Measurement of Kidney Function of Wistar Rats treated with Ethanol Leaf Extract of *Justicia schimperi* (Acanthaceae)

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ABSTRACT

Over the years, several plants have been reported to be of immense medicinal value in the treatment and management a number of disease conditions. In this study, the effect of ethanol extract of *Justicia schimperi* on kidney function of albino wistar rats was investigated. 30 matured male and female albino rats were used for the study. The rats were divided into five groups of six animals per group. Group 1 served as control and was administered with rat pellets and water *ad libitum*. Groups II, III, IV, and V were treated with 273.8mg/kg, 547.7mg/kg, 821.5mg/kg, and 1095.4mg/kg bw of ethanolic extract of *Justicia schimperi* respectively for 14 days. The rats were sacrificed on the 15th day after 12 hours of fasting and serum obtained and analyzed for the level of creatinine, urea, sodium, bicarbonate, potassium and chloride. The results revealed that groups 3, 4 and 5 showed significant ($p< 0.05$) increases in all the parameters assayed when compared to control except sodium level for group 4. Ethanol leaf extract of *Justicia schimperi* in the present study appear to increase the levels of electrolytes, creatinine and urea. Therefore care must be applied in consumption of the leaf.

Keywords: *Justicia schimperi* , Kidney function, Electrolytes, Albino Wistar Rats.

INTRDUCTION

Plant derived substances have recently become of great interest owing to supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.,* 2008). Medicinal plants are the most important life saving drugs for the majority of the world's population (Tripathi, 2003). The World Health Organization (WHO) estimates that up to 80% of people still rely on traditional remedies such as herbs for their medicine.

However, traditional use of any plant for medicinal purposes warrants the safety of such plant (Ashafa et al., 2009). *Justicia schimperi* belongs to the Acanthaceae family, which belongs to the order Scrophulariales. Acanthaceae comprises almost 250 genera with 2500 species. Its species including Justicia *schimperi* are widespread in tropical regions of the world (Wasshausen and Wood, 2004), and are poorly represented in temperate regions (Mabberley, 1997).

Justicia schimperi is locally referred to as mmeme (Ibibio) and kpahunmarogu (Igbo) Adams, 2007). *Justicia schimperi* is an herbaceous and perennial plant of 30-75cm high, its leaves are simple, opposite and the flowers are pink or purple (Berhant, 2005), the leaves are used to make vegetable soup or eaten cooked as spinach. In Western Cameroon, it is added to groundnut soup, while extracts of the boiled leaves are given to babies to loosen their bowels and the leaves are applied to wounds to promote healing in Togo land and North East Ghana (Hepper, 2008).

Ethanol extract of the leaf has been shown to posses antioxidant effect (Blois, 2009). Studies undertaken revealed the presence of flavonoids, alkaloids and glycosides in their leaves. Aqueous extract of *Justicia schimperi* has been proven in series of studies to induce ovarian steroidogenesis and folliculogenesis in female rats (Telefo *et al*., 2004). But up to date, no information is documented in the literature on the effect of *Justicia schimperi* on kidney function. Thus, this study was carried out to assess the effect of ethanolic extract of this leaf on kidney function

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MATERIAL AND METHODS

Sample Collection and Preparation

Leaves of *Justicia schimperi* commonly called "mmeme" by the Ibibio people of Akwa Ibom State, Nigeria, were collected from Uyo-metropolis, Akwa Ibom State, Nigeria and identified by Dr (Mrs) Margaret Bassey of the Department of Botany and Ecological studies of the University of Uyo. A voucher specimen was deposited at Department of Botany herbarium, University of Uyo.

Fresh leaves of *Justicia schimperi* were properly washed with distilled water to remove accumulated dirt and were further air dried for two (2) weeks at room temperature. The sample was ground to powder using a grinder. Absolute ethanol (99.7%) was used for the extraction of the ground leaves sample. The filtrate was then placed in a water bath and was slowly evaporated to dryness at 50˚C for 72 hours. The dried extract obtained was stored in an air- tight covered container to prevent contamination and placed in a refrigerator at 4˚C.

Determination of LD_{50}

LD_{50} of the ethanolic leaf extract of *Justicia schimperi* was determined according to Lorke's method 1983 which is the square root of the product of the maximum dose of the extract that does not cause mortality and minimum dose that cause mortality.

Preparation of Sample Stock

3g of the extract was dissolved in 10ml of distilled water to make a concentration of 300mg/ml. the stock solution was prepared daily throughout the period of administration. The extract was administered orally to the experimental rats with their respective dosages based on their body weight.

Experimental Animals

The research involved the use of 30 adult albino Wistar rats with weight ranging between 160g and 210g which were divided into 5 groups. The animals were obtained from the College of Health Science Animal House, University of Uyo. The animals were fed with rat pellets and clean drinking water *ad libitum*. The rats were housed in standard cages under laboratory conditions. They were allowed to acclimatize for 14 days before commencement of administration.

Experimental Design

Group I: Normal control group, received rat pellets and water only.

Group II: Received 272.8 mg/kg of the extract

Group III: Received 541.7 mg/kg of the extract

Group IV: Received 821.5 mg/kg of the extract

Group V: Received 1095.4 mg/kg of the extract

The doses were chosen based on the result of the LD_{50} obtained and different percentages of the LD50 (10%, 20%, 30% and 40% for groups 2, 3, 4 and 5 respectively) were administered to the treatment groups. The rats were fasted twenty four hours after the last administration and then sacrificed under chloroform anaesthesia. Blood samples were collected through cardiac puncture using sterile needles and syringes into labeled plain sample bottles and allowed to stand for two (2) hours and thereafter centrifuged at 2000g for ten minutes to obtain the serum. The serum obtained was preserved and used for biochemical assay.

Biochemical Assay

Serum concentration of potassium, sodium and chloride were estimated according to the method of Tietz, (1976). Serum concentration of bicarbonate was estimated according to the method Young (1990). Serum concentration of creatinine was estimated according to the method of Henry (1974). Serum concentration of urea was estimated according to the method Wybenga *et al*., (1971).

Statistcal Analysis

The results were expressed as mean $\pm$ standard error of mean (SEM). The statistical evaluation of data was performed using SPSS 7.5 for Windows. Student t-test and ANOVA were carried out on the data with value of $P < 0.05$ accepted as significant.

RESULTS

Acute Toxicity Study Result

After the last treatment, the animals were sacrificed and the results of the highest dose that caused no mortality and the lowest dose that caused mortality were used to calculate the LD_{50} which was 2738.6mg/kg bw.

Effect of Ethanol Extract of *Justicia schimperi* on Kidney Function of Albino Wistar Rats

The results of the study on the effect of ethanol extract of *Justicia schimperi* on kidney function of albino wistar rats are represented below.

Table 1: Effect of Ethanol Extract of *Justicia schimperi* on Kidney Function

Groups	Creatinine	Urea	Bicarbonate	Sodium	Potassium	Chloride
Group 1	0.39 ± 0.68	14.00 ± 0.68	23.30 ± 1.47	144.50± 1.73	3.92 ± 0.05	99.00 ± 0.86
Group 2	0.44 ± 0.04	14.30 ± 0.76	26.80 ± 0.95	142.80± 1.25	3.88 ± 0.03	98.83 ± 1.51
Group 3	0.53 ± 0.03*	16.17 ± 0.79*	31.80 ± 1.68*	148.30± 1.69*	5.09 ± 0.11*	101.00 ± 1.61*
Group 4	0.56 ± 0.02*	19.70 ± 0.49*	30.20 ± 1.30*	143.20± 2.55	4.51 ± 0.14*	102.20 ± 1.82*
Group 5	0.52 ± 0.03*	18.70 ± 0.76*	31.50 ± 0.76*	147.70± 1.33*	4.17 ± 0.07*	104.50 ± 1.06*

Data expressed as mean ± SEM at $p < 0.05$, n = 6, * Significantly different at $P < 0.05$ when compared to control.

Results obtained showed that there were no significant differences in all assayed parameters when group 2 was compared with group 1 (control). Groups 3, 4 and 5 showed significant ($p< 0.05$) increases in all the parameters assayed compared to control with the exception of sodium level of group 4.

DISCUSSION

The basic activities of the kidneys that are measurable by chemical means are glomerular filtration, tubular reabsorption excretion and renal blood flow (Methfessel, 2000). When glomeruli or tubules are damaged, they are not replaced. The ability of the glomeruli to filter soluble constituents from the blood can be measured by clearance tests. Two clearance tests generally employed are those for urea and creatinine (Sterens and Levey, 2005). Urea and creatinine are the most abundant end product of protein metabolism. They are excreted from the body by kidneys; the clearance of these waste products is a measure of function, principally the effectiveness of glomerular filtration.

Elevated levels of creatinine and urea observed in rats treated with higher doses of the extract may not be totally attributed to kidney problem which is noticed in blocked urinary tract, kidney damage, failure or infection (Rose, 2011). Increased body weight and high protein intake may also play a role in elevated levels of creatinine and urea observed in this study.

It has been reported in literature that *Justicia schimperi* contains 22.95% protein (Blois, 2009). Thus, the increased serum concentration of urea and creatinine which are the major end products of protein breakdown may be attributed to high concentration of protein in the leaf extract. It has also been established that high intake of protein increases serum creatinine and urea concentration (Blois, 2009). However, this does not indicate kidney dysfunction.

Electrolytes also served as measure or biomarkers of renal function, since they are filtered and reabsorbed by the kidney. Electrolytes are regulated by hormones such as anti duiretic hormone, aldosterone and parathyroid hormone with the kidney flushing out excess levels. Serum level of creatinine, urea and electrolytes provide a valuable tool for clinical diagnosis of kidney damage.

The result of the present study indicates increase in serum concentrations of potassium, chloride, bicarbonate and sodium at high doses of the extract. This shows the ability of *Justicia schimperi* in improving the effectiveness of tubular reabsorption. The increase in potassium and bicarbonate ions clearly show a greater improvement of the activity of intercalated cells of the kidneys which reabsorb bicarbonate (HCO_3^-) and potassium in exchange for the secretion of hydrogen ion (Blois, 2009). Also the increase in HCO_3^- concentration demonstrates that *Justicia schimperi* induces buffering capacity of the plasma. When hydrogen ion is secreted in excess of the HCO_3^- filtered into the tubular fluid, only a small part of the excess H^+ can be excreted in the ionic form (H^+) in the urine (Brown, 2014). When there is excess H^+ in the urine, it combines with buffers other than HCO_3^- and this leads to generation of new HCO_3^- (Blois, 2009) that can enter the blood. Thus, when there is excess H^+ in the extracellular fluid, the

kidneys not only reabsorb all filtered HCO_3^- but also generate new HCO_3^-, thereby helping to replenish the HCO_3^- lost in the extracellular fluid in acidosis. The increase in sodium and chloride concentrations at higher doses indicate improvement in the activity of the renal tubules (especially proximal tubules) in which reabsorption of sodium occurs. The increase in serum chloride is in line with increase in sodium concentration. Therefore, the consistency and stability of serum electrolytes is an indication of the effectiveness of the activity of renal tubules in reabsorption and secretion.

Ethanol leaf extract of *Justicia schimperi* in the present study appears to increase the levels of electrolytes, creatinine and urea. This may be attributed to high content of protein and other bioactive substances present in this leaf as indicated in previous studies. These increased levels of assayed parameters at higher doses are also suggestive of the inability of the kidney to perform its function and hence indicates a compromise of kidney integrity. This may suggest mild toxicity of the extract as the LD_{50} obtained (2738.6mg/kg bw.) was a beat low knowing that plant extracts with LD_{50} above 5000mg/kg is relatively non toxic (Lorke, 1983). Caution should therefore be taken in its consumption as further studies need to be done to ascertain its toxicity.

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