

Full Length Research Paper

Effects of Leech Salivary Extract (Lse) on Indices of Kidney Function in Rats, For Safe Application in Clinical Medicine

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Abstract

Sub-chronic toxicity test of LSE was carried out by oral administration of 25, 50 and 100mg/kgbw to healthy wister rats (*Rattus norvegicus*) for 28 days, with appropriate immune suppressant (Dexamethasone) and immune stimulant (Jobelyn) and negative controls. Blood samples were collected from the rats and analysed for metabolite parameters including, Sodium (Na), Chloride (Cl), Potassium (K), urea and Creatinine, following standard procedures. Histopathological examination of kidney harvested from the rats, post sacrifice, involved grossing and tissue processing. The results showed that sub-chronic tests even at high concentration (100mg/kgbw) did not cause mortality nor elicited behavioral aberrations in rats. LSE had no significant ($p>0.05$) effect on concentrations of Na (range = 115.60 ± 8.29 to 135.80 ± 3.24 mmol/L) and K (7.20 ± 0.20 to 8.48 ± 1.10 mmol/L). However, Chloride, Urea and Creatinine significantly ($p<0.05$) increased from control concentration of 82.40 ± 5.53 to 133.60 ± 6.40 mmol/L, 12.09 ± 1.41 to 25.5 ± 4.20 mmol/L and 2.25 ± 0.49 to 2.84 ± 0.72 mmol/L, respectively, at 50mg/kgbw of LSE, where significant peak concentrations were attained. The histopathological examination of kidney of rats showed a dose dependent moderate tubule-intestinal inflammation with congestion of the renal vessels. The findings of this study clearly indicated the potential physiological hazards inherent in LSE and should guide its refinement and applications in clinical conditions.

Keywords: Blood metabolites, Creatinine, Histopathology, Sub-chronic toxicity and wister rats.

INTRODUCTION

Kidney function parameters are valuable tools used in assessing the integrity of the various parts of the kidney (Singh *et al.*, 2011). The kidney functions in regulating the excretion of urea and the reabsorption of electrolytes into the blood (Mayne, 2005). The level of creatinine, electrolytes, urea and serum total protein provides

relevant information regarding the effects of xenobiotics on the tubular and glomerular region of the kidney (Yakubu and Musa, 2012).

An increase in the levels of creatinine and blood urea results in loss of renal function, also, referred to as kidney failure (Rodriguez, 2000). Creatinine is a breakdown product from a muscle: creatine used during muscle contraction. Serum creatinine is an important indicator of renal ill-health since; it is a measured byproduct of muscle metabolism that is excreted exclusively by the

kidney (Rodriguez, 2000). This invariably means that once the kidney is damaged, there will be inefficient excretion of creatinine thereby causing accumulation in the blood. High level of serum creatinine is an indication of kidney damage (Ryan, 2009). Urea is the final degradation product of protein and amino acid metabolism. The ammonia formed from this degradation process is converted to urea in the liver. Urea is the detoxification product of ammonia from the deamination of amino acids. Thus, urea is the end-product of protein catabolism. Urea is released into the blood and carried to the kidney where it is filtered out of the blood and released into the urine.

Blood urea is usually measured as levels of blood urea nitrogen (BUN) which is a poor index of renal function (Rodriguez, 2000). Electrolytes such as sodium, potassium and chlorides are dissolved salts found in body tissues and blood. These salts acquire the ability to conduct electrical impulses in the body when they become ions in solution. They are essential for normal function of organs and cells and they serve as biomarkers for renal and cardiac function. The kidney, therefore, helps in maintaining proper levels by reabsorption or elimination of these electrolytes into the urine. Sodium is a major cation (positive ion) in fluid outside the cell; they are required electric signals for communication in many processes in the brain, muscles and nervous system.

These electric signals are generated from the movement of sodium ions. Sodium regulates the total amount of water in the body, thus excess sodium in relation to water results in increased blood sodium also known as hypernatremia which may be caused by reduced fluid intake or loss due to diarrhea. This suggests that inadequate water intake and dehydration can lead to high blood sodium level. Low level of sodium is usually due to excess fluid accumulation in the body, i.e., edema (Burtis *et al.*, 2011).

MATERIALS AND METHODS

Laboratory animals

Healthy Wister rats (*Rattus norvegicus*) of the same weight group (120-200g) were used in acute and sub-chronic investigations. The animals were obtained from Animal House, Department of Biochemistry, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University Lapai, Nigeria. They were housed in stainless steel cages bedded with dry clean wood shavings, maintained at a temperature of $25\pm 2^{\circ}\text{C}$ and observed under 12-hour light/dark cycle, in a well ventilated room, for 2 weeks before the commencement of the experiment. They were fed with standard animal feeds (Bendel feeds and flour mills, Edo State, Nigeria) and tap

water ad libitum. The cages were cleaned and disinfected regularly. Soiled wood shavings were replaced often. The feed, water containers were washed regularly. The animals were housed and cared for in accordance with Good Laboratory Practice (GLP) regulations of WHO (1998). The principles of Laboratory Animal Care (NIEHS, 1985) were also followed throughout the study.

Toxicological studies

The sub-chronic toxicity study of the LSE was conducted as described by Aniagu *et al.* (2005). The studies included the evaluation of the effects of LSE on plasma biochemical parameters.

Sub-chronic toxicity studies of active leech salivary extract

The method described by Aniagu *et al.*, (2005) was employed in the sub-chronic toxicity of the LSE. Thirty rats (30) were selected and divided into six (6) groups of five rats each. The first three groups were respectively given 25mg/kgbw, 50mg/kgbw and 100mg/kgbw of LSE orally for 28days. The 4th group served as immune suppressant group which was administered with Dexamethasone dose; the 5th as immune stimulant group administered with Jobelyn dose and the 6th as Control which was only fed with the standard water. The body weight of the rats was taken once before dosing commenced, once weekly during dosing and on sacrifice day. The effect of the LSE on plasma biochemical parameters (Electrolytes, Urea and Creatinine levels) were also determined using commercial kits obtained from Radox laboratory, UK. The kidney was removed from each rat and weighed (absolute organ weight). They were then observed macroscopically.

Collection of blood, serum and organs

The collection of blood, serum and organs were as described by Shittu *et al.*, (2015). At the end of the four weeks treatment period, the animals were starved but still had water ad libitum for 24hours before they were sacrificed under diethyl ether anesthesia. The blood was collected in a clean, EDTA- free (plain) tubes which were allowed to stand for 10 minutes at room temperature before been centrifuged at 1000rpm for 15minutes, to obtain the serum. The animals were thereafter quickly dissected and the kidney was removed, cleaned and weighed. The kidney was then fixed in 10% formalin solution.

Histopathological studies

The histopathological examination of the kidney was done as described by Krause (2001). This involved two

Table 1: Effects of leech salivary extract on blood metabolite indicators of kidney function in rats

Treatments (mg/kgbw)	Sodium (mmol/L)	Chloride (mmol/L)	Potassium (mmol/L)	Urea (mmol/L)	Creatinine (mg/dL)
Control	122.00±7.41a*	82.40±5.53a	7.20±0.20a	12.09±1.41a	2.25±0.49b
25	135.80±3.24a	126.60±22.41b	7.38±1.88a	24.52±1.78c	2.85±0.78c
50	115.60±8.29a	133.60±6.40b	6.70±1.32a	25.67±4.20c	2.84±0.72c
100	121.00±10.03a	117.40±3.70ab	7.95±0.69a	25.28±2.41c	2.16±0.46a
Dexamethasone (3)	125.20±5.20a	118.20±4.15ab	7.62±1.32a	18.64±1.28b	1.27±0.26a
Jobelyn (4.17)	125.40±10.90a	84.20±5.64a	8.48±1.10a	14.55±0.32ab	3.23±0.63c

Values are means ± SEM for n=5

*Values along the same column with different superscripts are significantly different from each other (p < 0.05).

mg/kgbw: Milligram per kilogram body weight

mmol/L: Millimol per liter

mg/dL: Milligram per deciliter

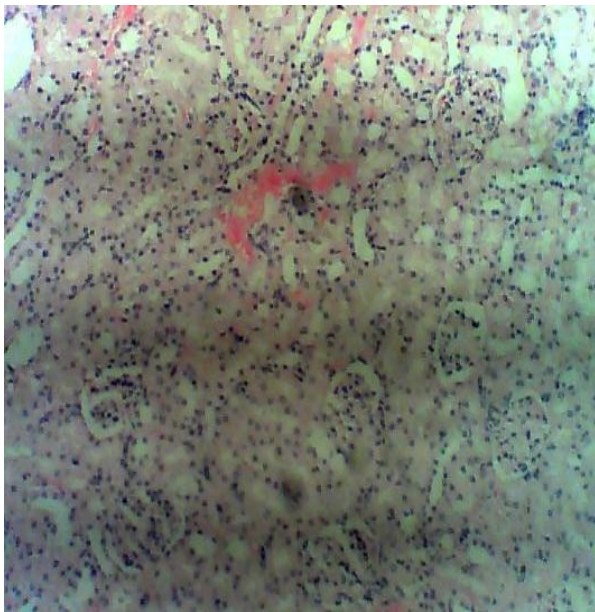


Plate I: KD1

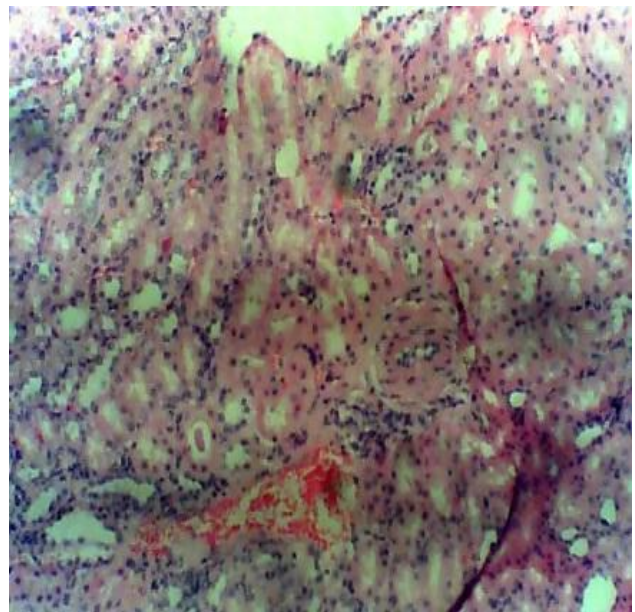


Plate II: KD2

main stages: grossing and tissue processing.

Statistical Analysis

Results were expressed as mean value ± standard error mean (SEM). Among groups, comparisons of means were performed by the Analysis of Variance (ANOVA) test, for statistical significance of differences, at p=0.05. The means were subsequently separated using Duncan Multiple Range Test (DMRT). All data analysis were done using the statistical package SPSS version 19.0.

RESULTS

The effects of Leech Salivary Extract (LSE) on blood metabolite indicators of kidney in rats are presented in

Table 1. On the whole, increasing concentrations of the LSE had differential effects on the metabolite parameters investigated, with respect to the Controls. The LSE had no significant (p>0.05) effect on concentrations of Sodium (Na) and Potassium (K), and, thus, varied within narrow limits among the various rat treatment groups (range = 115.60±8.29 to 135.80±3.24 mmol/L for Na and 7.20±0.20 to 8.48±1.10 mmol/L for K). The other three metabolites namely, Chloride, Urea and Creatinine were however, significantly (p<0.05) affected by LSE. Urea concentration was does independently significantly increased by LSE than in the group administered with the standard drug, Dexamethasone (18.64±1.28 mmol/L). At 25 and 50mg/kgbw LSE treatments, chloride concentrations were increased (126.60±22.41 and 133.60±6.40 mmol/L), compared with the Control (82.40±5.53 mmol/L), the higher 100mg/kgbw treatment

had no significant effect on Chloride level; in a way similar to the effects of the Dexamethasone and Jobelyn drugs. LSE treatment significantly affected the production of Creatinine in the rats. However, while lower concentrations (i.e., 25 and 50mg/kgbw) of LSE significantly increased Creatinine level to as much as 2.85 ± 0.78 mg/dL relative to the Control (2.25 ± 0.49 mg/dL), the highest concentration tested, i.e., 100mg/kgbw significantly reduced it to 2.16 ± 0.46 mg/dL.

DISCUSSION

The results of the present study showed that there was a significant increase in creatinine levels in rats administered 25mg/kgbw and 50mg/kgbw when compared with the control. This increase may be due to the fact that the extract has similar properties with certain antibiotics like famotidine, ranitidine and cefoxitin that can elevate serum creatinine levels. The report of the present study corroborates findings of Inker and Perrone (2012), which some drugs like trimethoprim can interfere with creatinine secretion and therefore result in a self-limited and reversible rise in the serum creatinine without changing the true glomerular filtration rate (GFR). On the other hand, there was a significant decrease in the level of serum creatinine in rats administered 100mg/kgbw LSE. This finding is in consonance with the reports of Ryan (2009), who established that an increase in creatinine levels may result from reduced renal blood flow due to dehydration, heart failure or complications of diabetes. The significant elevation in urea levels across the LSE treatment groups observed in the present study may be due to renal tissue toxicity, an indication of impaired kidney. This finding agrees with the reports of Onu *et al.*, (2013) that the marked elevation in serum urea level of rats administered with aqueous stem bark extract of *Khaya senegalensis* may suggest that the extract caused renal impairment in the rats. The results from this study showed that LSE did not exert any significant ($p > 0.05$) influence on sodium levels in the treated groups when compared with control. Although, groups administered 50mg/kgbw and 100mg/kgbw LSE showed a slight reduction in blood sodium levels, this reduction was not significantly ($p > 0.05$) different from the control. This may be beneficial to patients with high blood pressure since reduction in sodium intake is known to lower blood pressure. These findings is in corroboration with the reports of Akpanabiatu *et al.*, (2005) whose study revealed that leaf extract of *Sarcocephalus latifolius* had no significant difference on serum sodium.

CONCLUSION

The present study revealed that, upon chronic

administration of the LSE, there was an increase in the Urea and Creatinine concentration. However, LSE did not have significant effect on the Sodium and Potassium, but significantly increased Chloride levels in rats.

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