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TOXICITY STUDIES AND EFFECT OF AQUEOUS LEAF EXTRACTS OF ACALYPHA WILKESIANA ON THE RENAL FUNCTION IN MALE ALBINO RATS.

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ABSTRACT

The toxicity of aqueous leaf extract of Acalypha wilkesiana in rats was investigated. Acute toxicity was carried out to determine the *LD*50 of the plant's extract. The extracts were administered orally up to a dose of 5000mg/kg for the *LD*50 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 the 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 the management of diseases involved in high sodium and potassium ions in the serum. High doses should be avoided as may affect electrolytes balance in body fluids.

Keywords: Acalypha wilkesiana, Toxicity, Kidney function, Electrolytes

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 et al. 2015). *Acalypha wilkesiana* belongs to the family Euphorbiaceae (Madziga, 2010). They comprise of evergreen shrubs, trees, and annuals from tropical to subtropical areas, primarily in African, American, and Asian tropics (Ahmed et al.2012). A boiling decoction of *A. wilkesiana* 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).Medicinal plants should be safe but there have been records of many dangerous and fatal side effects (Bussmann et al. 2011), which may be direct adverse effects, allergic reactions, contaminant effects, and/or medication and other herbal interactions. Many times phytotherapeutic products are considered less toxic, wrongly so because they are 'natural.' Nonetheless, such goods contain bioactive components with the potential to cause adverse effects (Ekor, 2014). Some herbal medications 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 assess the renal functional parameters of aqueous leaf extract of *Acalypha wilkesiana in male albino rats.*

MATERIALS AND METHODS

Plant 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 the shed to avoid loss of phytochemicals.

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.

Animals:

Animal grouping and administration of the extract

About 20 rats weighing 115–145g 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.

Determination of acute toxicity

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, one 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'smethod (1983) of determining 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.

Phase Two:

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.

Sample collection and preparation

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.

Analytical methods

Serum creatinine was determined using Jaffe's kinetic method reported by Hyneck *et al*. (1981) whereas serum urea was determined using the method of Machodo and Horizonte (1958).

Serum potassium concentration was measured using the method described by Terri et al. (1958) whereas chloride was determined using the method of (Sobel and Fernandez, 1963). Serum bicarbonate was assayed by the enzymatic method of Forrester *et al.* (1976).

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 AND DISCUSSION

Oral administration of aqueous extract of *Acalypha wilkesiana* did not produce any mortality in rats up to a dose level of 5000mg/kg (tables 1 and 2). The oral LD50 was indeterminable up to a dose level of 5000mg/kg. This result was in agreement with an earlier study that reported the non-toxic effect of *Acalypha wilkesiana up to a dose of3000* mg/kg but found a dose of 1,600 mg/kg decreases the level of neutrophils and lymphocytes (Olukunle et al. 2015).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 (Table 3). 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 (Table 3 and 4). Earlier reports show that caliphate wilkesiana extract does 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 et al. 2013). Indeed, creatinine is known as a good indicator of renal function (Nwankpa et al. 2018). From the present study, creatinine was found to decrease 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. Creatinine and urea are non-protein nitrogen metabolites that are cleared after glomerular filtration from the body by the kidney. Thus, the evaluation of serum urea, creatinine, and electrolytes (Na^+, K^+, Cl^-, HCO_3^-) is a critical and delicate biochemical marker generally used in the diagnosis of renal failure (Nwankpa et al. 2018). Any rise in creatinine levels is only observed if there is marked damage to functional nephrons (Mukinda and Eagles, 2010). 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 increased with increase in doses in phase two. This implies that serum concentrations of bicarbonate and chloride were not significant at ($p<0.05$) when compared with the control group. Urea was found to increase at a dose of 100 and 1000mg/Kg in phase one. The extract administration of *Acalypha wilkesiana* was also reported to cause dose-dependent increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels that differed significantly (Olukunle et al. 2015).

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

Phase 1 (Group)	Number of animals	Dose (mg/kg)	Mortality recorded
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)	Number 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

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

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 may be the cause. Some of the more common causes of medication-induced hyponatremia are diuretics (Spital, 1999). Diuretics cause hypovolemic hyponatremia (Goh, 2004) and induce weight loss through the excretion of water (Cadwallader et al., 2010).

It can be hypothesized that prolonged use of this extract at 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. A recent study supports our findings and reported that the administration of *Acalypha wilkesiana* leaf extracts in rabbits fed with salt-loaded diets resulted in substantially lower serum sodium, chloride, bicarbonate, and AST activities (Omage and Azeke, 2019). This implies that *Acalypha wilkesiana* leaf extracts lower some electrolytes in body fluids that may be either beneficial or toxic to the body system.

CONCLUSION

Acalypha wilkesiana leaf extracts 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 in alternative medicine may be due to a potential diuretic feature of the extract in the treatment of hypertension. Based on these

findings, oral administration of the aqueous leaf extract in the treatment of various ailments in folklore medicine may be done with caution. Accordingly, *A. wilkesiana* might be considered relatively safe and can be employed in the management of diseases involved in high sodium. High doses should however be avoided, as it may affect electrolyte balance in the body fluids.

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