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Full Length Research Paper

Study of the renal function of Wistar albino rat treated with three different herbal bitters (Confam, G. Winco and 1960 Roots)

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This study evaluates the renal function of Wistar albino rats treated with three different herbal bitters (confam, G.Winco and 1960 roots). A total of 40 rats were randomly divided into 4 groups labeled A, B, C and D and kept in a well-ventilated room. Group A served as control and these rats were treated with distilled water. Rats in groups B, C, and D were treated with 3 different doses of the bitters (20, 30 and 40 mL/Kgbw) respectively. The drugs were administered once daily for 10 and 21days consecutively. Animals were sacrificed 24 h after the last treatment. Blood samples were collected into heparinized sample bottles for analysis. There was no significant difference in the serum urea concentration of the results obtained. Histology results indicated normal kidney tissue architecture, with the exception of G. Winco herbal bitters at a dose of 40 ml/kg at both 10 and 21 days of administration respectively which showed distorted kidney tissue. This study had demonstrated that the three herbal bitters have minimal negative side effects on kidney function of the experimental Wistar albino rats when used at moderate dose concentrations.

Keywords: Renal function, Wistar albino rats, histology, herbal bitters, distorted kidney

INTRODUCTION

From time immemorial, herbal remedies have been used to treat diverse conditions with relative success before the advent of modern medicines. This practice gradually waned with the development of synthetic drugs; however, there has been resurgence in the use of herbal medicines all over the world including sub- Saharan Africa (Oreagba et al., 2011). Self-treating illnesses with herbal medicine is encouraged by belief in local complementary and alternative medicines (CAM) such as massage, meditation and traditional medicines like “Agbo” and herbal bitters (HB) in Nigeria (Osamor and Owumi, 2010). Eighty percent of people in developing countries rely on herbal medicines to treat different ailments (McCrea and Pritchard, 2011).

Herbal medicines are readily available everywhere; the increase in their use, either alone or in combination with modern medicines is augmented by the belief that all herbal medicines are safe (Falodun, 2010). Although, unlicensed in many countries, in Nigeria, the National Agency for Food Drug Administration and Control (NAFDAC) recognizes these products and approves them for sales (Oguntade and Oluwalana, 2011). Herbal bitters are usually poly-herbal liquid preparations which contain bitter herbs. There are other dosage forms, like capsules, tablets and tinctures which have been labeled by their manufacturers as bitters. They are commonly used to induce flatulence, stimulate appetite and to improve digestion.

In Nigeria today, there is a preponderance of these products claiming to meet majority of the health need of the populace. The kidney plays a major role in the metabolism and excretion of substances and this makes it vulnerable to various types of injuries from some of these agents (Oforibika et al., 2016). For this reason, this paper focuses on the effects of three herbal bitters on the urea and kidney histology of some albino Wistar rats exposed to different concentrations for different lengths of time.

MATERIALS AND METHODS

Confam, G. Winco and 1960 roots bitter used in this study was gotten from Mile 3 market, Diobu Port Harcourt. Specimen (animal) used for the experiment: forty(40) albino rats were purchased from animal house of the Department of Biochemistry, University of Port Harcourt, Choba Park.

The animals were fed with rat pellets, water and libitum as recommended by Oforibika *et al.* (2016). Chemicals and reagents: all chemicals and reagents used in this study were obtained from Randox Laboratories UK. Preparation of Drug solution for administration: 20 ml/kg, 30 ml/kg and 40 ml/kg of the preparation was given to the rats each day after weighing depending on their respective groups.

Experimental procedure: a total forty (40) albino rats of weight range (124-194g/BW) were randomly divided into four groups labeled A, B, C, D and E where group A served as control and rats (n=2rats/dose) were treated with distilled water. Rats in groups B, C and D (n = 2 rats/dose) were orally treated with 3 different doses of Confam (20, 30 and 40ml/kgBW) , G. Winco (20, 30 and 40 ml/kgBW) and 1960 (20, 30 and 40 ml/kgBW) roots for 10 and 21 days respectively. Animals were sacrificed twenty four (24) hours after last treatment.

Collection of blood and preparation of serum

The rats were withdrawn from the cages in each of the group twenty four (24) hours after the last administration of the drugs for 10 and 21 days and placed in a desiccator containing cotton wool soaked in chloroform to anaesthetize the rats using the method of Aniago *et al.* (2005).

The blood samples were obtained by cutting the jugular vein of the rat on the neck by means of surgical blade and put in anticoagulant sample bottles smeared with lithium-heparin and fluoride oxalate. The blood samples were spun at 5000 rpm using MSE Centrifuge to obtain plasma. The animal was dissected and only the liver was collected for pathological studies.

Measurement of urea

Ten (10) ul of distilled water was dispensed into an empty test tube while another test tube contains the standard solution with the third test tubes for the samples. Then 100 ul of reagent I solution was added into the three test tubes, mix immediately and incubate at 37°C for 10 min. 250 ml of reagent two was then added to the three test tubes, mixed thoroughly and incubated in a water bath at 37°C for 10 min. Absorbance of the sample and standard were measured against the blank at 546 nm using a spectrophotometer.

Urea concentration = A Sample x (0.467 mg) standard concentration

Histological procedures and analysis

The kidney was cut on slabs about 0.5cm thick and fixed in 10 percent normal saline for a day after which they were transferred to 70 percent alcohol for dehydration. The tissues were passed through 90 percent alcohol and chloroform for different durations before they were eventually transferred into two changes of molten paraffin wax for 20 min each in an oven at 57 percent. Several sections of the 5 µm thick were obtained from a solid block of tissue and were stained with hematoxylin and eosin staining after which they were passed through a mixture of equal concentration of xylene and alcohols, following clearance of xylene, the tissues were oven dried. Photomicrographs were taken with a JVC colour video digital camera (JVC China) mounted on an Olympus light microscope (Olympus UK Ltd Essex, UK) to demonstrate cytoarchitecture of the kidney.

RESULTS AND DISCUSSION

The results in Figure 1 showed that there was no significant difference in serum urea concentration for all three bitters irrespective of dose and duration. The exception to this was 40ml/kg G. Winco at 10 days of administration which showed a drastic reduction. Histology also showed normal kidney tissues with the exception of 40 ml/kg G. Winco at 21 days of administration which showed distorted kidney tissue. This result shows that these bitters don't interfere with the excretory capacity of the rats when taken in moderate amounts. This is same as what was reported by Aniagu *et al.* (2005) and Anionye *et al.* (2017). They reported no significant difference in the urea levels of the treated rats and the control rats. The findings corroborated that of Showande and Amokeodo, (2014) who evaluated the extent and pattern of use of herbal bitters among students in tertiary institutions in southwestern Nigeria with similar results. A limitation in this work is that the serum creatinine and electrolyte levels were not accessed. The photomicrograph of kidney tissue treated with distilled water showed normal tissue having glomeruli with tuffs made up of glomerular cells, mesangial matrix and glomerular capillaries (Slide 1). Glomeruli are surrounded by bowman's capsular spaces. Renal tubules are lined by simple epithelial cells (control). Slides 2-10 show the results for 10 days of administration. The photomicrograph of kidney tissue treated with confam 20 ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 2). The photomicrograph of kidney tissue treated with confam 30

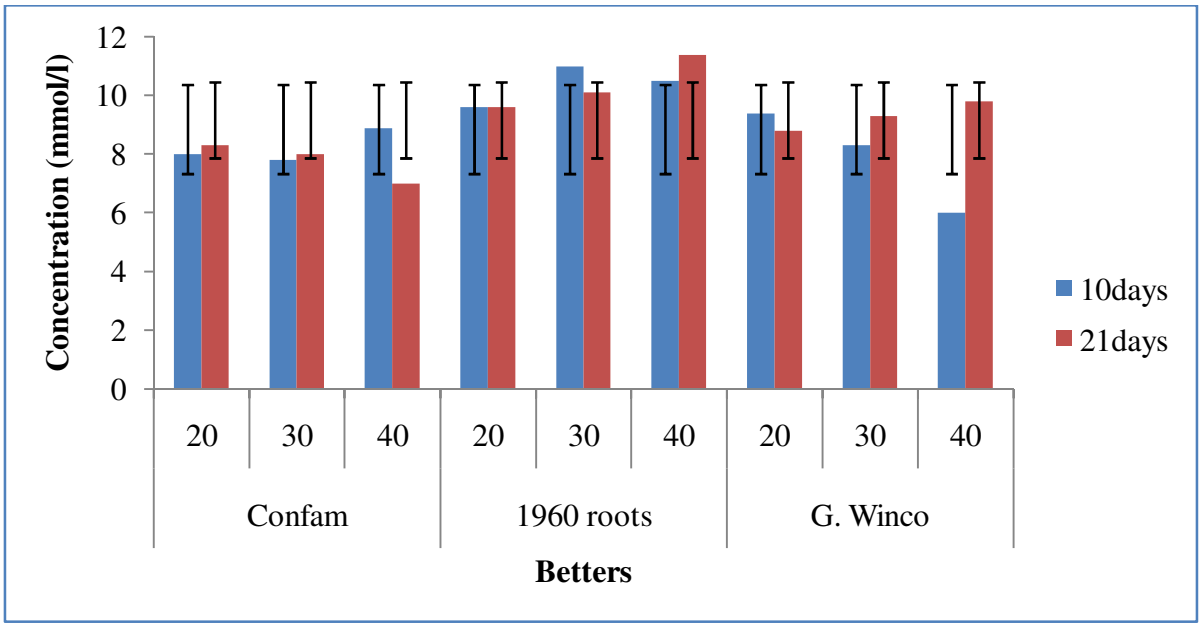
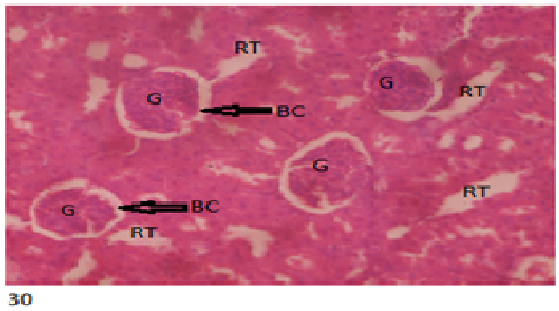
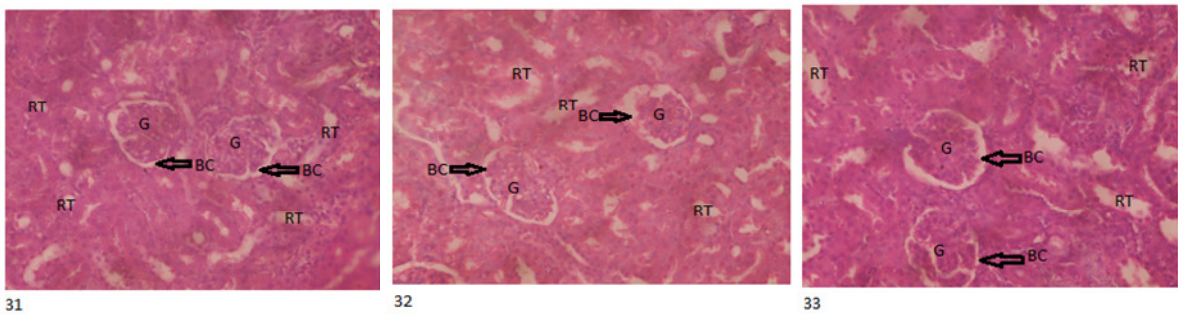


Figure 1. Effect of three local bitters (Confam, 1960 roots and G. Winco) on urea.



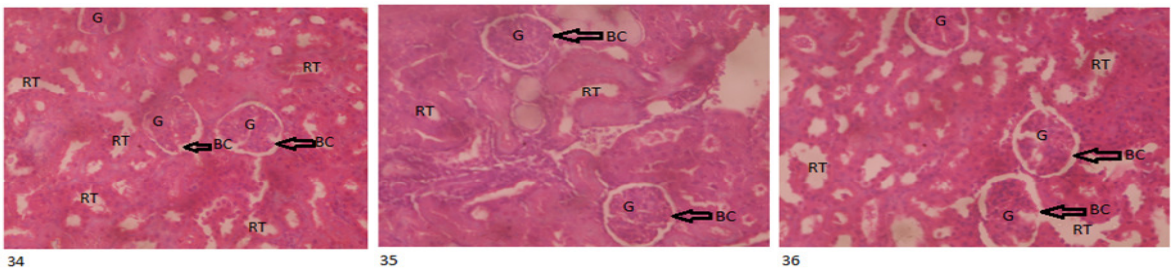
Slide 1. Control



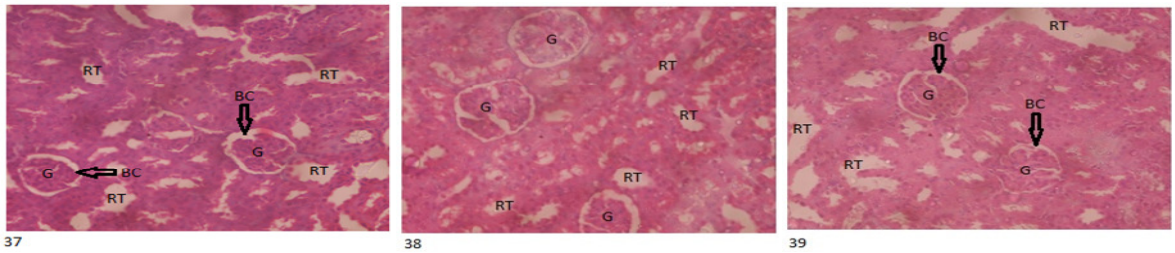
Slides 2, 3 and 4 L-R

ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 3).

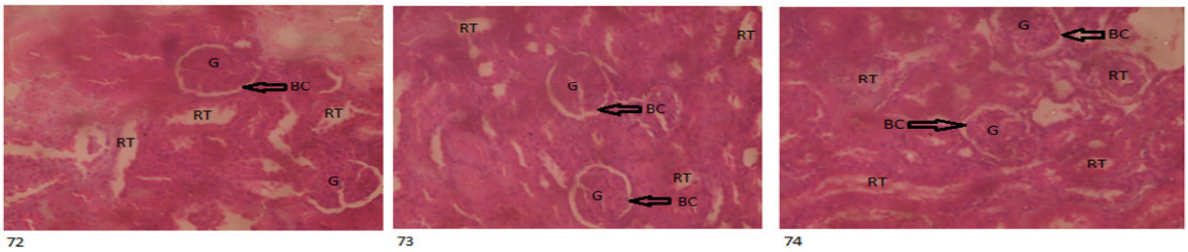
The photomicrograph of kidney tissue treated with confam 40 ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 4). The photomicrograph of kidney tissue treated with 1960 roots 20 ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 5). The photomicrograph of kidney tissue treated with 1960 roots 30 ml/kg showed



Slides 5, 6 and 7 L-R



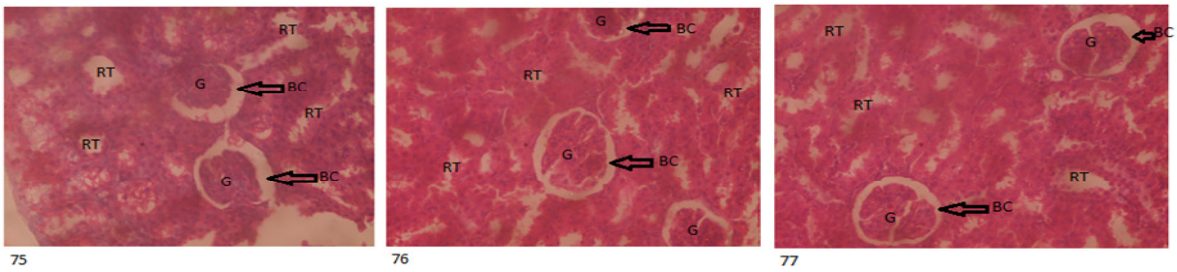
Slides 8, 9 and 10 L-R



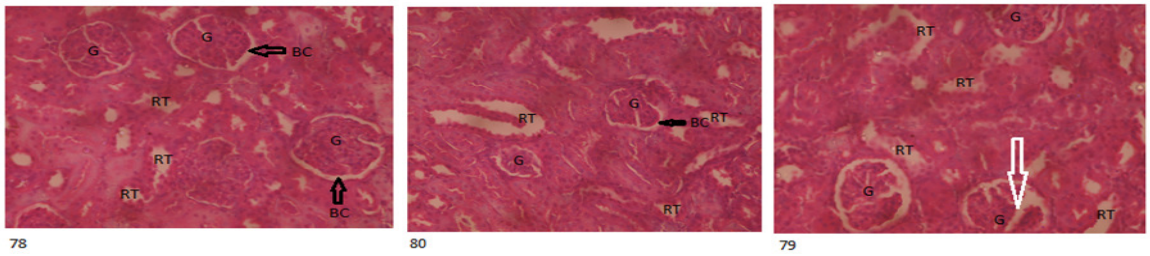
Slides 11, 12 and 13 L-R

histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 6). The photomicrograph of kidney tissue treated with 1960 roots 40 ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slides 2-7).

The photomicrograph of kidney tissue treated with G. Winco 20 ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 8). The photomicrograph of kidney tissue treated with G. Winco 30 ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 9). The photomicrograph of kidney tissue treated with G. Winco 40 ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slides 8-10). The results for 21 days of administration are presented in (slides 11-19). The photomicrograph of kidney tissue treated with confam 20 ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 11). The photomicrograph of kidney tissue treated with confam 30 ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 12). The photomicrograph of kidney tissue treated with confam 40ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slides 11-13). The photomicrograph of kidney tissue treated with 1960 roots 20 ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 14). The photomicrograph of kidney tissue treated with 1960 roots 30ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 15). The photomicrograph of kidney tissue treated with 1960 roots 40ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's



Slides 14, 15 and 16 L-R



Slides 17, 18 and 19 L-R

capsular spaces (Slides 14-16). The photomicrograph of kidney tissue treated with G. Winco 20 ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 17). The photomicrograph of kidney tissue treated with G. Winco 30ml/kg showed histologically normal kidney tissue with normal glomeruli, renal tubules and patent bowman's capsular spaces (Slide 18). The photomicrograph of kidney tissue treated with G. Winco 30ml/kg showed histologically distorted kidney tissue with partitioned glomerula tuft, renal tubules and patent bowman's capsular spaces (Slides 17-19).

Conclusion

This study demonstrates that herbal bitters have little negative effect on kidney function when used at moderate amounts. Within the limits of experimental error, the researcher discovered that, at the dosage of the bitters used, there was little evidence of kidney damage at high dosages and longer duration of consumption. There was no significant difference in the serum urea concentration of the results obtained. Histology results indicated normal kidney tissue architecture, with the exception of G. Winco herbal bitters at a dose of 40ml/kg at both 10 and 21 days of administration which showed distorted kidney tissue.

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Authors' declaration

We declared that this study is an original research by our research team and we agree to publish it in the journal.

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