European Journal of Medicinal Plants
2(4): 348-355, 2012

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Hyponatremic Effect of Aqueous Leaf Extract of *Acalypha wilkesiana* in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author IOM designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author EO managed the laboratory analysis of the study. Author OEB managed the literature searches. All authors read and approved the final manuscript.

Research Article

Received 2nd January 2012
Accepted 21st March 2012
Published 12th November 2012

ABSTRACT

Aims: To evaluate the hyponatremic effect of aqueous leaf extract of *Acalypha wilkesiana* in male wistar rats.
Study Design: *In vivo* study.
Place and Duration of Study: Department of Biochemistry, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma Nigeria, between August 2011 and October 2011.
Methodology: Thirty two male wistar rats of average body weights (167.50 ± 5.56 g) were grouped into four (A-D), of eight rats each. Group A received distilled water (control), while constituted doses of 2500, 5000 and 10000 mg/kg body weight of the extract were administered once daily for 14 days to animals in group B, C and D respectively. The effect of administration of this extract on serum sodium ions and weight parameters was evaluated. Serum activities of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase; serum proteins, bilirubin, creatinine, urea, uric acid, potassium, calcium and phosphate ion concentrations were determined.
Results: Significant reductions ($p<0.05$) were observed in serum sodium ion at doses above 2500 mg/kg body weight and this reduction was significantly dose-dependent up to 10000 mg/kg body weight of the extract. No significant differences ($p>0.05$) were obtained

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in all other serum and weight parameters determined.
Conclusion: This extract at the administered doses is safe, and its hyponatremic action suggests that it could be used as a diuretic.

Keywords: Acalypha wilkesiana; hyponatremic; diuretic; sodium ion.

1. INTRODUCTION

The use of plants for healing purposes has always been part of human culture and it is getting increasingly popular in Nigeria. *Acalypha wilkesiana* is one of several medicinal plants used in Nigeria and it has various ethnobotanical uses. *Acalypha wilkesiana* belongs to the family *Euphorbiaceae*. It is propagated by stem cuttings at any time of the year. Under ideal conditions, it grows as a spreading evergreen shrub with upright branches that tend to originate near the base and can get up to 3.1 m tall with a similar spread. It has leafs (12.7-20.3 cm long) that are alternate, elliptic to oval, serrate and multicoloredans small inconspicuous flowers (10.2-20.3 cm) that hangs in catkin-like racemes beneath the foliage (Al-Attar, 2010).

In some parts of southern Nigeria, the use of diuretics in the treatment of hypertension has been traditionally substituted for aqueous leaf extract of *Acalypha wilkesiana*. Diuretics cause a hypovolemic hyponatremia (Goh, 2004), thus the hyponatremic ponentials of this extract was investigated in male wistar rats. Acute changes in body mass over a short time period can frequently be assumed to be due to body water loss or gain; 1 ml of water has a mass of 1 g and therefore changes in body mass can be used to quantify water gain or loss. Over a short time period, no other body component will be lost at such a rate, making this assumption possible (Shirreffs, 2003), thus weight parameters were evaluated and used as makers of hydration status of the male wistar rats.

In order to detect any sign of toxicity of the extract in the liver and kidney, serum activities of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase; serum proteins, bilirubin, creatinine, urea, uric acid, potassium, calcium and phosphate ion concentrations were determined.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material for analysis

Fresh mature plants of *A. wilkesiana* were collected from the natural habitat in August within Ambrose Alli University, Ekpoma, Edo State, Nigeria. The plant was authenticated in the Department of Botany, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The leaf were picked, washed in distilled water and spread in the sun. The dried leaf was ground to powder using an electric grinder. The leaf powder (1 kilogram) was suspended in four liters of distilled water and boiled for one hour. The suspension was allowed to attain room temperature and filtered, using Whatman No 1 filter paper. The filtrate was evaporated to dryness at 50^0C using a heater and the percentage yield of the extract was 8.54% (w/v). The pasty residue was used to prepare a standardized solution of the leaf extract in calculated

amount of distilled water with standardized concentration of 2.96 g/ml. The standardized solution (the stock solution) was stored air tight in plastic bottles and kept frozen.

2.1.2 Quantitative assay kits

Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Total Protein, Albumin, Bilirubin, Creatinine, Urea and Uric Acid were products of LABKIT, CHEMELEX, S.A. Pol. Canovelles-Barcelona, Spain.

All the chemicals and reagents used in the study were of analytical grade and were purchased from the Bristish Drug House (BDH) Poole England and Sigma Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A.

2.1.3 Laboratory animals

Thirty two male wistar rats weighing 145-175 g were obtained from the Animal Care Facility, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The rats were fed with rat pellet (product of Bendel Feeds and Flour Mills Ltd, Ewu, Edo State, Nigeria). The Animal Ethics Committee of Department of Biochemistry, Faculty of Natural Sciences of Ambrose Alli University, Ekpoma, Nigeria approved all experimental protocols.

2.2 Methods

2.2.1 Experimental animals and procedure

The Thirty two male wistar rats were randomly grouped into four, comprising of eight rats per group. The rats were housed in cages made of wooden frames and metal netting, and were fed ad libitum with rat pellet and tap water with 12-hours light/dark cycle. The cages were cleaned every morning and disinfected at intervals of 3 days. The rats were allowed to acclimatize for 10 days before extract administration was commenced. Calculated amount of aqueous leaf extracts of *A. wilkesiana* were constituted in distilled water from the stock solution of 2.96g/ml to give doses of 2500, 5000 and 10000 mg/kg body weight and administered to the various groups as illustrated:

Group A: control, received 1.0 ml distilled water
Group B: received 2500 mg/kg body weight of the extract
Group C: received 5000 mg/kg body weight of the extract
Group D: received 10000 mg/kg body weight of the extract

Prior to the administration of aqueous leaf extract of *A. wilkesiana* and every interval of 7 days, the body weights of the animals were recorded. Administration of aqueous leaf extract of *A. wilkesiana* was performed orally once daily between 9:30 am ± 30 minutes, using metal cannula attached to a 2 ml syringe. Administration lasted for 14 days, after which the rats were fasted for 12 hours, body weights recorded and were sacrificed by anaesthesia using chloroform. Blood was collected by cardiac puncture and placed in bottles with no anticoagulant and the organs of interest were excised, cleansed of tissues, washed and the weights recorded.

2.2.2 Liver and kidney function tests

The determination of serum Aspartate transaminase, Alanine transaminase, Alkaline phosphatase, total protein albumin, bilirubin, urea, uric acid, creatinine, phosphate and calcium concentrations were done according to the manufacturer's instruction on the assay kits. The determination of serum sodium and potassium ion concentrations was by flame photometry.

2.2.3 Statistical analysis

The results were expressed as mean ± standard error of mean (SEM) and were subjected to one way analysis of variance (ANOVA), using statistical package for social sciences (SPSS-15) at 95% level of confidence. The least square difference (LSD) was performed for the pair-wise mean comparisons, to determine the significant treatment dose at 95% level of confidence. Values were considered statistically significant at ($p<0.05$) and denoted by different alphabets.

3. RESULTS AND DISCUSSION

The acute administration of the aqueous leaf extract of *A*. *wilkesiana* did not result in obvious signs of morphological changes or death of male rats throughout the experimental period. The average body weights of male rats following the acute administration of aqueous leaf extract of *A*. *wilkesiana* is shown in Table 1. No significant differences ($p>0.05$) were obtained in the average body weight of animals administered with the extract as compared with the control. The organ weight and organ-body ratio also elucidated no significant differences ($p>0.05$) in the liver, kidney, brain, heart, lung, pancreas and testes (Tables 2 and 3). Acute administration of the aqueous leaf extract at all doses did not change significantly ($p>0.05$) the serum activities of ALP, ALT and AST (Table 4). In addition, the liver function indices, such as the serum concentrations of total and conjugated bilirubin, total protein and albumin were not significantly changed ($p>0.05$) following the administration of the aqueous leaf extract (Table 5). The kidney function indices: serum concentrations of urea, uric acid, creatinine, calcium, phosphate and potassium were not significantly changed following acute administration of aqueous leaf extract ($p>0.05$). However, there was a progressive significant reduction ($p<0.05$) in serum sodium ions concentration with increasing doses of the extract above 2500 mg/kg when compared with control group (Table 6).

Table 1. Effect of aqueous leaf extract of *A. wilkesiana* on body weight of male wistar rats

Body weight (g)	Control	2500mg/kg	5000mg/kg	10000mg/kg
Day 0 (g)	167.50 ± 5.56[a]	167.50 ± 5.56[a]	167.50 ± 5.78[a]	167.50 ± 5.57[a]
Day 7 (g)	170 ± 10.00[a]	168.00 ± 3.21[a]	163.75 ± 7.25[a]	164.17 ± 6.42[a]
Day 14 (g)	176.67 ± 9.85[a]	161.00 ± 6.05[a]	162.33 ± 4.82[a]	162.50 ± 9.79[a]
Mortality	Nil	Nil	Nil	Nil

(Values are mean ± S.E.M: n = 8)

Table 2. Effect of aqueous leaf extract of *A. wilkesiana* on organ weight of male wistar rats

Parameters	Control	2500mg/kg	5000mg/kg	10000mg/kg
Liver weight (g)	6.92 ± 0.23[a]	7.09 ± 0.88[a]	6.44 ± 0.31[a]	5.81 ± 0.49[a]
Kidney weight (g)	0.63 ± 0.01[a]	0.56 ± 0.03[a]	0.60 ± 0.02[a]	0.55 ± 0.03[a]
Lungs weight (g)	1.35 ± 0.11[a]	1.19 ± 0.08[a]	0.93 ± 0.17[a]	1.05 ± 0.09[a]
Testes weight (g)	1.05 ± 0.07[a]	0.88 ± 0.18[a]	1.12 ± 0.04[a]	1.04 ± 0.06[a]
Pancreas weight (g)	0.81 ± 0.09[a]	0.74 ± 0.07[a]	0.77 ± 0.06[a]	0.63 ± 0.09[a]
Brain weight (g)	1.43 ± 0.04[a]	1.29 ± 0.06[a]	1.16 ± 0.09[a]	1.28 ± 0.04[a]
Heart weight (g)	0.57 ± 0.04[a]	0.61 ± 0.02[a]	0.83 ± 0.18[a]	0.54 ± 0.04[a]

(Values are mean ± S.E.M: n = 8)

Table 3. Effect of aqueous leaf extract of *A. wilkesiana* on organ/body weight ratio of male wistar rats

Organ	Control	2500mg/kg BW	5000mg/kg BW	10000mg/kg BW
Lungs	0.008 ± 0.0002[a]	0.007 ± 0.0002[a]	0.006 ± 0.0002[a]	0.007 ± 0.0002[a]
Liver	0.040 ± 0.0002[a]	0.044 ± 0.0001[a]	0.036 ± 0.0002[a]	0.036 ± 0.0002[a]
Brain	0.008 ± 0.0001[a]	0.008 ± 0.0002[a]	0.007 ± 0.0002[a]	0.008 ± 0.0001[a]
Heart	0.003 ± 0.0003[a]	0.004 ± 0.0002[a]	0.005 ± 0.0002[a]	0.002 ± 0.0002[a]
Kidney	0.008 ± 0.0001[a]	0.008 ± 0.0002[a]	0.007 ± 0.0002[a]	0.007 ± 0.0002[a]
Pancreas	0.005 ± 0.0002[a]	0.005 ± 0.0002[a]	0.004 ± 0.0002[a]	0.004 ± 0.0002[a]
Testes	0.012 ± 0.0002[a]	0.012 ± 0.0002[a]	0.013 ± 0.0002[a]	0.013 ± 0.0002[a]

(Values are mean ± S.E.M: n = 8)

Table 4. Effect of aqueous leaf extract of *A. wilkesiana* on serum activities of ALP, ALT and AST in male wistar rats

Parameters	Control	2500mg/kg	5000mg/kg	10000mg/kg
Serum ALP (u/L)	146.83 ± 5.11[a]	158.40 ± 0.69[a]	147.17 ± 6.13[a]	157.00 ± 1.20[a]
Serum ALT (u/L)	29.17 ± 1.94[a]	31.80 ± 3.40[a]	28.83 ± 1.23[a]	34.40 ± 2.30[a]
Serum AST (u/L)	77.33 ± 6.34[a]	77.00 ± 5.62[a]	74.50 ± 6.17[a]	101.40 ± 16.35[a]

(Values are mean ± S.E.M: n = 8)

Table 5. Effect of aqueous leaf extract of *A. wilkesiana* on serum proteins and bilirubin level in male wistar rats

Parameters	Control	2500mg/kg	5000mg/kg	10000mg/kg
Total protein (g/l)	7.33 ± 0.27[a]	7.18 ± 0.19[a]	7.41 ± 0.17[a]	7.36 ± 0.21[a]
Albumin (g/l)	3.55 ± 0.04[a]	3.62 ± 0.07[a]	3.55 ± 0.07[a]	3.42 ± 0.06[a]
Globulin (g/l)	3.78 ± 0.24[a]	3.60 ± 0.21[a]	3.87 ± 0.13[a]	3.94 ± 0.17[a]
Total bilirubin (mg/dl)	0.60 ± 0.05[a]	0.60 ± 0.29[a]	0.67 ± 0.04[a]	0.62 ± 0.03[a]
Conjugated bilirubin (mg/dl)	0.32 ± 0.04[a]	0.35 ± 0.02[a]	0.30 ± 0.03[a]	0.36 ± 0.03[a]

(Values are mean ± S.E.M: n = 8)

Table 6. Effect of aqueous leaf extract of *A. wilkesiana* on serum creatinine and urea level in male wistar rats

Parameters	Control	2500mg/kg	5000mg/kg	10,000mg/kg
Sodium (mmol/l)	145.83±0.91[a]	144.00 ± 0.84[ac]	142.50 ± 0.37[bc]	140.67 ±0.37[bd]
Potassium(mmol/l)	4.60 ± 0.36[a]	4.14 ± 0.10[a]	4.67 ± 0.15[a]	4.28 ± 0.11[a]
Calcium (mg/dl)	9.60 ± 0.14[a]	10.02 ± 0.18[a]	9.65 ± 0.18[a]	9.76 ± 0.07[a]
Phosphate (mg/dl)	5.32 ± 0.15[a]	5.06 ± 0.10[a]	4.93 ± 0.80[a]	4.92 ± 0.11[a]
Creatinine (mg/dl)	0.35 ± 0.03[a]	0.40 ± 0.03[a]	0.37 ± 0.03[a]	0.38 ± 0.03[a]
Urea (mg/dl)	35.17± 1. 06[a]	26.75 ± 2.33[a]	36.33 ± 3.7 8[a]	37.60 ± 6.35[a]
Uric acid (mg/dl)	4.37 ± 0.48[a]	3.34 ± 0.22[a]	3.73 ± 0.33[a]	4.10 ± 0.53[a]

(Values are mean ± S.E.M: n = 8)

The observation in the physical appearance of male rats following the acute administration of the aqueous leaf extract of *A. wilkesiana* suggested the extract is not toxic at the administered doses as no obvious morphological changes and death were observed. Since there were no changes in the average body weight, organ weight and organ-body weight index of the rats at the various doses administered (Tables 1, 2 and 3 respectively), then the extract did not compromise the cellular integrity of the organs. That is, the acute administration of extract did not result in tissue inflammation (possibly tissue damage) or dehydration due to the alteration in osmotic pressure (Megges et al., 2002).

The trends depicted in the serum ALP, ALT and AST activities of rats administered with the aqueous leaf extract (Table 4) suggests that the extract did not disrupt the integrity of plasma membrane of tissues and thus, there were no loss of cellular components into the serum (Malbica and Hart, 1971). AST and ALT are well known enzymes which serve as biomarkers capable of predicting tissue toxicity. AST is present in a wide variety of tissues which includes heart, kidney, skeletal muscle and liver whereas ALT is primarily localized in the liver (Mukinda and Syce, 2007). The analysis of these parameters is important since there are several reports of liver toxicity related to the use of phytotherapeutic products. Since there was no leakage of aminotransferases into serum, therefore, the biosynthesis of some crucial proteins in cells was not impaired and the organs would be in the normal functional state. The result of the liver function indices of rats administered the aqueous extract (Table 5) may indicate that the aqueous leaf extract did not damage the hepatocytes and is consistent with the result of serum enzyme activity (Table 4). In addition, the trend presented in the albumin concentration supported the result of the organ weight and organ-body weight index (Tables 2 and 3) as albumin is responsible for the maintenance of the osmotic pressure between systemic and localized circulation. Alterations in serum albumin concentration are seen in localized oedema or plasmolysis, due to induced inflammation or dehydration in tissues respectively. The result obtained in the serum bilirubin concentrations (Table 5) suggest that the aqueous leaf extract did not cause the lysis of erythrocytes and the disruption of secretion of conjugated bilirubin into the bile.

The trends obtained for kidney function indices following the administration of the aqueous extract of *A. wilkesiana* (Table 6) where there was no significant changes in serum potassium, calcium and phosphate ion, creatinine, urea and uric acid, suggests that the extract did not interfere with the renal capacity to excrete the metabolites. Indeed, creatinine is known as a good indicator of renal function. Any rise in creatinine levels is only observed if there is marked damage to functional nephrons (Mukinda and Eagles, 2010). Similarly, serum creatinine level is also a good indicator of the kidney function. Uric acid has been shown to be a very strong reducing agent *in vivo*. It has comparable antioxidant capabilities

as ascorbic acid and is often used as a biomarker for oxidative stress (Glantzounis et al., 2005). In humans, over half the antioxidant capacity of blood plasma comes from uric acid. However, there is increase in the demand for uric acid as an antioxidant, when the concentration of Glutathione in the liver is low, in conditions such as oxidative stress, liver diseases and chronic inflammation (Becker, 1993).

The observed progressive decrease in serum sodium ions (imminent hyponatremia) as the dose of the extract exceeds 2500 mg/kg might be an indication of this extract could cause hyponatremia. Some of the more common causes of medication-induced hyponatremia are diuretics (Spital, 1999). Diuretics cause a hypovolemic hyponatremia (Goh, 2004). Diuretics induce weight loss through the excretion of water (Cadwallader et al., 2010). The weight of the animals as observed in Table 1 showed that the mean weight of the animals decreased when the extract was administered though this decrease was not significant. It can be hypothesized that prolonged use of this extract at doses greater than 10,000 mg/kg body weight may lead to hypovolemic hyponatremia and a significant weight loss of the animals. This may be a probable mechanism of action of the use of this extract in treatment of hypertension and may also be useful in inducing weight loss especially in obese individuals.

4. CONCLUSION

The aqueous leaf extract of Acalypha wilkesiana may be considered relatively safe, as it did not cause either mortality or obvious morphological toxic effect, or compromise the functional state of the liver and kidney. It can be hypothesized that this extract is a potential diuretic and can induce hypovolemic hyponatremia which could lead to loss in weight. The use of this extract in the treatment of hypertension may be due to a possible diuretic characteristic of the extract. Therefore, based on these findings, the oral consumption of the aqueous leaf extract as employed in the treatment of various ailments in folklore medicine is recommended.

ACKNOWLEDGEMENTS

Special thanks to Prof. (Mrs) S.O. Malomo of Biochemistry Department, University of Ilorin, Ilorin, Nigeria, for the fundamental technical assistance received.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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