different doses of this plant on cardiorenal function in both normotensive and hypertensive male Wistar rats.

Materials and Methods

C. aconitifolius leaves were harvested from a

garden in Ibadan, Nigeria and was identified and authenticated at the herbarium of Botany Department, University of Ibadan with a voucher number: UIH-22694. The leaves were air-dried and blended into powder (1.2 kg) to be extracted by percolation at room temperature with 70% ethanol. Leaf extract of *C. aconitifolius* was concentrated under reduced pressure (bath temperature 50°C) and finally defatted with n-hexane. The extract was evaporated to dryness. The dried mass (yield=62.5 g which is 6.25% of the dried leaf). The concentrated ethanolic fraction was used for this experiment. Doses administered were 200 mg/kg, and 800 mg/kg body weight, stock solution was prepared by dissolving the extract in distilled water according to the methods of Olivia *et al.* (16).

Forty-two healthy male Wistar rats weighing between 120 and 150 grams were used for the study. The rats were procured from Central Animal House, College of Medicine, University of Ibadan. The animals were kept in plastic cages throughout the duration of the experiment and housed under standard animal housing conditions at the Physiology Department, Postgraduate Animal House, College of Medicine, Ibadan and the ethical approval for use of animals was granted by University of Ibadan Ethical Committee (UI-ACUREC/18/0140) and animal handling were in accordance with the institution's animal handling guidelines. The metabolic cages used for the urine collection were fabricated by the Central Technological Laboratory and Work Department (CTLW) of the Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. After two weeks of acclimatisation of the animals to laboratory condition, the study animals were selected into two groups; the normotensive group and the hypertensive group. Each group were randomly subdivided into three subgroups (n=5) as follows: Normotensive group: Group 1 (Normal diet (ND) and water *ad libitum*), Group 2 (Normal diet and water with 200 mg of the extract), and Group 3 (Normal diet and water with 800 mg of the extract). Hypertensive group: Group 1 (high salt diet (HSD) and water *ad libitum*), Group 2 (HSD and water with 200 mg of the extract), and Group 3 (HSD and water with 800 mg of the extract).

The animals in hypertensive group were fed with high salt diet for six weeks before treating with 200 mg and 800 mg doses of the extract for 4 weeks. However, the normotensive group were fed with normal diet and treated with 200 mg and 800 mg of the extract for 4 weeks. Prior to sacrifice, the heart rate and blood pressure were also measured. The animals were put in metabolic cages to collect 24 h urine samples and after sacrifice using ketamine

(70 mg/kg), blood samples were drawn from the left ventricle (through cardiac puncture), centrifuged at 10,000 rpm revolutions per minute at 4°C for 15 minutes and the serum was then decanted. Sodium and creatinine concentrations were measured from the urine and serum samples. The kidneys and the heart were excised, cleared of fat, weighed and fixed in 10% formalin for histological investigation.

Hypertension was induced by salt-loading with 8% sodium chloride diet as described by Sofola *et al.* (17) and modified by Asiwe *et al.* (1). Sodium and creatinine were measured in serum and urine under standard laboratory procedures using their respective kits. They were measured using the microplate reader SpectraMAX PLUS (a molecular Device product, USA). Serum and urine concentrations of sodium and creatinine were used to determine the clearance values of creatinine and fractional excretion of sodium. The creatinine clearance (CrCl) was gotten from the multiplication of urine creatinine concentration (in mg/dL) and urine volume (in mL) divided by the multiplication of serum creatinine concentration (in mg/dL) and time (in minute) as follows:

$$\text{Creatinine clearance} = \frac{\text{Ucr} \times \text{Uvol}}{\text{Scr} \times \text{time (mins)}}$$

The fractional excretion of sodium was provided from the multiplication of urine concentration of sodium and serum concentration of creatinine divided by the multiplication of serum concentration of sodium and urine concentration of creatinine as follows:

$$\text{Fractional Excretion of Sodium (Na)} = \frac{\text{UNa} \times \text{SCr}}{\text{SNa} \times \text{UCr}} \times 100.$$

The blood pressure and heart rate were recorded in conscious animals. The values of heart rate, systolic and diastolic blood pressures were measured by the tail-cuff method using the CODA

non-invasive method (Kent Scientific Co., USA). The principle of the machine was reported before by Usman *et al.* (18).

The 10% neutral-buffered formalin fixed kidney and heart and were then dehydrated in increasing concentration of ethanol, cleared with xylene and embedded in molten paraffin wax using embedding system (Leica EG 1160). They were later sectioned with microtome at 4 micrometer thickness. The sections were then floated on water using water bath at temperature of 45°C and then picked on frosted end slide. The slides were fixed on hot plate for about thirty minutes. The sections were then stained by Haematoxylin and Eosin. To analyse the results, data were expressed as mean±standard error of mean. All data were analysed using one way analysis of variance (ANOVA) and *Post hoc* test and comparison of groups were performed by Tukey nonparametric test using GraphPad prism 7.0 (GraphPad software, San Diego, CA, USA). *p*<0.05 was considered statistically significant.

Results

There were significant differences in heart rate and body weight, when compared with the negative control; while in hypertensive animals, systolic, diastolic, mean arterial pressure (MAP) and heart rate differed significantly, when compared with positive control. There was a significant change in body weight. However, the organ weights did not change significantly in both normotensive and hypertensive groups that are presented in Table 1. Urine sodium, urine creatinine and fractional excretion of sodium were significantly different from the negative control; while urine sodium and fractional excretion of sodium were significantly different from the positive control. However, urine creatinine and creatinine clearance showed a significant difference at dose of 200 mg of *C. aconitifolius* that were exhibited in Table 2.

Table 1: The effect of *C. aconitifolius* on cardiac function markers as well as tissue and body weights.

Variable	-Ve cntl	Normotensive Animals		Hypertensive Animals		HSD+800 mg
		ND+200 mg	ND+800 mg	+Ve cntl	HSD+200 mg	
Systolic (mmHg)	117±3.0	112±2.59	109±2.29	156±1.25	135±2.92 [#]	128±2.16 [#]
Diastolic (mmHg)	75.5±4.35	68±3.24	80±3.56	147±2.36	89.8±2.17 [#]	108±1.03 [#]
MAP (mmHg)	89±4.38	82.3±2.56	89.5±3.28	149±3.86	105±1.44 [#]	114±1.87 [#]
Heart rate (b/min)	244±4.63	277±4.14*	304±4.87*	323±1.31	309±5.31 [#]	325±2.22
Heart weight (g)	0.37±0.01	0.36±0.01	0.34±0.01	0.37±0.02	0.39±0.02	0.36±0.02
Kidney weight (g)	0.31±0.01	0.32±0.02	0.34±0.01	0.29±0.01	0.34±0.01	0.32±0.02
Final weight (g)	171.3±4.70	179±11.00*	180.5±6.10*	172.5±3.50	148±7.39 [#]	175±5.07 [#]

**p*<0.05 is significant when compared with negative control while [#]*p*<0.05 is significant when compared with positive control (n=5). -ve cntl=negative control (feed and water only), ND=Normal diet with 200 mg and 800 mg of extract wile +ve cntl=positive control (high salt diet and water only), HSD=high salt diet with 200 mg and 800 mg of extract MAP=mean arterial blood pressure.

Table 2: The effect of *C. aconitifolius* on renal function markers.

Variable	-Ve cntl	Normotensive Animals		Hypertensive Animals		HSD+800 mg
		ND+200 mg	ND+800 mg	+Ve Cntl	HSD+200 mg	
Serum sodium (mg/dL)	107±2.09	114±3.04	104±2.62	107±1.7	110±0.88	105±0.83
Urine sodium (mg/dL)	106±1.66	116±2.57*	115±0.92*	157±5.16	114±3.83 [#]	122±2.83 [#]
Serum creatinine (mg/dL)	0.92±0.13	1.23±0.20	1.15±0.09	0.94±0.07	0.68±0.12	0.96±0.09
Urine creatinine (mg/dL)	333±4.25	206±40.2*	132±63.3*	460±14.6	651±21.8 [#]	487±57.3
Urine volume (mL)	2.13±0.09	2.55±0.17	1.63±0.20	5.85±1.27	2.45±0.53	5.65±0.71
Creatinine clearance	0.61±0.22	0.77±0.11	0.34±0.06	1.38±0.07	1.01±0.08 [#]	1.61±0.02
Fractional excretion of sodium	0.43±0.03	0.22±0.02*	0.05±0.01*	0.34±0.03	0.18±0.03 [#]	0.21±0.01 [#]

* $p < 0.05$ is significant when compared with negative control while [#] $p < 0.05$ is significant when compared with positive control (n=5). -ve cntl=negative control (feed and water only), ND=Normal diet with 200 mg and 800 mg of extract wile +ve cntl=positive control (high salt diet and water only), HSD=high salt diet with 200 mg and 800 mg of extract.

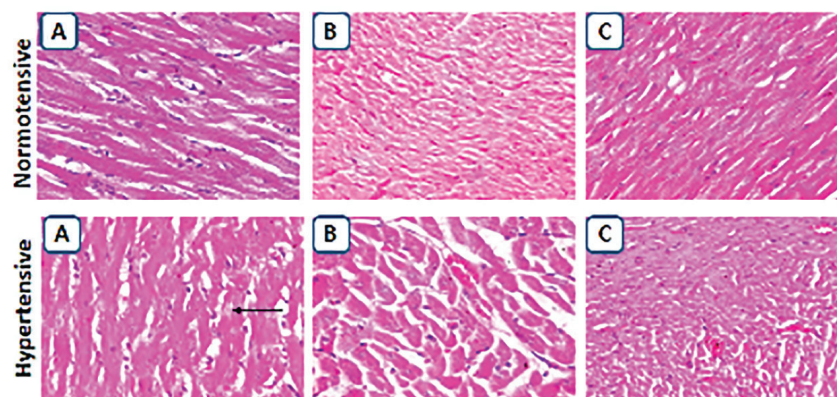


Figure 1: Histomorphology of the heart. Normotensive (A=negative control, B=ND+200 mg and C=ND+800 mg), Hypertensive (A=positive control, B=HSD+200 mg and C=HSD+800 mg), ND=Normal diet, HSD=high salt diet.

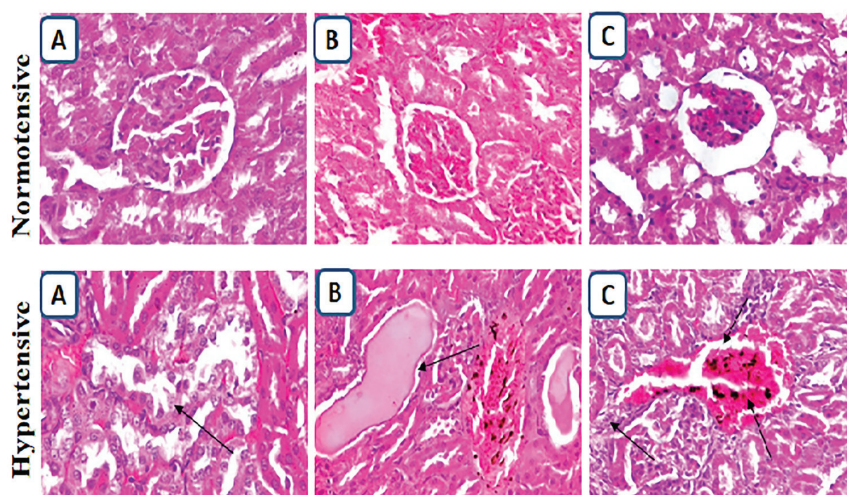


Figure 2: Histomorphology of the kidney. Normotensive (A=negative control, B=ND+200 mg and C=ND+800 mg), Hypertensive (A=positive control, B=HSD+200 mg and C=HSD+800 mg), ND=Normal diet, HSD=high salt diet.

The photomicrograph of Cardiac tissue section showed normal architecture and cellularity of the myocytes and no significant lesion in normotensive groups was observed; while *C. aconitifolius* extracts (200 mg and 800 mg) restored the normal architecture of the heart tissue, when compared with the positive control group that is characterized by slight infiltration of inflammatory cells (thin arrow) into the myocardium (Figure 1). The micrograph of the renal tissue shows a normal architecture

in the normotensive groups, while there were moderate perivascular inflammation, moderate periglomerular inflammation, mild thrombosis and mild tubular degeneration (black arrow) in positive control group. However, haemorrhagic lesions, mild peritubular inflammation and presence of eosinophilic materials in the tubules (black arrow) were noted in plate B and C in hypertensive groups (Figure 2).

Discussion

This study was designed to investigate the effect of different doses *C. aconitifolius* on cardiorenal biomarkers in both normotensive and hypertensive male Wistar rats. The result of this finding showed no significant difference in the kidney and heart tissue weights in both doses of normotensive and hypertensive animals. However, there were progressive increase in body weight of both normotensive and hypertensive animals. These changes suggested that *C. aconitifolius* leaves contain some nutritional components that increases the appetite which will increase food intake and subsequently resulted into weight gain. It has been previously reported that *C. aconitifolius* is toxic to the cardiovascular system as it increases the functional biomarker enzymes (19). However, our study revealed a beneficial effect of the extract at dose of 200 mg/kg body weight to decrease the systolic and diastolic blood pressure which contributes significantly to the mean arterial blood pressure. There were no significant changes in normotensive animals which further support the beneficial effect of *C. aconitifolius* in managing cardiovascular diseases, such as hypertension. Excess salt loading has been reported to cause not only water retention, but also presents a major challenge to the kidney in excreting this large amount of salt thereby increasing the risk of cardiovascular diseases such as hypertension, stroke, heart attack, heart failure and renal disease (20-23).

The present study showed that *C. aconitifolius* enhanced the excretion metabolic wastes in both normotensive and hypertensive animals as shown in increased urine sodium and creatinine concentrations. Bad state of muscle or inflammation has been linked with high salt intake-induced kidney disease which resulted in increased creatinine clearance and urine concentration of creatinine in normotensive animals (24). However, *C. aconitifolius* was able to increase urine concentration of these metabolites that further supported the beneficial effects not only on cardiac functions, but also in the kidney as well as increasing creatinine clearance and a decrease in fractional excretion of sodium in both normotensive and hypertensive animals. Electrolytes such as sodium and potassium have been the frequently used clinical techniques to screen for acid-base balance and monitor renal functions. However, the decrease in fractional excretion of sodium when compared with the control groups suggest the protective role of *C. aconitifolius*, since high fractional excretion of sodium has been reported to be associated with sodium wasting due to acute tubular necrosis or other causes of intrinsic kidney failure (25).

The photomicrograph of the heart tissue showed regenerative and remodelling ability of *C. aconitifolius* in plate B and plate C, when compared with plate A which is characterised by high salt induced infiltration and inflammation (Figure 1). However, both the cardiac and renal tissues of normotensive animals showed no significant lesion. In Figure 2 plate A, hypertensive kidney showed perivascular, periglomerular and tubular degeneration, as well as mild haemorrhagic lesions. These observations were consistent with other reports which showed periglomerular and tubular degeneration of renal tissue in animals fed with high salt diet (1). Unlike the cardiac tissue, different doses *C. aconitifolius* was not able to ameliorate the changes as eosinophilic substances were also observed within the tubules in plate B. This could be attributed to the duration of treatment as many studies has reported a protective effect of this plant.

Conclusion

This study suggests that *C. aconitifolius* leaf extract ameliorate cardiovascular diseases by decreasing systolic, diastolic as well as mean arterial blood pressure. However, it also plays a role in kidney functions by increasing sodium excretion, creatinine clearance as well as decreasing fractional excretion of sodium. The effect of this plant at dosage of 200 mg/kg was observed to be more potent as shown in the cardiorenal markers and histology of normotensive and hypertensive animals. Further studies are on-going to further elucidate the mechanism of action of *C. aconitifolius* on heart other than its anti-oxidant properties.

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Conflict of Interest

None declared.

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