Serum aminotransferase activities and bilirubin levels in salt loaded experimental rabbits treated with aqueous and ethanol extracts of *Acalypha wilkesiana*

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Abstract

Background: *Acalypha wilkesiana*, commonly called Irish petticoat, is native to the South Pacific Islands and belongs to the family Euphorbiaceae. In traditional medicine practice, the leaves of *A. wilkesiana* are eaten as vegetables for the management of hypertension, being a diuretic plant. **Aim:** This study was conducted to evaluate the effects of extracts of *A. wilkesiana* leaves on activities of serum aminotransferases and bilirubin levels, which are indicators of liver function, in salt loaded rabbits. **Materials and Methods:** A total of 30 rabbits were randomly divided into five Groups (A to E) of six rabbits each and treated with salt loaded diet, aqueous extract (Group B) and ethanol extract (Group C) of *A. wilkesiana* leaves; continuous salt loading (Group A); salt loaded and nontreated (D); nonloaded (with salt) and nontreated (with extract) (E). **Results:** Salt loading resulted in a significantly ($P < 0.05$) higher serum alanine aminotransferases (ALT) and aspartate aminotransferases (AST), and a significantly ($P < 0.05$) lower serum direct bilirubin, when compared with the control. Treatment with *A. wilkesiana* leaf extracts, at a dose of 300 mg/kg body weight, resulted in a nonsignificantly ($P > 0.05$) lower serum total bilirubin and direct bilirubin, as compared with the control, in the salt loaded rabbits. **Discussion and Conclusion:** The use of *A. wilkesiana* leaf could be relatively safe considering its effects on serum ALT, AST, total and direct bilirubin of the experimental animals. Thus, it may be useful in the management of any possible deleterious effect of salt load to the liver.

Key words: *Acalypha wilkesiana*, alanine aminotransferase, aspartate aminotransferase, bilirubin, salt load

INTRODUCTION

There are considerable human and animal experimental studies implicating excessive dietary salt intake in cardiovascular diseases especially hypertension.[1,2] Evidence from many sources suggests a possible relationship between excess salt ingestion and human hypertension.[3-5] Increase in blood pressure leads to damages to the kidney, heart, blood vessels, brain, and the eyes. Salt excess also had deleterious renal effects in spontaneously hypertensive rat (SHR) reflected by a massive proteinuria after 8 week of 8% salt loading.[6] It has been demonstrated that salt loading enhanced ventricular and renal fibrosis in normotensive and SHR rats.[6,7] Clinical studies have also provided evidence that a high-salt diet was an independent determinant of renal injury.[8-10] Increase in blood pressure leads to damages to the kidney, heart, blood vessels, brain, and the eyes. These deleterious effects of excess salt can be managed with the use of medications or medicinal herbs.

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Medicinal herbs are plants, which contain active principles that are effective for therapeutic purposes and can serve as precursors for the synthesis of drugs.[11] *Acalypha wilkesiana*, also known as Irish petticoat or Jacob's coat, is indigenous to the South Pacific Islands and belongs to the family Euphorbiaceae. The plant has been reported to contain sesquiterpenes, monoterpenes, triterpenoids and

polyphenols.[12] The leaves reportedly contain saponins, tannins, anthraquinones and glycosides.[13] The plant has antimicrobial and antifungal properties and in traditional medicine, the leaves are eaten as vegetables for the management of hypertension, being a diuretic plant.[13] The plant has been shown to be a potential source of useful drugs.[14]

MATERIALS AND METHODS

Plant Materials

Fresh *A. wilkesiana* leaves were obtained from local gardens within Benin City and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The leaves were properly washed, air-dried and ground into fine powder.

Preparation of Ethanol Extract

Portion (100 g) of the powdered leaves was soaked in 400 ml of ethanol (95%) for 72 h (3 days), with occasional stirring using a magnetic stirrer to ensure proper mixture of the vessel content. The content was then filtered using a sintered funnel, (which is equivalent to four-fold of bandage or sheet of cheese cloth). The extract (filtrate) was then concentrated using rotary evaporator and weighed.

Preparation of Aqueous Extract

Portion (100 g) of the powdered leaves was soaked in 400 ml of distilled water for 72 h (3 days), and treated as described above.

Preparation of Salt-Loaded Diet (Feed)

The salt-loaded diet (8% NaCl) was prepared by mixing 8 g of analytical NaCl (from BDH Chemicals, England) with 92 g of the feed. The mixture was then fed to the experimental animals as described in the experimental design below.

Experimental Animals

Thirty adult rabbits of the New Zealand strain, weighing between 0.9 and 1.5 kg, purchased from local dealers at Aduwawa Cattle Market Benin City, were used for the study. The animals were kept in the animal house of the Department of Biochemistry, University of Benin and maintained on a 12-h light and dark cycle in clean disinfected cages. They were allowed free access to feed (standard pelletized growers feed from UAC - Vital Feed, Jos, Plateau State) and water throughout the duration of the experiment. The animals were treated according to the International guidelines for the care and use of laboratory animals and allowed to acclimatize to the new environment for a period of 3 weeks. They were then randomized into five groups (Groups A-E) of six rabbits each.

Experimental Design

Groups A-E rabbits were treated as follows;

Group A: Continuous salt-loading
Group B: Salt-loaded and treated with aqueous extract
Group C: Salt-loaded and treated with ethanol extract
Group D: Salt-loaded and nontreated
Group E: Nonloaded (with salt) and nontreated (with extract) (control).

The animals in Groups A-D were fed with the salt-loaded diet for a period of 70 days, after which Groups B and C were treated with aqueous and ethanol extracts, respectively, for a period of 7 days. Group A animals were given the salt-loaded diet continuously until the 77th day, while Group E animals were neither given salt-loaded diets nor treated with the extract (i.e., it served as control). The extracts were administered orally at a dose of 300 mg/kg body weight.

Administration of Extracts

Five grams of the dry mass (concentrated extracts) were suspended in distilled water for administration to the experimental animals. The extracts (aqueous or ethanol) were administered orally at a dose of 300 mg/kg body weight for a period of 7 days.

Collection of Blood

After 70 days of salt loaded diets and prior to treatment (day 71) with the extracts, blood samples were collected from the veins located on the dorsal side of the ear lobes of the experimental animals (rabbits), using sterilized hypodermic needles. At day 78 after treatment with the extracts, blood samples were also collected. Samples were collected into fluoride oxalate and plane universal bottles immersed in ice. Immediately after collection of blood, the tubes were centrifuged at 3,500 rpm for 10 min to obtain clear plasma (fluoride oxalate) and serum (plane bottles) for further analysis.

Assay Methods

Alanine aminotransferase (ALT) was by the method of Reitman and Frankel[15] where ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl hydrazine at 546 nm. Aspartate aminotransferase (AST) was also by the method of Reitman and Frankel[15] where AST was measured by monitoring the concentration of oxaloacetate formed with 2,4-dimtrophenyl hydrazine at 546 nm. Bilirubin was determined by the method of Jendrassik and Grof.[16] The principle involves the reaction of direct (conjugated) bilirubin with diazotized sulfanilic acid in alkaline medium to form a blue colored complex. Total bilrubin was determined in the presence of caffeine, which releases albumin bound bilrubin, by the reaction with diazotized sulfanilic acid and absorbance was read at 578 nm.

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM) (n = 6). Significance of difference was tested by Student's t-test, ANOVA and Turkey-Kramer test, using the GraphPad Instat Version 3 (GraphPad Software Inc. San

Diego, California USA). Statistical Significance was set at $P < 0.05$.

RESULTS

The effects of oral administration of extracts (aqueous and ethanol) of *A. wilkesiana* leaves on some serum parameters in salt loaded experimental rabbits, are as described below.

After 70 days of salt loading, serum ALT activity were shown to be significantly ($P < 0.05$) higher in all the salt loaded groups, as compared with the nonloaded group (control). This trend was maintained 1 week after [Table 1]. At day 78, Group A showed a significantly ($P < 0.05$) higher values, Groups C and D showed nonsignificantly ($P > 0.05$) higher value, while Group B showed nonsignificantly ($P > 0.05$) lower ALT activity (U/L).

Aspartate aminotransferase activity were also significantly ($P < 0.05$) higher in all the groups given salt load as compared with the control [Table 2]. But at day 78, only Group A (given continuous salt load), maintained the increase ($P > 0.05$) as compared with the control. Other Groups (B-D) showed nonsignificantly ($P > 0.05$) lower AST activity. Still at day 78, after administration of the extracts (aqueous or ethanol), there were significantly ($P < 0.05$) lower AST activity in the treated groups (day 78 compared with day 71), as compared with that of the untreated group. The ethanol extract was, however, shown to be more effective.

As shown in Table 3, salt loading and/or treatment with aqueous or ethanol extract of *A. wilkesiana* leaves had no effect on levels of serum total bilirubin of the experimental animals.

Serum direct bilirubin levels of the animals given salt load showed slight differences when compared with the control, after 70 days of salt load [Table 4]. Group B showed significantly ($P > 0.05$) higher levels of direct bilirubin while Group C showed a significantly ($P < 0.05$) lower levels as compared with that of Group E. However, at day 78, after 1 week of treatment, there was no significant difference ($P > 0.05$) in the levels of direct bilirubin in all the groups.

DISCUSSION AND CONCLUSION

Aminotransferases are found in many tissues throughout the body, including the liver, heart, muscles, kidney, and brain. If any of these organs or tissues is affected by disease or injury, AST is released into the bloodstream. Serum aminotransferase activities are sensitive indicators of parenchymal liver damage. Both ALT and ASTs have been implicated in infective hepatitis, infectious mononucleosis, and hepatocellular damage. Exposure to toxic substances such as carbon tetrachloride poisoning, therapeutic

Table 1: Serum ALT (U/L) of salt-loaded rabbits; treated with extracts of *Acalypha wilkesiana* leaves

Group	Serum ALT level (U/L)	
	71 days	78 days
A (continuous salt)	46.33 ± 3.77^{ax}	54.17 ± 4.28^{cx}
B (salt+aqueous extract)	38.17 ± 2.59^{ax}	33.17 ± 4.34^{dx}
C (salt+ethanol extract)	34.67 ± 8.01^{ax}	40.00 ± 1.04^{dx}
D (salt+no extract)	37.83 ± 5.70^{ax}	44.00 ± 5.75^{dx}
E (control)	25.33 ± 1.45^{bx}	28.67 ± 5.26^{ex}

Data represent means ± SEM ($n = 6$), Means with different letter (a, b, c, d, and e) superscripts, along column, are significantly different ($P < 0.05$), Means with different letter (x) superscripts, along row, are significantly different ($P < 0.05$), ALT = Alanine aminotransferases, SEM = Standard error of the mean

Table 2: Serum AST (U/L) of salt-loaded rabbits; treated with extracts of *Acalypha wilkesiana* leaves

Group	Serum AST level (U/L)	
	71 days	78 days
A (continuous salt)	74.50 ± 14.50^{ax}	67.00 ± 12.70^{cx}
B (salt+aqueous extract)	64.00 ± 4.58^{ax}	33.17 ± 3.71^{dy}
C (salt+ethanol extract)	72.83 ± 8.97^{ax}	35.67 ± 2.74^{dy}
D (salt+no extract)	37.00 ± 5.51^{ax}	34.00 ± 0.58^{dx}
E (control)	30.67 ± 6.62^{bx}	35.83 ± 7.08^{dx}

Data represent means ± SEM ($n = 6$), Means with different letter (a, b, c, and d) superscripts, along column, are significantly different ($P < 0.05$), Means with different letter (x, y) superscripts, along row, are significantly different ($P < 0.05$), AST = Aspartate aminotransferases, SEM = Standard error of the mean

Table 3: Serum total bilirubin (mg/dl) of salt-loaded rabbits; treated with extracts of *Acalypha wilkesiana* leaves

Group	Serum total bilirubin concentration (mg/dl)	
	71 days	78 days
A (continuous salt)	1.33 ± 0.29^{ax}	0.95 ± 0.09^{bx}
B (salt+aqueous extract)	1.26 ± 0.45^{ax}	0.73 ± 0.12^{bx}
C (salt+ethanol extract)	1.18 ± 0.17^{ax}	0.59 ± 0.09^{bx}
D (salt+no extract)2	0.94 ± 0.20^{ax}	0.95 ± 0.16^{bx}
E (sontrol)	1.20 ± 0.45^{ax}	0.92 ± 0.04^{bx}

Data represent means ± SEM ($n = 6$), Means with different letter (a, b, c) superscripts, along column, are significantly different ($P < 0.05$), Means with different letter (x, y) superscripts, along row, are significantly different ($P < 0.05$), SEM = Standard error of the mean

Table 4: Serum direct bilirubin (mg/dl) of salt-loaded rabbits; treated with extracts of *Acalypha wilkesiana* leaves

Group	Serum direct bilirubin concentration (mg/dl)	
	71 days	78 days
A (continuous salt)	3.42 ± 0.33^{ax}	1.35 ± 0.12^{dx}
B (salt+aqueous extract)	4.79 ± 0.28^{bx}	1.53 ± 0.15^{dy}
C (salt+ethanol extract)	2.23 ± 0.89^{cx}	1.29 ± 0.06^{dx}
D (salt+no extract)	4.01 ± 0.23^{ax}	1.35 ± 0.17^{dx}
E (control)	4.10 ± 0.24^{ax}	1.38 ± 0.10^{dx}

Data represent means ± SEM ($n = 6$), Means with different letter (a, b, c) superscripts, along column, are significantly different ($P < 0.05$), Means with different letter (x, y) superscripts, along row, are significantly different ($P < 0.05$), SEM = Standard error of the mean

substances such as chloramphenicol, cephalosporins, and paracetamol overdose have also resulted in elevated AST and ALT activities.

After 70 days of salt loading, serum ALT activities were shown to be significantly ($P < 0.05$) higher in all the salt loaded groups, as compared with the nonloaded group (control). This trend was maintained 1 week after [Table 1]. At day 78, Group A showed a significantly ($P < 0.05$) higher values, Groups C and D showed nonsignificantly ($P > 0.05$) higher values, while Group B showed nonsignificantly ($P > 0.05$) lower ALT activity (U/L).

This may portent the protective effect of the plant against the possible hepatocellular damage resulting from the salt load. AST activities were also significantly ($P < 0.05$) higher in all the groups given salt load as compared with the control [Table 2]. But at day 78, only Group A (given continuous salt load), maintained the increase ($P > 0.05$) as compared with the control. Other Groups (B-D) showed nonsignificantly ($P > 0.05$) lower AST activities. Still at day 78, after administration of the extracts (aqueous or ethanol), there were significantly ($P < 0.05$) lower AST activities in the treated groups (day 78 compared with day 71), as compared with that of the untreated group. The ethanol extract was, however, shown to be more effective. The sustained increase in AST activity of the group given continuous salt load as against the decrease in AST activity of the treated group is indicative of the possible adverse effect of the salt load on the functional status of the liver. This may be supported by the observed decrease in Group D after cessation of salt load. The decrease in AST activity in the treated groups, resulting from the extracts, is also indicative of the possible protective effect of the plant against the adverse effects of salt load on the liver.

As shown in Table 3, salt loading and/or treatment with aqueous or ethanol extract of *A. wilkesiana* leaves resulted in nonsignificant ($P > 0.05$) effects in levels of serum total bilirubin of the experimental animals. Total bilirubin concentration reflects the levels of both the conjugated and unconjugated fractions of bilirubin. Total bilirubin levels are elevated in various forms of liver disease such as cirrhosis, hepatitis, and obstructions of the hepatobiliary system such as gallstones or tumors. Elevated total bilirubin levels are also observed in cases of intravascular hemolysis.[17] Serum direct bilirubin levels of the animals given salt load showed slight differences when compared with the control, after 70 days of salt load [Table 4]. Group B showed significantly ($P > 0.05$) higher levels of direct bilirubin while Group C showed a significantly ($P < 0.05$) lower levels as compared with that of Group E. However, at day 78, after 1 week of treatment, there was no significant difference ($P > 0.05$) in the levels of direct bilirubin in all the groups. The lower levels of direct (conjugated) bilirubin in some groups probably caused by salt load may be protective as lower levels indicate decrease hemoglobin lyses.

A. wilkesiana leaf can be seen to be relatively safe considering its effects on serum ALT, AST, total and direct bilirubin of the experimental animals.

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