Int.J.Curr.Microbiol.App.Sci (2018) *7*(2): 2759-2766

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 02 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.702.336

Effect of Ethanol Root and Leave Extracts of *Sida acuta* on Some Kidney Function Indices and Electrolytes in Albino Wistar Rats

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Keywords

Sida acuta, Wistar rats, Toxicity, Electrolytes, Renal dysfunction

Article Info

Accepted:
24 January 2018
Available Online:
10 February 2018

A B S T R A C T

This study investigates the effect of ethanol root and leaf extracts of *Sida acuta* on some kidney function indices and electrolytes in albino wistar rats. The rats were divided into 4 groups of 7 rats each. Group 1 was the control group, group II received an oral dose of 100 mg/kg leaf extract of *Sida acuta* daily, group III received an oral dose of 100 mg/kg root extract of *Sida acuta* daily while group IV received 50 mg/kg each of leaf extract and root extract of *Sida acuta* daily. After 21 days of treatment, the rats were sacrificed and standard analytical methods were used to assay the biochemical indices. The result of creatinine (mol/L) showed a significant ($P<0.05$) increase in a dose dependent manner in the treatment groups (10.20 ± 0.25, 11.8 ± 0.26, 12. 55 ± 0.16) compared to the control (5.48 ± 0.76) while urea (mol/L) showed a significant ($P<0.05$) decrease in treatment groups (2.54 ± 0.43, 3.75 ± 0.29, 4.08 ± 0.61) compared to the control (8.53 ± 0.33). The electrolytes (Na^+, Cl^- and HCO_3^-) showed a significant ($P<0.05$) decrease in the treatment groups compared to the control while K (mmol/L) was not significant ($P>0.05$) compared to the control. The kidney ALT and ALP (lU/L) in treatment groups revealed a significant ($P<0.05$) decrease while AST (lU/L) showed a significant, ($P<0.05$) increase compared to control. The result of this study has shown that the administration of root and leaf extracts of *Sida acuta* may be toxic to the kidney which may induce renal dysfunction.

Introduction

Herbal medicine has generated a considerable lot of interest worldwide for its contribution to the overall health care delivery (Ahmed and Hussain, 2013). This is predicated on the fact that estimated 80% of the population in developing countries depend on natural products or herbal medicine (Bodekar and Wilcox, 2000) and also natural products and their derivatives represent almost half of the drugs approved since 1994 (Harvey, 2008). Expectedly in Africa and Nigeria in particular, the increasing cost of these drugs has decreased its accessibility to poor communities who cannot afford them. *Sida*

acuta plant is known across Africa, Asia, Mexico, Central America and has spread to other continents of the world constituting an important ethnomedicinally group of plants (Wake, 2012). *Sida acuta* belong to the family Malvacae. It is a perennial shrub which grows up to one metre and is propagated by seeds and stem cuttings. It grows on waste areas, fields and road sides with erect stem, shiny alternate leaves and strong tap root (Mann *et al.,* 2013).

In southern Nigeria, *Sida acuta* has become a household herb. Native names include Udo (Igbo), Iyeye (Yoruba) and Nsukere (Efik). It is used in the treatment of diarrhea, asthma, headache, cold fever, malaria, paralysis and skin infections (Edeoga *et al.,* 2005; Kayode, 2006; Wake, 2012). The plant posses antifertility potential and has been used as a contraceptive to cause abortion (Prakash *et al.,* 1987; Londonkar *et al.,* 2009). Hepatoprotective, anti-inflammatory, antibacterial and hypoglycaemic potential of *Sida acuta* have been reported (Iroha *et al.,* 2009; Sreedevi *et al.,* 2009; Okwuosa *et al.,* 2011). Various parts of the plant like roots, leaves and stem are used singly or in combination for the treatment of diseases. The leave is most frequently used against infections compared to the root. Reports by Benzouzi *et al.,* (2004) and Nwankpa *et al.,* (2015) have shown the presence of phytochemicals like alkaloids, saponins, flavonoids, anthraquinones, polyphenols and tannins in the leave of *Sida acuta.* Bonjean *et al.,* (1998) reports that cryptolepine (5-methylindole [2,3b]-quinoline) is the major alkaloid and possesses antiplasmodial and antibacterial activity. The micronutrient composition and antioxidative potential of the plant leaves extract have been documented (Rami *et al.,* 2014, Nwankpa *et al.,* 2015).

The increasing health challenges coupled with unavailability and unaccessibility of orthodox medicine have triggered tremendous awareness and acceptability of herbal medicine such as *Sida acuta* by rural communities without recourse to its effects on the kidney. Therefore, this study is carried out to investigate the likely effects of ethanol roots and leave extracts of *Sida acuta* on some kidney function indices in albino wistar rats.

Materials and Methods

Plant material: The fresh leaves and roots of *Sida acuta* were harvested from farms and roadsides in Owalla Uratta Owerri North L.G.A of Imo State. The plants were authenticated by Dr. F. Mbagwu of the department of Plant Science and Biotechnology, Imo State University Owerri. The roots and leaves were chopped to pieces with knife, washed under running tap water and dried under shade at room temperature for 4 weeks. The dried roots and leaves were ground to a fine powder with mechanical grinder and kept in labeled airtight containers under dry conditions until required for extraction.

Ethanol extraction *of Sida acuta* leaves and roots

The extraction of *Sida acuta* roots and leaves was done using modified method of Abdulrahman *et al.,* (2004). Two hundred grams each of grounded *Sida acuta* leaves and roots were macerated in 100 ml absolute ethanol for 72 hours properly labelled and covered.

The extracts were then filtered with sterile filter paper (Watman No. 1). The sample filtrates were evapourated to dryness at 40°C in a vacuum using a rotary evaporator and stored at 5°C in a refrigerator until required for use approximate concentrations of the sample extracts were made in 100 ml of 10% ethanol for the treatment of the animals.

Animals

Male albino wistar rats weighing between 160-200 g were used for the experiment. The rats were kept in the animal house of department of Biochemistry, Imo State University Owerri. They were acclimatized to daily handling for *5* days and were fed ad-libitum with normal rat chow (Product of Vital feed Nig. Ltd) and water.

Experimental design

Twenty-eight rats were used in this study. They were randomly assigned into four groups of seven rats each.

Group 1

The animals in this group were fed with normal rat chow and had free access to water. They were orogastrically given 1ml of 10% ethanol daily for 21 days. They serve as the control.

Group II

The rats in this group were fed with normal rat chow and water was provided ad-libitum. They received I ml equivalent to 100 mg/kg body weight of *Sida acuta* leave extract daily for 21 days using orogastric tube.

Group III

This group was fed with normal rat chow and water was provided ad-libitum. They received 1 ml equivalent to 100 mg/kg body weight of *Sida acuta* root extract daily for 21 days using orogastric tube.

Group IV

This group was fed with normal rat chow and water was provided ad-libitum. They received 0.5ml equivalent to 50 mg/kg body weight of *Sida acuta* leave extract and 0.5 ml equivalent to 50 mg/kg body weight of *Sida acuta* root extract daily for 21 days using orogastric tube.

Analytical procedure

After 21 days of treatment, the rats were anaesthetized with chloroform and their thoracic cavities were cut open to expose the heart. By cardiac puncture of each rat, blood sample was collected with a sterile syringe into a plain sterile test tube and allowed to clot for 10 minutes. The serum was separated by spinning at 1000 rpm for 10 minutes with Wisperfuge model 1384 centrifuge (Samson Halland) and collected with a pastuer pipette into clean labeled test tubes. The serum was used for biochemical analysis.

Preparation of Kidney homogenate

The kidney homogenate of each rat was prepared under chloroform anesthesia by removing the kidney from the rats and the surrounding fatty tissues. The kidney of each rat was blended separately in 2 ml of 1% glucose solution until a smooth homogenate solution was obtained. The homogenate of each rat kidney was centrifuged for 10 minutes and the liquid homogenate was extracted into a sterile plane test tubes. This is used to estimate the kidney function enzymes.

Biochemical analysis

Creatinine concentration was measured by the alkaline picrate method (Tietz *et al.,* 1986) while urea concentration was determined by using the diacetyl monoxime method of Marshal (1957). Determination of serum sodium and potassium concentration were done using reagent set (Tietz *et al.,* 1986). Serum bicarbonate concentration was determined titrimetically, while mercuric nitrate method was used to determine the concentration of chloride (Schales and

Schales, 1969). Kidney alanine transaminase (ALT) and asparate transaminase (AST) activities were assayed by the method of Reitman and Frankel (1957) while alkaline phosphatase activity was assayed by the method of King and Amstrong (1934) using Randox Kit.

Statistical analysis

Data were presented as mean ± SD of four determinations. Statistical analysis was carried out using one-way analysis of variance of the SPSS version 21.0. This was followed by student's t-test of significance. Values at $P<0.05$ were considered statistically significant.

Results and Discussion

The effect of ethanol root and leaf extracts of *Sida acuta* on serum urea and creatinine on wistar albino rats were shown in table 1. There were significant ($P<0.05$) increase in creatinine concentration on rats treated with 100 mg/kg of root, 100 mg/kg of leaf and 50 mg/kg of root plus 50 mg/kg of leaf extracts compared to control.

Conversely, urea concentration showed a significant ($P<0.05$) decrease on rats treated with root, leaf and root plus leaf extracts compared to control.

Table 2 showed the effects of ethanol root and leaf extracts of *Sida acuta* on serum electrolytes of albino wistar rats. The result revealed a significant ($P<0.05$) decrease on sodium, chloride and bicarbonate concentrations of test groups compared to control while the concentration of potassium showed insignificant ($P>0.05$) decrease compared to control.

The effect of ethanol root and leaf extracts of *Sida acuta* on kidney enzymes are shown in table 3. The result reveals that root and leaf extracts decreased significantly ($P<0.05$) the concentration of ALT and ALP compared to control while AST showed a significant ($P<0.05$) increase compared to control.

Kidney serves as the excretory organ for urea and creatinine while in the tubules electrolytes are reabsorbed. Thus determination of serum urea, creatinine and electrolytes (Na^+, K^+, HCO_3^- and Cl^-) are important and sensitive biochemical markers in the diagnosis of renal damages (Yakubu *et al.,* 2003). Metabolism of protein produces urea which in the major nitrogen containing catabolite of protein. In this study, the result showed a significant ($P<0.05$) decrease in urea on the administration of root and leaf extracts of *Sida acuta* compared to the control. This suggest that the urea cycle may have been affected leading to the reduction to the production of urea (Yakubu *et al.,* 2003). The plant extract may have affected the catabolism of protein which results in reduced synthesis of urea and can be attributed a non-renal factor. Creatinine is an endogenous compound produced in the muscles by non enzymic action on creatinine phosphate and its clearance in the glomerulus of the kidney is useful in determining the functionality of kidney (Devlin, 2011; Ifeanacho, 2017). The result of this study revealed a significant ($P<0.05$) increase in creatinine concentration in administration of ethanol leave and root extracts of *Sida acuta* compared to the control. Similar results have been reported by Nwanjo *et al.,* (2007) on administration of chloroquine and aspirin and Kayode *et al.,* (2012) on administration of ethanolic leave extracts of *Piliostigma thonningii* on creatinine concentration.

The observation of this study suggest that ethanol root and leave extracts of *Sida acuta* may have caused both impaired glomerular and tubular function which may lead to tissue damage although the exact mechanism is not covered by this study.

Table.1 Effect of ethanol root and leaf extracts of *Sida acuta* on serum urea and creatinine

Group	Treatment	Creatinine MoI/L	Urea MoI/L
1	lml of 10% ethanol (control	5.48 ± 0.76	8.53 ± 0.33
II	1ml of 100 mg/kg leaf of Sida *acuta*	10.20 ± 0.25*	2.54 ± 0.43*
III	1 ml of 100 mg/kg root of *Sida acuta*	11.82 ± 0.26*	3.75 ± 0.29*
IV	**1 ml of 50 mg/kg root and 50 mg/kg leaf of Sida *acuta***	12.55 ±0.16*	4.08±0.61*

Values are mean of four determinations ± SD. (n=7) * significantly different from control (P<0.05).

Table.2 Effect of ethanol root and leaf extracts of *Sida acuta* on serum electrolytes

Group	Treatment	Na^+ mmol/l	K^+ mmol/l	Cl^- mmol/l	HCO_3^- mmol/l
1	1ml of 10% ethanol (control)	150.50±3.59	6.58±0.49	91.75±1.55	30.52±2.68
II	1ml of 100 mg/kg leaf of *Sida acuta*	134.15±3.16*	6.15±0.36	86.50±4.86*	20.24±2.32*
III	1 ml of 100 mg/kg root of *Sida acuta*	13 1.48±3.71*	6.23±0.08	87.46±2.06*	22.53±0.63*
IV	**1ml of 50 mg/kg root and 50 mg/kg leaf of *Sida acuta***	130.75±4.99*	7.12±0.17	86.92±1.89*	24.75±2.49*

Table.3 Effect of ethanol root and leaf extracts of *Sida acuta* on kidney enzymes

Group	Treatment	ALT (lU/L)	AST(IU/L)	ALP (lU/L)
1	lml of 10% ethanol (control	498.52±2.5 1	21.52±0.53	2996.4±4.2
II	lml of 100mg/kg leaf of *Sida acuta*	385.29±2.67*	29.41±0.33*	1663.26±4.11*
III	1 ml of 100mg/kg root *of Sida acuta*	*360.57±3.46**	28.56±1.22*	1702.36±3.28*
IV	**1 ml of 50 mg/kg root and 50 mg/kg of leaf of *Sida acuta***	391.63±3.56*	24.68±1.67*	1811.38±2.29*

Values are mean of four determinations ± SD. (n=7) * significantly different from control (P<0.05).

Extracellular and intracellular fluids comprise majorly of inorganic electrolytes which readily dissociate into their constituent ions and this comprises the most important factor in the transfer and movement of water and electrolytes between these compartments (Chatterjea and Sainde, 2012). In this study, the administration of ethanol root and leave extracts of *Sida acuta* significantly reduced (P<0.05) the serum concentrations of Sodium, chloride, and bicarbonate compared to the control. The hyponatraemic effect of the extract may be due to excessive loss of heat from the body fluid. Also Na^+ K^+ - ATPase which regulates efflux of Na^+ and influx of K^+ may not be primarily involved in this case since potassium concentration is not significantly increased but can be linked to Na^+/H^+ exchanger (Ganong, 2001; Kayode *et al.*, 2012) which can be achieved by membrane-bound aldosterone which regulates the absorption of sodium. Thus Na^+/K^+ pump may have been impaired on the administration of root and leave extract of *Sida acuta*. This is

supported by the significant decrease ($P<0.05$) in chloride and bicarbonate concentration observed in this study, suggesting that the extract may have induced renal damage resulting to impairment on renal function.

Specific marker enzymes for the plasma and endoplasmic reticulum are frequently used to access the integrity of plasma membrane. Their alteration in the tissue and serum would indicate likely damage to the external boundary of the cell, (Sallie *et al.,* 1991). These enzymes include alkaline phosphatase (ALP) aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The significant decrease ($P<0.05$) in kidney ALP on administration of ethanol root and leave extracts may suggest the deleterious effect on the kidney thus resulting in the leakage of this enzyme into the serum. Consequent upon this, other metabolic processes where enzyme is involved such as synthesis of nuclear protein, phospholipid and nucleic acid may also be hampered. Similar results have been obtained by Nwanjo *et al.,* (2007) on administration of halofantrine on liver and Kayode *et al.,* (2012) on administration of *Piliostigma thonningii* leaves on kidney. The result of this study showed a significant decrease ($P<0.05$) in kidney ALT and significant increase ($P<0.05$) in kidney AST on administration of ethanol root and leave extracts of *Sida acuta.* This may be an indication to the damage of the lysosomal membrane resulting to the leakage of ALT into the serum. Alternatively, the extract may also have increased the rate of synthesis of AST in the tissue.

The alterations of the kidney parameters evaluated in this study suggest deleterious effect of root and leave extracts of *Sida acuta* on the kidney. This may pose glomerular and tubular dysfunction of the nephron indicating that herbal preparation may contribute to kidney failure.

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How to cite this article:

Nwankpa, P., C.C. Etteh, C.N. Ekweogu, P.C. Chikezie, O.G. Chukwuemeka and Egwurugwu, J.N. 2018. Effect of Ethanol Root and Leave Extracts of *Sida acuta* on Some Kidney Function Indices and Electrolytes in Albino Wistar Rats. *Int.J.Curr.Microbiol.App.Sci.* 7(02): 2759-2766. doi: https://doi.org/10.20546/ijcmas.2018.702.336