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RESEARCH ARTICLE

PRESERVED RENAL CYTO-ARCHITECTURE AND ELECTROLYTE BALANCE IN WISTAR RATS TREATED WITH GONGRONEMA LATIFOLIUM

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ABSTRACT

Many herbal concoctions are criticized for causing electrolyte imbalance and kidney damage. This research understudied the kidney handling of two fractions of *Gongronema latifolium,* a leaf with reputable medical reports on serum electrolytes and kidney architecture of normal Wistar rats. Eighten (18) male Wistar rats were divided into three groups of six rats each. Group 1, the control group, was administered 0.9% normal saline placebo. Group 2 was administered 200 mg/kg ethanol extract of *Gongronema Latifolium.* Group 3 received 200 mg/kg ethyl acetate fraction of same extract. Administration was via oral gavage and lasted for 14 days. The rats were sacrificed and blood samples collected into plain bottles, and centrifuged for serum. Laboratory tests were done for serum concentrations of electrolytes. The kidneys were also harvested for organ weight and histological studies. Result shows that both extracts decreased ($p<0.01$) serum levels of sodium and chloride ions, and increased ($p<0.01$) potassium and bicarbonate ions, all within normal values. There was no significant change in the weight of kidneys across the groups. Histology of the kidneys showed no derangement in their cellular architecture following administration of extracts. This therefore implies that *Gongronema latifolium* does not result in electrolyte imbalance, and does not impact negatively on the kidney.

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INTRODUCTION

Essential pharmaceutical compounds like quinine, artemisinin, salicylate, digitalis, and a lot others are of botanical origin[1]. Till date, research into alternative medicine continues and either whole plants or plant parts are still employed in pharmaceutical industries as raw materials and forms integral part of modern medicine[2]. This follows a global increase in the use of herbal medicines in both developed and developing countries, leaving a global turnover of about $US60 billion, representing 20% of the overall drug market[3]. This drift comes with an economic burden, one of which is lack of standardization of these herbal medicines and supplement. They lack consistent prerequisites for safety, efficacy and purity, and thus often result in irreparable damage to some vital organs, typically the kidney and liver[4]. The kidney is the route of excretion of most active substances present in herbs. It is thus predisposed to varying damages like acute kidney injury, chronic kidney diseases, tubular function defects, renal papillary necrosis, and altered electrolytes levels in blood[5,6,7]. Evidence abound that some medicinal plants alter serum electrolytes concentration, with consequent kidney damage and mortality.

Examples of such plants are Liquorice root (*Glycyrrhiza glabra*), *Senna*, *Rheum* officinale[8,9], *Morinda citrifolia*, *Taraxacum officinale*, *Urtica dioica*, Star fruit and *Medicago sativa*[10]. To avoid adverse effects resulting from treatment with plant extracts, evaluation of serum electrolytes and kidney cytoarchitecture following administration of plant extracts is imperative. *Gongronema latifolium* Benth. (family *Apocynaceae*) is a tropical plant with proven medicinal properties in reducing oxidative stress and lipid peroxidation[11,12]. It is widely explored in the traditional folk medicine for various health conditions including hypertension, diabetes, diarrhea, ulcer and dyspepsia[13,14]. It also possesses antiulcer activity[15] and increases white blood cells count and hemoglobin concentration[16]. The plant has anti-inflammatory, anti-malarial, and anti-microbial activities[13,17,18]. *G. latifoilium* is reported to have a cardioprotective and hypotensive potential[19,20]. Phytochemicals present in the leaves are alkaloids, saponins, flavonoids, tannins, glycosides polyphenols and reducing sugars[20]. Given the hypotensive effect of G. latifolium described by our group, and considering the popular use of the plant for its widely acclaimed medicinal properties, study on effect of this plant on serum electrolytes and kidney cytoarchitecture is very limited. The present study aims at evaluating the effect of G. latifolium on some serum electrolytes and the cyto-architecture of the kidney.

MATERIALS AND METHODS

Experimental animals/ design of the study: Eighteen (18) male Wistar rats (200- 250 g, n=18) were used for the study. They were divided into 3 groups of 6 rats each. Group 1 served as the normal control placed on normal saline placebo. Group 2 was administered 200 mg/kg of ethanol extract, group 3 received 200 mg/kg ethyl acetate fraction. Administration was via oral gavage, and lasted for 14 days. The animals were kept in plastic cages and controlled environment (12h light/dark cycles at 27 ± 2°C) one week for acclimatization before commencement of the study. The rats had free access to normal rat chow and tap water *ad libitum*. Approval for the study was obtained from the Faculty of Basic Medical sciences Animal Research Ethics Committee, University of Calabar with Approval No: 019PY20317.

Plant material and extraction: *Gongronema latifolium* leaves were bought from a local market in Calabar, Nigeria. A voucher specimen was identified at the herbarium unit of the Department of Botany and deposited (GLB 4612) in the herbarium of the University of Calabar, Calabar, Nigeria. The ethanol extract of the leaves were prepared as previously described[20]. Briefly, the leaves were washed with clean water, air-dried and ground into coarse powder. The powder (1.65 kg) was suspended in 2 litres of ethanol (BDH Ltd Poole, England) for 24 hours at room temperature. The suspension was thereafter filtered with Whatman No. I filter paper and evaporated by hot air oven at 40-45°C to a thick dark gummy crude extract giving a yield of 66 g (4.8%). For ethyl acetate fraction, 5g of the ethanol extract was inserted into a mortar with warm distilled water, then stirred to form a suspension, and transferred into a separating funnel. Ethyl acetate (200 ml) was added to the suspension and shaken to form two layers. The ethyl acetate soluble fraction was filtered over Whatmann No. 1 filter paper. The filtrate was also evaporated following same procedure for the ethanol extract. The extracts were refrigerated at -4°C until required for use.

Estimation of serum electrolytes: At the end of the feeding period, the rats were fasted overnight, and then sacrificed under chloroform anaesthesia. Blood was collected via cardiac puncture into sterile plain tubes, and allowed to clot at room temperature over one hour period. The blood was centrifuged at 4000rpm for 10 minutes and serum was collected into clean sample bottles for serum electrolytes estimation. Potentiometric procedure[21] was used to determine serum sodium and enzymatic method[22] was used to determine potassium concentrations. Serum chloride concentration was determined using standard method[23] while serum bicarbonate concentration was by the enzymatic method[24].

Histological studies: After sacrifice, the kidneys were harvested, weighed using a digital weighing balance and washed in normal saline, and fixed in 10% buffered formalin. The organs were later transferred to 250mls of 70% alcohol, 95% alcohol, and absolute alcohol to dehydrate the tissues and processed following routine procedure. The fixed specimens were sliced, processed, and embedded into paraffin blocks. The blocks were cut into 5 µm paraffin sections by a rotator microtome. The sections were stained with Hematoxylin and Eosin (H&E). The sections were then viewed under the light microscope (ECLIPSE E400, and model 115, Japan). Photomicrographs were performed using Olympus digital camera (DP20) fitted in the microscope.

Statistical Analysis: The results are presented as mean ± standard error of the mean. Data were analyzed using GraphPad prism software version 6.00 for Windows (GraphPad Software, San Diego, CA, USA). One-way analysis of variance with Tukey's post-test was performed, and probability level of $p<0.05$ was considered statistically significant.

RESULTS

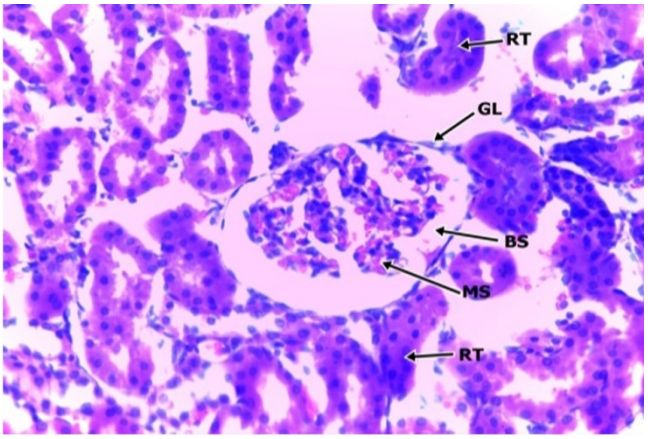
Effect of *Gongronema latifolium* extracts on serum sodium concentrations: The serum sodium ion concentration in the control, ethanol extract, and ethyl acetate fraction groups was 139 ± 0.70mmol/L, 134 ± 0.8mmol/L, and 136 ± 0.9mmol/L respectively. The result showed a decrease ($p<0.01$) in levels of sodium ion concentrations in both ethanol and ethyl acetate fraction of *GL* treated groups compared with the control. This is presented in table 1.

Effect of Gongronema latifolium leaves extracts on serum chloride concentrations: The serum concentration of chloride in the control, ethanol extract, and ethyl acetate fraction groups was 99.0 ± 2.12mmol/L, 95.0 ± 1.32mmol/L, and 96.0 ± 0.53mmol/L respectively. The result showed a reduction ($p<0.01$) in the serum chloride concentrations in the extract treated groups compared with the control. This is presented in table 1.

Effect of *Gongronema latifolium* extracts on serum potassium concentrations: The serum concentration of potassium ion in the control, ethanol extract, and ethyl acetate fraction groups was 4.10 ± 0.06mmol/L, 4.61 ± 0.11mmol/L, and 4.41 ± 0.12mmol/L respectively. The result showed increase ($p<0.01$) levels of potassium ion in the extract treated groups when compared with the control. This is presented in table 1.

Effect of ethanol extract and ethyl acetate fraction of *GL* on serum bicarbonate concentrations: The serum bicarbonate concentration in the control, ethanol extract, and ethyl acetate fraction groups were 20.0 ± 0.20mmol/L, 23.00 ± 0.35mmol/L, and 22.01 ± 0.33mmol/L respectively. The result showed increase ($p<0.01$) levels of bicarbonate in the extract treated groups when compared with the control. This is presented in table 1.

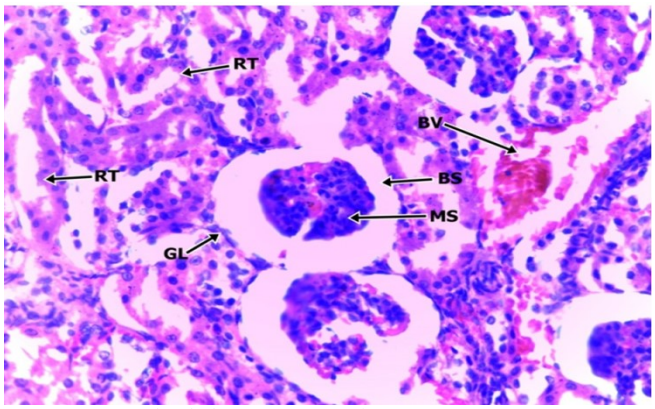
Effect of ethanol extract and ethyl acetate fraction of *GL* on absolute kidney weight: The weight of the kidneys across the groups was 0.0063 ± 0.0001g, 0.0065 ± 0.0003g and 0.0064 ± 0.0001g respectively. Result showed no statistical difference in weights of the organs across the groups. This is presented in table 1.



RT=Renal Tubules, MS= Mesangium, BS=Bowman Space,
GL= Glomeruli, BV= Blood Vessel

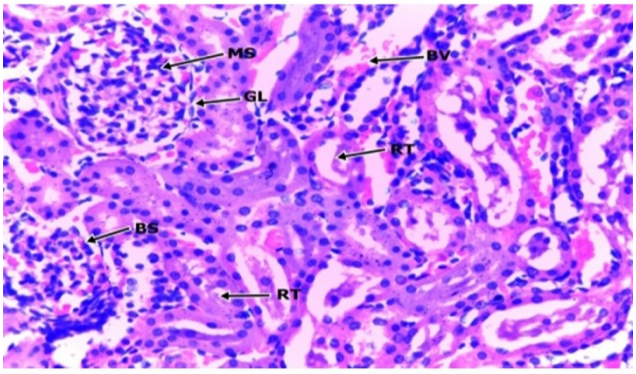
Plate 1. Photomicrographs of a cross section of the kidney in the control. *X400*

Photomicrographs of the kidney in control, ethanol extract and ethyl acetate fraction of *GL* treated rats: Plates 1, 2, and 3 present the photomicrograph of the kidney in the control, ethanol extract, and ethyl acetate fraction groups respectively. The photomicrographs in all the groups show normal histological structure of the kidneys. The glomeruli have intact Bowman's spaces lined by flattened epithelial cells, and an acellular mesangium composed of mesangial cells and arterioles. The renal tubules are also intact with prominent lumen. Their basement membranes are lined by columnar to cuboidal epithelium.



RT=Renal Tubules, MS= Mesangium, BS=Bowman Space, GL= Glomeruli, BV= Blood Vessel

Plate 2. Photomicrographs of a cross section of the kidney in the ethanol extract of *GL.x400*



RT=Renal Tubules, MS= Mesangium, BS=Bowman Space, GL= Glomeruli, BV= Blood Vessel

Plate 3. Photomicrographs of a cross section of the kidney in the ethyl acetate fraction of *GL. x400*

The intervening interstitium has congested blood vessels. There is no difference in the histological architecture of the kidneys in the extract treated groups and the control group.

Table 1. Effect of ethanol extract and ethyl acetate fraction of *GL* on some serum electrolytes and kidney weight

Parameters	Control	Ethanol Extract	Ethyl Acetate Fraction
Na^+ (mmol/L)	139 ± 0.70	134 ± 0.8**	136 ± 0.9*
Cl^-(mmol/L)	99.0 ± 2.12	95.0 ± 1.32*	96.0 ± 0.53*
K^+ (mmol/L)	4.10 ± 0.06	4.61 ± 0.11**	4.41 ± 0.12*
HCO^-_3 (mmol/L)	20.0 ± 0.20	23.00 ± 0.35**	22.01 ± 0.33**
Kidney Weight (g/g)	0.0063 ± 0.0001	0.0065 ± 0.0003^{ns}	• ±0.0001^{ns}

* = p<0.05; ** = p<0.01; ns = not significant, all compared with the control.

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