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RESEARCH PAPER

EFFECTS OF CRUDE ETHANOL EXTRACT OF *TAPINANTHUS GLOBIFERUS* A. RICH ON FUNCTIONAL AND STRUCTURAL INTEGRITY OF THE RATS KIDNEY

[1]Abdullahi, M.H.*, [2]Danjuma, N.M., [1]Aliyu, M. and [3]Abubakar, S.
[1]Department of Pharmacology, Bayero University, Kano; [2]Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria; [3]Department of Histopathology, Aminu Kano Teaching Hospital, Kano
*Corresponding author: *E*-mail: am.huguma@yahoo.com ; *Tel*: +234-806-554-1850

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ABSTRACT

The present study was carried out to investigate the effects of crude ethanol extract of *Tapinanthus globiferus*on rat kidney. The toxic effects of the extract on rats kidney after 28 days of oral administration were evaluated on serum levels of urea, creatinine, sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO_3^-) while histopathology was evaluated on sections of the kidney. Results from the acute toxicity studies on the extract were found to be greater than 5,000 mg/kg body weight in rats after oral administration. The biochemical analysis of the extract showed significant ($p<0.05$) decrease in serum urea, creatinine and sodium levels, and significant ($p<0.05$) increase in serum potassium and chloride levels at doses of 175 and 350 mg/kg, while serum bicarbonate remained insignificant at tested doses. However, histological observations showed no significant structural changes in the kidney architecture at doses of 87.5 and 175 mg/kg extract compared to control, but at 350 mg/kg dose of extract showed areas of degeneration of Bowman's capsule.The present work has revealed the non-toxicity of the ethanol extracts of *Tapinanthus globiferus* at low dose but suggests that its prolonged usage at higher dose should be monitored.

Keywords: *Tapinanthus globiferus*, Creatinine, Electrolytes, nephrotoxic, Bowman's capsule

INTRODUCTION

Medicinal plants have both positive and negative values, especially their usage as food and therapeutic applications on one hand, and their fatal toxic capabilities on the other respectively. These potentials have been well authenticated and documented (Borodo, 2001). Most of the bi-products of medicinal plants in the body are excreted primarily by the kidneys and some with nephrotoxic potentials, have been shown to be deleterious to kidney's structure and function (Contran *et al.,* 2005). The consequent impairment of kidney functions usually results in raised blood levels of urea, creatinine, and electrolytes -sodium, potassium, bicarbonate and chloride (Contran *et al.,* 2005), and such kidneys present cytoarchitectural distortions that are of histopathological importance (Yunusa, 2014).

Tapinanthus globiferus is the most common mistletoe that grows on *Vitellaria paradoxa* tree (host) in West Africa and it's a major cause of *Vitellaria paradoxa* mortality in the Northern limit of the savannah (Watson, 2001). *Tapinanthus globiferus* is locally known as mistletoe (English), *Kauchin kadanya* (Hausa), *Eme-emi afomo* (Yoruba), and *Osisi/Okwuma osa* (Igbo) in Nigeria, and belongs to the family of *Loranthaceae* (Burkill, 2000). *Tapinanthus globiferus* is a semi-parasite with glabrous pendulous stems up to 1.2 m long with presumably roots that mostly grows on the branches ofa large number of tree species such as *Vitellaria paradoxa, Kola*, *Citrus, Combretum, Acacia,Aloe* and *Terminalia* as host trees (Waterberg *et al.,* 1989). *Tapinanthus globiferus* is used locally by traditional herbalist for the treatment of various diseases including diabetes and stroke (Odugbemi, 2006).

In Sudan, *Tapinanthus globiferus* is used to make a lotion for the treatment of itching (Burkill, 2000). In Saudi Arabia, fresh *Tapinanthus globiferus* (local name, *Hadhal*) is given orally to all types of livestock for the treatment of fever and removal of placenta after parturition (Sher and Alyemeni, 2011). *Tapinanthus globiferus* is used in traditional medicine to treat inflammations, malaria, bacterial infections, ulcer, headaches, diabetes mellitus, stroke, stomach problems, as well as convulsions (personal communication). *Viscum album* (mistletoe) has been reported to possess a number of therapeutic uses for managing a wide range of diseases such as diabetes mellitus, stroke, stomach problems, heart palpitations, high blood pressure and breathing difficulties (Karakas *et al*., 2008). *Loranthus micranthus* (mistletoe) have also been used in the treatment of epilepsy, infertility, menopausal syndrome and rheumatism (Osadele and Ukwueze, 2004). This study investigates the toxic effects of ethanol extract of *Tapinanthus globiferus* on the functional and structural integrity of the kidney in rats.

MATERIALS AND METHODS

Plant collection and extraction: Fresh *Tapinanthus globiferus* was collected from Huguma village of Takai Local Government Area of Kano State, Nigeria. Plant was identified and authenticated in the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, by comparing with voucher specimen number 1052. The mistletoe was air dried under the shade at room temperature (27^oC) for 28 days and then ground into a fine powder using pestle and mortar. Powdered material (500g) was used for the extraction in ethanol (75%) and the extraction was carried out using Soxhlet extractor. The extract was concentrated on water bath at temperature of 60^oC.

Experimental Animals: Rats (weighing 150 – 180 g) of either sex were used for the experiments. The animals were obtained from animal House of the Department of Pharmacology, Bayero University Kano. Animals were kept in a well-ventilated room, fed with a pelletized grower mash (Vital feeds, Plc, Jos) and water provided *ad-libitum*.

Animal Grouping: Twenty four (24) Wister albino rats of either sex were randomly divided into four (4) groups of six (6) rats each. The first group served as control and was treated with distilled water (1 ml/kg body weight), while the second, third and fourth groups were treated with 87.5, 175 and 350 mg/kg body weight of the ethanol extract of *Tapinanthus globiferus* orally respectively on daily basis for twenty eight (28) days, after which rats were sacrificed on the 28^{th} day.

Acute toxicity study: The determination of oral median lethal dose (LD_{50}) of the ethanol extract of *Tapinanthus globiferus* was conducted using the method of Lorke (1983).

Biochemical studies: Rats were sacrificed following chloroform anaesthesia and blood was collected by cardiac puncture from rats for test of renal function indices. Serum urea and creatinine were determined using the method of Weatherburn (1967) and Bartels and Bohmer (1972) respectively. Serum sodium (Na^+) and potassium (K^+) were assayed using the method of Henry (1974). Serum chloride (Cl^-) and bicarbonate (HCO_3^-) were determined according to the method of Schales and Schales (1941) and Forrester *et al.,* 1976 respectively.
Histopathological studies: Method of Auwioro (2010) was used. Rats were sacrificed at the end of the study period following chloroform anaesthesia. The rats Kidneys were harvested and preserved in 10% formalin solution. The diagnoses on kidney of the rats were fixed with 10% normal saline, dehydrated with ascending grade of alcohol, cleared with toluene, infix-treated with molten paraffin wax and embedded with paraffin wax. The microtome sections of the tissues were stained with haematoxylin and eosin staining technique. Slides were prepared and observed using a standard light microscope in the Histopathology Department, Aminu Kano Teaching Hospital, Kano, Nigeria.

Statistical analysis: Results were expressed as Mean ± Standard error of mean (Mean ± SEM). Data was analyzed using one - way analysis of variance (ANOVA) followed by Post - hoc tests (Bonferroni, Dunnet's, and Tukey HSD) and the differences between means were considered significant when $P \leq 0.05$.

RESULTS

Acute toxicity evaluation: The median lethal dose (LD_{50}) of ethanol extract of *Tapinanthus globiferus* after oral administration was found to be greater than 5,000 in rats.

Biochemical analysis: The groups treated with doses of 175 and 350 mg/kg extract showed significant ($p < 0.05$) decrease in serum concentration of urea, creatinine and sodium, however serum concentration of potassium and chloride significantly ($p < 0.05$) increased compared to control at the same doses. However there wasno significant change in serum biocarbonate concentrationat the tested doses compared to control (Table 1).

Histological analysis: The histopathology evaluation of the rat kidney sections treated with doses of 87.5 and 175 mg/kg extracts showed normal kidney architecture with the cortex containing the glomerulus and the medulla containing renal tubules compared to control (Fig. 1, 2 and 3). The kidney section of rat that received 350 mg/kg dose of extract showed area of local necrosis or degeneration of Bowman's capsule (Fig. 4).

Table 1: Effect of ethanol extract of *Tapinanthus globiferus* on serum urea, Creatinine and electrolytes in rats

Treatment (mg/kg)	Mean ± SEM (mmol/L)					
	Urea	Creatinine	Na^+	K^+	Cl^-	HCO_3^-
Control (1ml/kg)	6.50±0.27	85.40±13.52	143.40±1.00	3.74±0.12	104.20±1.36	23.20±1.24
TgE 87.5	6.30±0.25	84.00±8.24	138.80±1.60 [a]	4.10±0.29	104.20±1.46	23.20±1.40
TgE 175	5.28±0.44 [a]	80.40±8.10	138.40±1.03 [a]	4.40±0.31 [a]	108.40±0.40[a]	22.60±0.75
TgE 350	4.00±0.76[a]	53.33±8.91 [a]	124.67±1.93[a]	4.07±0.10 [a]	109.17±0.91[a]	20.00±1.52

[a]P< 0.05, one way ANOVA followed by Bonferroni and Dunnet's Post hoc test, Mean ± SEM, n =6, TgE = *Tapinanthus globiferus* extract.

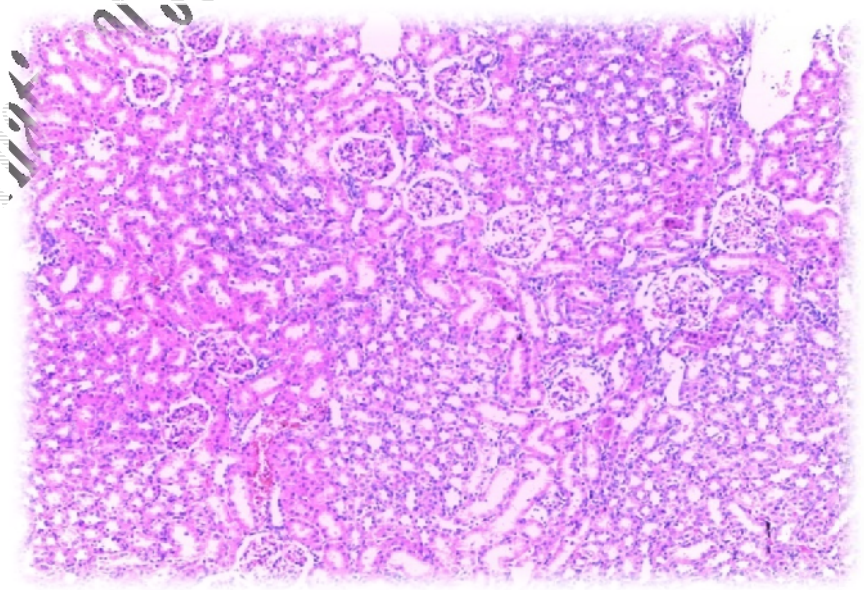

Fig. 1: Photomicrograph of transverse section of the kidney of rat that treated with 1ml/kg normal saline (Control). Section shows no significant pathology (H and E stain x100).

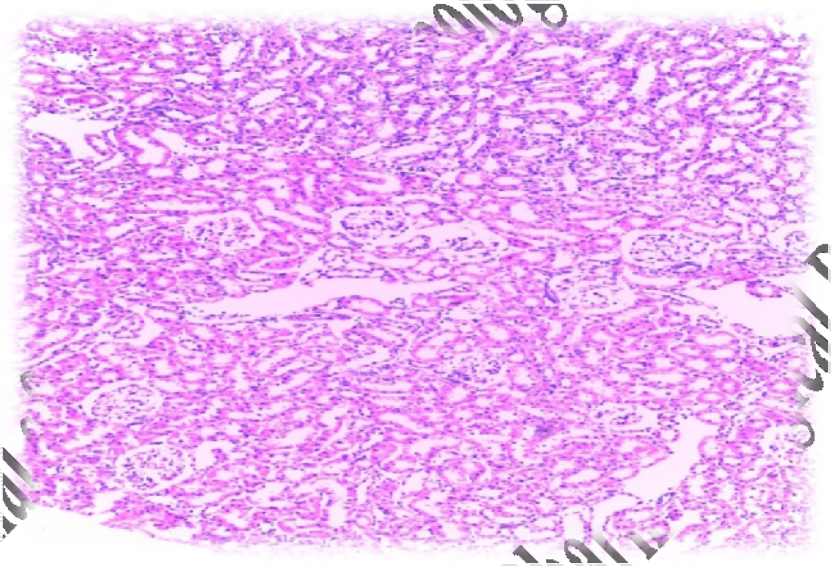

Fig. 2: Photomicrograph of transverse section of the kidney of rat that received a dose of 87.5 mg/kg extract. Section shows no significant pathology (H and E stain x100).

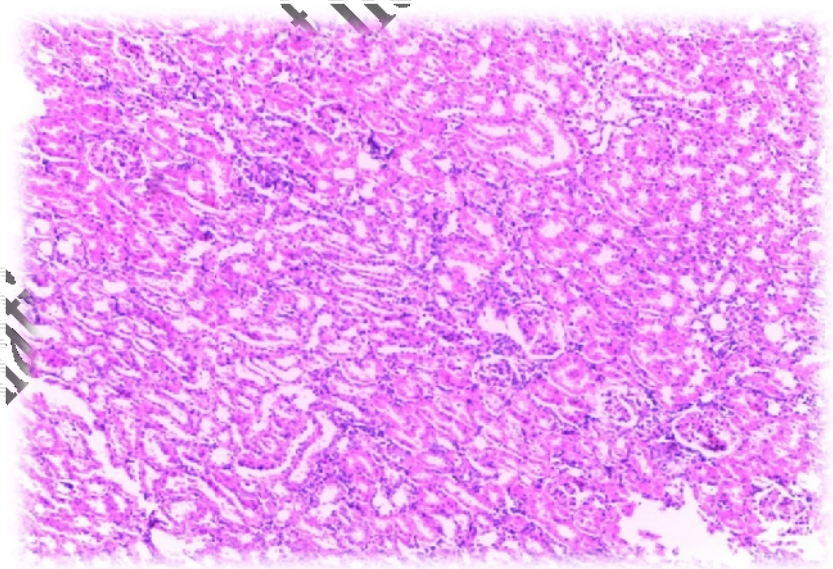

Fig. 3: Photomicrograph of transverse section of the kidney of rat that received a dose of 175 mg/kg extract. Section shows no significant pathology (H and E stain x100).

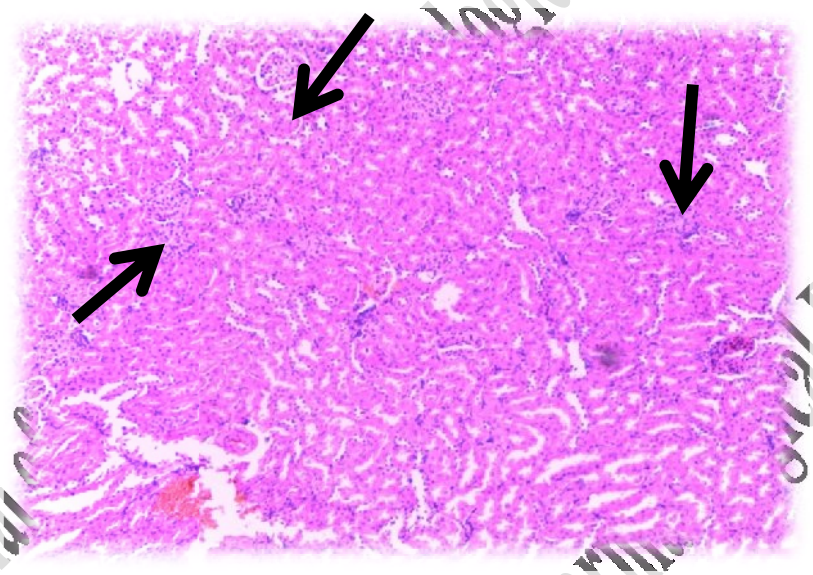

Fig. 4: Photomicrograph of transverse section of the kidney of rat that received a dose of 350 mg/kg extract. Section shows areas of degeneration of Bowman's capsule (H and Estain x100).

DISCUSSION:

In the present study, the effects of ethanol extract of *Tapinanthus globiferus*on functional and structural integrity of the rat kidney was studied. The result of acute toxicity studies (LD_{50}) of the extract in the rats showed that the extract was practically non-toxic via oral administration and can be used for folkloric medicine. According to Lorke (1983), the proposed scale of acute toxicity study (LD_{50}) are; LD_{50} < 1.0 mg/kg-very toxic, LD_{50} < 10 mg/kg- toxic, LD_{50} up to 100 mg/kg- less toxic, LD_{50} up to 5,000 mg/kg- slightly toxic and substances with LD_{50} values greater than 5,000 mg/kg are practically non-toxic.

Generally, acute toxicity studies are designed to determine the dose that will produce either mortality or serious toxicological effects or high level safety when given once and also serve to provide information regarding doses that would be used in sub-chronic or chronic studies. Thus, the significant ($p < 0.05$) increase and decrease in biochemical levels observed at doses of 175 and 350 mg/kg may be as a result of the body trying to maintain the electrolytes level. There was significant decrease in Na^+ level at doses of 175 and 350 mg/kg, this could not be as a result of renal toxicity since there was a significant increase in K^+ level at the same doses. This may result in physiological action of the Na^+-K^+ ATPase pump in trying to maintain normal electrolytic balance. The decrease in the serum concentration of urea and creatinine and increase in serum K^+ showed clearly that the extract is not causing any damage to the kidney. The significant changes observed in biochemical levels might be due to the maintenance of electrochemical neutrality by the body system (Ibrahim *et al*., 2006).

Furthermore, the histological observations made from the sections of the rat kidney showed that the extract produced signs of toxicity at the highest dose of extract but no pathological findings were observed in the section of rat kidney treated with low doses of extract and normal saline. The kidneys of the rat, treated with ethanol extract of *T. Globiferus* showed maintenance of normal function and the kidney architecture of rats treated at low doses compared control. The plants *T. Globiferus* have been reported to contain alkaloids, flavonoids, saponins, tannins and cardiac glycosides (Bassey, 2012). The extract was observed to maintain the functional and structural integrity of the rat kidney may be due the presence of phytochemical constituents and it was observed not to be toxic at lower dose but showed tendency to predispose to kidney toxicity at higher dose.It can be concluded that the extract was fairly non-toxic at low dose. Moderate doses up to 175 mg/kg body weight appeared to be safe. Therefore, *Tapinanthus globiferus* should be used with caution, bearing in mind that higher dose could induce kidney damage.

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AUTHOR'S CONTRIBUTION

This work was carried out in collaboration between all authors. All the authors played significant roles and no conflict of interest is declared.