TOXICITY STUDIES AND ASSESSMENT OF RENAL FUNCTIONAL PARAMETERS OF AQUEOUS LEAF EXTRACT OF ACALYPHA WILKESIANA IN MALE ALBINO RATS

ABSTRACT

Toxicity of aqueous leaf extract of *acalypha wilkesiana* in rats was investigated. An acute toxicity was carried out to determine the $LD50$ of the plant's extract. The extracts were administered orally up to a dose of 5000mg/kg for the $LD50$ determination. No death was recorded during the acute toxicity test, which may imply that the plant is practically non-toxic. There was a progressive significant reduction ($p<0.05$) in serum sodium and potassium ions concentration with increasing doses of the extract above 10 mg/kg when compared with control group in phase one. A significant change in urea was also observed at a dose of 100 and 1000mg/Kg respectively. In phase two, there was a slight increase in potassium, urea, and creatinine as well. Indeed, creatinine is known as a good indicator of renal function. The kidney functional indices: serum concentrations of creatinine, bicarbonate, and chloride were not significantly changed following acute administration of aqueous leaf extract ($p<0.05$) when compared with the control group in phase one. The observed progressive decrease in serum sodium and potassium ions as the dose of the extract exceeds 10mg/kg might be an indication of this extract could cause hyponatremia and hypokalemia. Accordingly, *a. wilkesiana* might be considered relatively safe and can be employed in management of diseases involved in high sodium and potassium ions in the serum. High doses should be avoided as may result in increase creatinine concentrations.

Keywords: Acalypha Wilkesiana, Toxicity, Kidney function

INTRODUCTION

Plants of the genus Acalypha are historically used in the treatment and/or management of various illnesses such as diabetes, jaundice, hypertension, cough, hepatic inflammation, schistosomiasis, dysentery, respiratory disorders like bronchitis, asthma, and pneumonia, and skin conditions such as scabies, eczema, and mycoses (Seebaluck, Gurib-Fakim & Mahomoodally, 2015). Acalypha wilkesiana belongs to the familyEuphorbiaceae. It is an evergreen tropical and subtropical shrub with greenish-yellow to white dotted green leaves Irregular black spots mottled over. It is native to South Africa but grows in different parts of Africa and the islands of

the Pacific. A boiling decoction of A leaves in west Nigeria is used in the treatment of infant fungal infections (Oyebode et al.,2018). This plant's leaves have anti-inflammatory, anti-microbial, and anti-pyretic roles (Ikewuchi et al.,2011). Researchers have been working over the last few years to define and verify plant-derived substances for the treatment of various diseases. Ironically, more than 25 percent of conventional drugs are known to be produced directly or indirectly from plants. It is worth noting that Indian medicinal plants are considered a large source of many concepts and compounds in pharmacology. Medicinal plants should be safe but there have been records of many dangerous and fatal side effects (Bussmann et al.,2011). Which may be directly adverse effects, allergic reactions, contaminant effects, and/or medication and other herbal interactions. Many times phytotherapeutic products are considered less toxic, wrongly because they are 'natural.' Nonetheless, such goods contain potentially bioactive components with the potential to cause adverse effects (Ekor, 2014). Some herbal medicines can exert kidney toxicity by their inherent properties (Asif, 2012). Toxicity may occur when a herb of unknown toxicity is ingested, when an incorrect diagnosis results in the replacement of a harmless herb with a toxic one, when preparations are contaminated with toxic non-herbal compounds or when a herb potentiates the nephrotoxic effect of traditional therapy (Jha, 2010). This paper aims to investigate the acute toxicity and assesses the renal functional parameters of aqueous leaf extract of acalypha wilkesiana in male albino rats.

MATERIALS AND METHODS

Materials

Collection and Identification of Plant Material

The leaves of the plant (*Acalypha wilkesiana*) were obtained within Dutsinma local government, Katsina state, Nigeria. The leaves were identification and authentication at the Department of Plant Biology, Bayero University Kano, by a botanist, Dr. Yusuf Nuhu. The leaves were thoroughly washed with distilled water and dried in the laboratory under a shed to avoid loss of phytochemicals.

METHODS

Preparation of the Aqueous Leave Extract

The dried leaves were crushed using laboratory mortar and pistol and then ground to powder using a laboratory grinder. 500g of the powdered sample was dissolved in 2000ml of distilled water and allowed to stay for 24 hours with periodic stirring. The sample was filtered using Whatman number 1 filter paper, the filtrate was then concentrated in a water bath at 400 C for 5 days. The crude slurry was placed in the ovum at 400 C, complete drying took two days.

Animal grouping and administration of the extract

20 rats weighing 115–145 g were purchased from the Biochemistry Department, University of Ilorin Kwara State, Nigeria. The rats were allowed to acclimatize for two weeks. Three of the rats were used as the control group. The experiment was divided into two phases, phase one and phase two.
Phase I of Lorke's method of determining acute toxicity requires 9 rats, three rats per each group. In phase II, 5 rats were used per each group. The rats were fed with starter mesh with full access to pure water. They were kept in a well-ventilated cage at the animal facility in Federal University Dutsinma. We use Lorke's method(1983) of determining the acute toxicity. The grouping and extract administration was administered as follows:

Phase one
Group 1(n=3): was administered 10mg/kg aqueous extract 24

Group 2 (n=3): was administered 100mg/kg aqueous extract
Group 3 (n=3): was administered 1000mg/kg aqueous extract
The rats were monitored for 24hours for mortality and general behavior.

In phase II, 5rats were used and grouped into 5 of 1 rat each. They were treated with the dose based on the findings of phase I

Group 1(n=3): 1250 mg/kg aqueous extract
Group 1(n=3): 2000mg/kg aqueous extract
Group 1(n=3): 2750mg/kg aqueous extract
Group 1(n=3): 3750mg/kg aqueous extract
Group 1(n=3): 5000mg/kg aqueous extract

The rats in phase two were monitored for 14 days.

Collection of Blood

A blood sample was collected from the jugular veins of the experimental rats, using sterilized hypodermic needles into heparinized tubes. The tubes were centrifuged to remove the serum. The serum was used to perform the kidney markers parameters analysis.

Assay procedures

Determination of Serum Creatinine

This was determined using Hyneck *et al*. (1981). Serum creatinine determination is mainly used for the diagnosis of renal disease. Creatinine is an endogenous NPN (Non-Protein Nitrogen) waste product of the body excreted through kidneys. The creatinine kit is based on Jaffe's Kinetic method. A drawback of Jaffe's endpoint reaction is the interference due to non-specific substances such as proteins, ascorbic acid, and ketoacids.

Principle of Test

Creatinine reacts with alkaline picrate to produce a red-colored complex; the rate of red-colored complex formation is directly proportional to the Creatinine concentration.

Procedure
In this assay procedure, 2.0 ml of the picric acid reagent in a tube was added to 0.2 ml of serum for deproteinization of the specimen, which was mixed well and centrifuged at 3000 rpm to obtain a clear supernatant.100µl of buffer reagent was added to 1.1 ml of supernatant, 0.1 ml of standard creatinine and 0.1 ml of distilled

water to prepare the test, stand and blank, respective. 1.0 ml of picric acid reagent was added to blank and standard. The test tubes were mixed well and kept at room temperature for 20 minutes. The alkaline picrate reacts with creatinine to form the orange-colored complex, which was read at 520 nm with the spectrophotometer.

$$\boldsymbol{Calculation\ of\ creatinine}(\boldsymbol{mg/dl}) = sample(test)/(Absorbance\ of\ standard) \times concentration$$

Determination of Serum Urea

This was determined using the method of Machodo and Horizonte (1958). Urea is the highest non-protein nitrogen compound in the blood and it is the major excretory product of protein metabolism. It is formed in the liver from free ammonia generated during protein catabolism. Protein metabolism produces amino acids that can be oxidized, this result in the release of ammonia, which is converted to urea (via urea cycle) and excreted as a waste product.

PRINCIPLE OF THE TEST

Urea is hydrolyzed in the presence of urease enzyme and water to yield ammonia and carbon dioxide.

$$NH2 - CO - NH2 + H2O \rightarrow 2NH3 + CO2$$

The ammonia reacts with α-ketoglutaric acid and reduced nicotinamide adenine dinucleotide (NADH) in the presence of glutamate dehydrogenase (GLDH) to yield glutamic acid and nicotinamide adenine dinucleotide (NAD).

$$NH3 + HOOC - (CH2)2 - CO - COOH + NADH + H+ \rightarrow HOOC(CH2)2CH(NH2)\,COOH + NAD + H2O$$

Procedure

Using the commercially available kit, 1000 µl of working reagent containing urea reagent, and a mixture of salicylate, hypochlorite, and nitroprusside was added to 10 µl of serum, 10 µl of standard urea (40 mg/dl) and 10µl of purified water to prepare the test, standard and blank, respectively. All the tubes were mixed wheel and incubated at 380C for 5 minutes. Then 1000µl of reagent-2 containing alkaline buffer was added to all the test tubes, which are incubated at 380C for 5 minutes. Urease catalyzes the conversion of urea to ammonia and carbon dioxide. The ammonia thus released reacts with a mixture of salicylate, hypochlorite, and nitroprusside to yield indophenol, a blue-green colored compound. The intensity of the color produced is directly proportional to the concentration of urea in the sample and is measured with a spectrophotometer at 578nm. The blood urea was calculated using the formula:

$$\boldsymbol{Calculation\ of\ Urea(mg/dl)} = sample(test)/(Absorbance\ of\ standard) \times concentration$$

Determination of Serum Electrolytes

Determination of Serum Sodium

This was determined using the method of Maruna (1958). Sodium is the major cation of extracellular fluid. It plays a major role in the maintenance of the normal distribution of water and osmotic pressure in the various fluid compartments. Hyponatremia (low serum sodium level) is found in a variety of conditions including the following: severe polyuria, metabolic acidosis, Addison'sdisease, diarrhea, and renal tubular disease. Hypernatremia (increased serum sodium level) is found in the following conditions: hyperadrenalism, severe dehydration, and diabetic coma after therapy with insulin, excess treatment with sodium salts.

Principle of The Test

The present method is based on modifications of those first described by Maruna in which sodium is precipitated as the triple salt, sodium uranyl acetate, with the excess uranium then reacting with Ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Procedure

In this assay procedure, the test tubes were labeled appropriately as blank, standard, and test. Then, 1.0 ml of filtrate reagent was pipette into all the tubes, 50µl of distilled water was added to the blank tube, 50µl of the standard to the standard tube, 50µl of the blood sample to the test tube. The tubes were shaken

vigorously and continuously for 3 minutes. The tubes were centrifuged for 10 minutes and the supernatant was transferred into another tube.
The test tube was labeled accordingly and 1.0 ml Acid Reagent was added into all the tubes and 50µl of color reagent was also added to all tubes. The absorbance was read on the spectrophotometer at 550 nm.

Calculations

$$Serum\ sodium\ (mEqL) = Absorbance\ of\ Blank - Absorbance\ of\ Sample \times Concentration\ of\ standard$$

Determination of Serum Chlorine (Cl^-)

This was determined using the method of (Sobel & Fernandez,1963)
Principle of Test
The chlorine ion displaces thiocyanate from non-ionized mercuric thiocyanate to form mercuric chloride and thiocyanate ions. The released thiocyanate ions react with ferric ions to form a color complex that absorbs light at 550 nm. The intensity of the color produced is directly proportional to the chloride concentration.

$$Hg(SCN)2 + 2Cl \rightarrow HgCl2 + 2SCN$$

$$3SCN + Fe + 3 \rightarrow Fe(SCN)3(red\ complex)$$

Procedure

In the determination of serum chloride, the test tubes were labeled appropriately as test and blank. 1.0 ml of chloride reagent was added to each tube, 10 µl of distilled water was added to the blank tube and 10µl of the sample was added to the test tube. It was incubated at room temperature for 5 minutes and the absorbance was read on the spectrophotometer at a wavelength of 550 nm.

Calculations

$$\boldsymbol{Calculation\ of\ Chloride}(\text{mEq/L}) = sample(test)/(Absorbance\ of\ standard) \times concentration$$

Determination of Serum Bicarbonate (HCO_3^-)

The bicarbonate reagent utilizes the enzymatic method developed by Forrester *et al.* (1976). This procedure bicarbonate ($HCO3^-$) and phosphoenolpyruvate (PEP) are converted to oxaloacetate and phosphate in the reaction catalyzed by phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MD) catalyzed the reduction of oxaloacetate to malate with the concomitant oxidation of reduced nicotinamide adenine dinucleotide (NADH). This oxidation of NADH results in a decrease in absorbance of the reaction mixture measured chromatically at 380/410 nm proportional to the Bicarbonate content of the sample.

$$PEP + HCO3 \rightarrow Oxaloacetate + H2PO4$$

$$Oxaloacetate + NADH + H+ \rightarrow Malate + NAD+$$

Procedure

In the determination of serum bicarbonate, the test tubes were labeled appropriately as blank and test. 1 ml of bicarbonate reagent was added in each test tube, 20 µl of the standard was added to blank, and 20µl of the sample was added to test, it was mixed vigorously and incubated for 3 minutes. The absorbance was read on the spectrophotometer at a wavelength of 520 nm.

Calculation

$$\boldsymbol{Calculation\ of\ Bicarbonate}\ (\mathrm{mEq/L}) = sample(test)/(Absorbance\ of\ standard) \times concentration$$

Statistical Analysis

Data are expressed as mean ± Standard deviation. Comparisons between different groups were done using one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test using the software SPSS 16.0. A probability level of less than 0.05 was accepted as statistically significant.

RESULTS

Acute toxicity

Oral administration of aqueous extract of *Acalypha wilkesiana* did not produce any mortality in rats up to a dose level of 5000mg/kg (table 1 and 2). The oral LD50 was indeterminable up to a dose level of 5000mg/kg.

Table 1: dose and mortality recorded following a 24hours administration of *Acalypha wilkesiana* leaf extract.

Phase 1	**No. of animals**	**Dose(mg/kg)**	**Mortality recorded**
(group)			
1	3	10	0/3
2	3	100	0/3
3	3	1000	0/3

Table 2: dose and mortality recorded following a 24hours administration of *Acalypha wilkesiana* leaf extract.

Phase 2 (group)	**No. of animals**	**Dose(mg/kg)**	**Mortality recorded**

1	1	12500	0/1
2	1	2000	0/1
3	1	2750	0/1
4	1	3750	0/1
5	1	5000	0/1

Table 3: kidney parameters profile of rats given different dosages of aqueous leave extract of *Acalypha wilkesiana* (phase one).

Parameter	Control	10mg/kg	100mg/kg	1000mg/kg
Sodium(Na)	141.3+2.08a	136.6+1.52b	138.6+2.08b	141.0+2.82a
Potassium(K)	15.83+10.92a	3.70+0.20b	5.03+0.31b	4.63+0.75b
Chloride(Cl)	98.67+3.05a	98.67+3.05a	100.33+2.08a	101.00+4.24a
Bicarbonate	24.67+0.57a	27.33+2.08a	28.33+2.0a	26.00+1.41a
Urea	4.36+0.37a	4.70+0.26a	6.20+0.30b	6.50+0.84b
Creatinine	92.67+4.04a	83.00+4.58b	85.67+5.6b	92.67+9.01a

Values are expressed as mean $\pm$ SD. Values along the row with the same superscript are not significantly different ($P < 0.05$)

Table 4: kidney parameters profile of rats given different dosages of aqueous leave extract of *Acalypha wilkesiana* (phase two).

Parameter	1250mg/kg	2000mg/kg	2750mg/kg	3750mg/kg	5000mg/kg
Sodium	138	141	139	143	139
Potassium	5.7	5.5	4.8	5.8	4.9
Chloride	98	102	99	105	98
Bicarbonate	27	25	24	28	27
Urea	7.2	6.8	7.2	7.8	8.4
Creatinine	94	99	101	102	94

One animal is used to determined the effect of the extract on electrolytes according to lorke's method, 1983

DISCUSSION

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 behavioral changes and death were observed. However, there was a progressive significant reduction ($p<0.05$) in serum sodium ions concentration with increasing doses of the extract above 10 mg/kg when compared with the control group in phase one. The trends obtained for kidney function indices following the administration of the aqueous extract of *A. wilkesiana* results in significant changes in serum potassium from all the experimental groups, and urea for

those treated with 100mg/Kg and 1000 mg/Kg, suggesting

that the extract interfered with the renal capacity to excrete substances.

Earlier reports show that acalipha wilkesiana extract do not significantly increase packed cell volume, hemoglobin, red blood cell count, white blood cell, neutrophil,

lymphocytes, platelets, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and erythrocyte sedimentation rate (Iniaghe, Egharevba & Oyewo, 2013)

. 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). From the present study, creatinine was found to increase significantly at doses of 10 and 100mg/Kg compared with the control in phase one and increases in the second phase at 2750 and 3750mg/Kg respectively. Similarly, the serum creatinine level is also a good indicator of kidney function. The acute administration of the aqueous leaf extract of *A. wilkesiana* did not result in a significant change in chloride and bicarbonate ions during the phase one but somehow increases with increase in doses in phase two, which implies that serum concentrations of bicarbonate and chloride were not significant at ($p<0.05$) when compared with the control group.

The observed progressive decrease in serum sodium and potassium ions (hyponatremia and hypokalemia) as the dose of the extract exceeds 10mg/kg might be an indication of this extract could cause hyponatremia and hypokalemia. Some of the more common causes of medication-induced hyponatremia are diuretics (Spital, 1999). Diuretics cause hypovolemic hyponatremia (Goh, 2004). Diuretics induce weight loss through the excretion of water (Cadwallader et al., 2010).

It can be hypothesized that prolonged use of this extract at doses greater doses may lead to hyponatremia. This may be a probable mechanism of action of the use of this extract in the treatment of hypertension.

CONCLUSION

Acalypha wilkesiana 's aqueous leaf extract may be considered relatively safe, as it did not cause either mortality or obvious toxicity signs. This extract could be suspected as a possible diuretic and could cause hypovolemic hyponatremia that may contribute to weight loss. The use of this extract may be due to a potential diuretic feature of the extract in the treatment of hypertension. Thus the oral intake of the aqueous leaf extract when used in the treatment of various ailments in folklore medicine may be used with caution, based on these results. Accordingly, *a. wilkesiana* might be considered relatively safe and can be employed in management of diseases involved in high sodium and potassium ions in the serum. High doses should be avoided as may result in increase creatinine concentrations.

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