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Irvingia gabonensis leaves mitigate arsenic-induced renal toxicity in wistar rats.

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Abstract

Arsenic has been reported to contaminate groundwater and agricultural soil in different parts of the World. The effect of ethanol leaf extract of *Irvingia gabonensis* (ELEIG) against sodium arsenite (SA)-induced renal toxicity was investigated in this study. Wistar albino rats of weights between 100 and 161 g were assigned to eleven (11) groups of five (5) animals each. Group 1 (control) was given feed and water *ad libitum*. Group 2 was exposed to SA at a dose of 4.1 mg/kg body weight (kgbw) for two weeks. Groups 3-11 were treated with ELEIG with or without SA. Treatment was done orally and lasted for 14 days. Serum concentrations of urea, creatinine, sodium ion (Na$^+$), potassium ion (K$^+$), bicarbonate (HCO$_3$-) and chloride ion (Cl-) as well as histological assessment of kidney tissues were used to assess kidney function. Results show that SA caused significant (p<0.05) increases in serum concentrations of all assayed parameters as well as acute tubular necrosis, vascular lesions of ulceration and stenosis. Treatment with ELEIG both simultaneously and 2 weeks after SA exposure reversed the deleterious effects of SA. Administration of the extract alone at various doses also produced similar results with those of the normal control. It may therefore be concluded that ELEIG possesses potent medicinal properties against SA-induced renal toxicity in Wistar rats.

Introduction

Humans and animals alike are inseparable from their environment since it contributes to their general wellbeing. However, when the environment is compromised by way of pollution, it facilitates the deterioration in the health of its occupants (humans and animals) rather than contributing to their overall wellbeing. Arsenic is an environmental pollutant and a carcinogen that has been reported to contaminate groundwater in many parts of the World, including Nigeria [1-7]. Several studies have linked arsenic exposures from contaminated drinking water to carcinogenesis among inhabitants of different countries [8-12]. Exposure to arsenic has also been reported to culminate in renal injury which may lead to the development of tubulointerstitial nephritis and acute tubular necrosis [13]. In addition, consumption of arsenic-contaminated drinking water enhances the development of renal injury and hypertension [14,15].

Most locals, especially those in low-income countries have been compelled to use medicinal plants for their healthcare needs due to unaffordable cost of orthodox drugs. *Irvingia gabonensis* O'Rorke Baill (also called bush mango or wild mango) is one of such medicinal plants. The stem bark of *I.* *gabonensis* is used in Cameroon to treat hunch back and infections [16]. The aqueous maceration of the leaves is utilized as an antidote to combat poisonous substances. The stem bark is also used in treating gonorrhea, hepatic and gastrointestinal disorders in Senegal [17]. The hematological, hepatoprotective, anti-diabetic and prophylactic effects of stem bark and leaf extracts of this plant have also been reported in animal models [18-21]. Similarly, the medicinal effect of leaf extracts of *I. gabonensis* under conditions of induced toxicities in Wistar rats have been documented [22-25]. However, there is little or no documented information on the renal protective effect of the leaf of this plant against sodium arsenite-induced toxicity. This information gap is what informed this study.

Materials and Methods

Chemicals and reagents

Sodium arsenite was purchased from British Drug House (BDH) chemicals, Poole, England. Absolute ethanol was purchased from JHD, China. Teco Diagnostic assay kits were used for kidney function analyses. All other chemicals/reagents used in this study were of analytical grade and standard.

Collection, authentication and preparation of plant extract

Fresh and mature leaves of *I. gabonensis* O'Rorke Baill were harvested from Itak Ikot Akap village in Ikono local government area of Akwa Ibom State, Nigeria. The samples were identified and authenticated by a taxonomist Mr. Daniel Etefia of the Department of Pharmacognosy and Herbal Medicine, University of Uyo, Akwa Ibom State, Nigeria with the voucher number James Daniel UUH 042116 (Uyo). They were washed using clean water to eliminate dust and other contaminants, prior to air-drying for 7 days on a clean table at room temperature in Biochemistry laboratory, University of Uyo, Uyo, Akwa Ibom State, Nigeria. They were then pulverized using a clean manual grinder and mortar and pestle, and stored in an air-tight container prior to extraction.

Approximately 850 g of pulverized leaves was macerated in absolute ethanol (JHD, China) and allowed to stay for 72 h with intermittent stirring to ensure proper extraction. The sample was filtered thrice through a clean muslin cloth and the filtrate was concentrated in stainless steel bowl using a water bath at 45°C. The paste-like gel extract obtained after continuous concentration was then transferred into pre-weighed transparent containers, weighed and stored in the refrigerator prior to use.

Experimental animals

Fifty-five (55) healthy and non-pregnant female Wistar albino rats of weights between 100 and 161 g were acquired at the animal house facility of Faculty of Basic Medical Sciences, University of Uyo, Nigeria. They were allowed access to feed and water *ad libitum*, and were acclimatized for seven (7) days in the same facility in a well-ventilated room under standard conditions.

Ethics

This study was carried out in accordance with internationally accepted principles of laboratory animal use and care (National Institute of Health (NIH) 85-23) and experiments were in accordance with Committee for the Purpose of Control and Supervision Experiments on Animals (CPCSEA) ethical guidelines.

Experimental design

At the end of seven days acclimatization and just before the commencement of treatment, the experimental animals were assigned to eleven (11) groups of five animals each in standard animal cages and weighed using a digital weighing balance (Camry electronic scale EK5350, China) after overnight fast to obtain their initial body weights. They were administered treatment as shown in Table 1 below:

Table 1. Experimental design.

Groups	Treatment
1. Negative control	Normal feed and water *ad libitum*
2. Positive control	4.1 mg/kgbw SA for 14 days
3. Post-treatment	4.1 mg/kgbw SA for 14 days, followed by 100 mg/kgbw extract for another 14 days
4. Post-treatment	4.1 mg/kgbw SA for 14 days, followed by 200 mg/kgbw extract for another 14 days
5. Post-treatment	4.1 mg/kgbw SA for 14 days, followed by 400 mg/kgbw extract for another 14 days
6. Simultaneous Treatment	4.1 mg/kgbw SA+100 mg/kgbw extract simultaneously for 14 days
7. Simultaneous Treatment	4.1 mg/kgbw SA+200 mg/kgbw extract simultaneously for 14 days
8. Simultaneous Treatment	4.1 mg/kgbw SA+400 mg/kgbw extract simultaneously for 14 days
9. Extract only	100 mg/kgbw extract only for 14 days
10. Extract only	200 mg/kgbw extract only for 14 days
11. Extract only	400 mg/kgbw extract only for 14 days

Note: SA: Sodium arsenite; mg/kgbw: milligram per kilogram body weight

Termination of treatment, collection of blood samples and extraction of kidney tissues

On the last day of treatment, all experimental animals were fasted overnight with access to water only, and their final body weights were taken. The experimental animals were sacrificed under chloroform anesthesia by lower abdominal incision about 24 h after the last treatment. Blood samples were obtained by cardiac puncture using sterile syringes and needles and collected in sterile plain sample bottles for analyses. Sera were obtained from clotted blood samples in the plain bottles

after centrifugation using a table top centrifuge (Model 800-1, Zeny Inc. Salt Lake, USA) at 3000 rpm for 15minutes. Separated sera were stored in the refrigerator at 40˚C prior to analyses. Kidney tissues were excised from the sacrificed experimental animals and rinsed with 1.15% ice cold potassium chloride (KCl, BDH, Poole, England) solution to remove traces of blood prior to weighing. A small portion of the excised kidney was fixed in 10% neutral buffered formalin for histological assessment.

Kidney function assays

Teco diagnostics assay kits (Anahaema, USA) were used for the determination of blood and serum concentrations of urea, creatinine, sodium ion (Na^+), potassium ion (K^+), bicarbonate (HCO_3^-) and chloride ion (Cl^-). Blood urea was determined according to the method of Wybenga et al., [26] while serum creatinine was assayed according to the method of Henry [27]. Serum sodium ion (Na^+), potassium ion (K^+) and chloride ion (Cl^-) were determined according to the method of Tietz [28], while serum bicarbonate (HCO_3^-) levels were determined using the method of Young [29].

Histological assessment of kidney tissues

The routine haematoxylin and eosin staining method (H&E) as described by Drury and Wallington [30] were used to prepare the kidney tissues for histological assessment. Sequel to staining of tissues for histological studies, sections was examined under a Leica DM500 microscope and results were reported by a Consultant Histopathologist. Photomicrographs were taken using an attached Leica ICC50 digital camera.

Data analysis

Results are presented as mean ± standard deviation (SD) and were analysed with one – way analysis of variance (ANOVA) for differences between groups with the aid of SPSS Software (IBM, version 20) (Figure 1). Values of $p < 0.05$ were considered statistically significant.

Results

Effect of ethanol leaf extract of I. gabonensis O'Rorke Baill on kidney function of experimental rats in presence or absence of sodium arsenite toxicity

Results obtained showed that administration of sodium arsenite (group 2) led to significant ($p < 0.05$) increases in serum concentrations of all assayed parameters, when compared with normal control. Post-treatment with the ethanol leaf extract at the various doses produced significant ($p < 0.05$) decreases in serum concentrations of urea, creatinine, Na+, K+, HCO_3^- when compared with group 2 except Cl^- concentrations which were not dose-dependent (Figure 2). Similarly, simultaneous treatment with the extract at various doses produced significant ($p < 0.05$) decreases in the assayed parameters in dose-independent manner when compared with group 2 except HCO_3^- concentration which significant ($p < 0.05$) decrease was dose-dependent. Furthermore, administration of extract only at various doses produced significant ($p < 0.05$) decreases in blood urea and serum K+concentrations and non-significant ($p > 0.05$) differences in serum concentrations of creatinine, Na+, HCO_3^- (at 200 mg/kgbw and 400 mg/kgbw) and Cl^- when compared with the normal control. The results are presented in Table 2 below.

Table 2. *Effect of ethanol leaf extract of I. gabonensis O'Rorke Baill on kidney function of experimental rats in presence or absence of sodium arsenite toxicity (Figures 3-11).*

Groups	Urea (mg/dL)	Creatinine (mg/dL)	Na+(mmol/L)	K+(mmol/L)	HCO3- (mmol/L)	Cl- (mmol/L)
Control	18.24 ± 2.55 [befghijk]	0.46 ± 0.18[b]	143.20 ± 9.73[be]	4.86 ± 0.73[eijk]	30.00 ± 3.74[bi]	98.20 ± 7.73[b]
Arsenite only	28.96 ± 3.72[acdefgh]	1.16 ± 0.35[acdefgh]	156.00 ± 14.35[adefgh]	5.59 ± 1.32[defgh]	47.00 ± 7.52ac[defghijk]	115.00 ± 11.77[adefh]
Post-Treatment at 100 mg/kg	19.62 ± 3.72[bef]	0.51 ± 0.07[b]	144.00 ± 4.85[e]	4.77 ± 0.54[e]	35.60 ± 8.38[bde]	106.67 ± 2.08[de]
Post-Treatment at 200 mg/kg	18.10 ± 1.04[beg]	0.37 ± 0.10[bg]	142.25 ± 5.68[be]	4.02 ± 0.20[b]	25.50 ± 2.08[bc]	92.25 ± 9.43[bcg]
Post-Treatment at 400 mg/kg	12.97 ± 0.49[abcd]	0.38 ± 0.14[b]	124.40 ± 16.41[abcd]	3.51 ± 0.47[abc]	25.20 ± 3.42[bc]	93.20 ± 11.52[bc]
Simultaneous at 100 mg/kg	12.78 ± 0.83[abcg]	0.44 ± 0.05[b]	137.80 ± 5.54[b]	4.25 ± 0.57[b]	29.60 ± 4.56[b]	100.40 ± 5.03[b]

Simultaneous at 200 mg/kg	25.05 ± 3.01^{abdfh}	0.61 ± 0.13^{bdh}	135.75 ± 7.14^{b}	4.65 ± 0.69^{b}	29.25 ± 5.38^{b}	107.50 ± 3.70^{d}
Simultaneous at 400 mg/kg	13.75 ± 1.70^{abg}	0.33 ± 0.06^{bg}	137.50 ± 5.45^{b}	4.11 ± 0.19^{b}	25.71 ± 1.85^{b}	98.25 ± 11.87^{b}
Extract only at 100 mg/kg	15.05 ± 3.01^{aj}	0.27 ± 0.04	140.20 ± 13.90	3.72 ± 0.58^{a}	23.60 ± 2.08^{a}	100.20 ± 8.41
Extract only at 200 mg/kg	9.34 ± 1.72^{aik}	0.31 ± 0.09	140.80 ± 8.79	3.99 ± 0.83^{a}	28.40 ± 6.80	102.40 ± 7.99
Extract only at 400 mg/kg	12.74 ± 1.34^{aj}	0.36 ± 0.18	138.00 ± 4.18	3.66 ± 0.38^{a}	24.00 ± 1.58	99.60 ± 8.44

Note: Data are expressed as mean ± SD, n=5; a=mean difference is significant at p ≤ 0.05 when compared with group 1; b=mean difference is significant at p ≤ 0.05 when compared with group 2 c=mean difference is significant at p ≤ 0.05 when compared with group 3; d=mean difference is significant at p ≤ 0.05 when compared with group 4 e=mean difference is significant at p ≤ 0.05 when compared with group 5; f=mean difference is significant at p ≤ 0.05 when compared with group 6; g=mean difference is significant at p ≤ 0.05 when compared with group 7; h=mean difference is significant at p ≤ 0.05 when compared with group 8 I=mean difference is significant at p ≤ 0.05 when compared with group 9; j=mean difference is significant at p ≤ 0.05 when compared with group 10 k=mean difference is significant at p ≤ 0.05 when compared with group 11.

Effect of ethanol leaf extract of *I. gabonensis* O'Rorke Baill on kidney histology of experimental rats in presence or absence of sodium arsenite toxicity

Exposure of the experimental animals to SA for a period of 14 days led to the induction of acute tubular necrosis, vascular lesions of ulceration and stenosis in the kidney of the experimental rats. Treatment with graded doses (100, 200, and 400 mg/kgbw) of ethanol leaf extract of *I. gabonensis* O'Rorke Baill both simultaneously and after 14 days of SA exposure (post-treatment) resulted in a dose-dependent ameliorative and therapeutic effects, with the low dose having the most potent effect and the therapeutic treatment giving a better result than the ameliorative (simultaneous) treatment. The results are shown in the photomicrographs below.
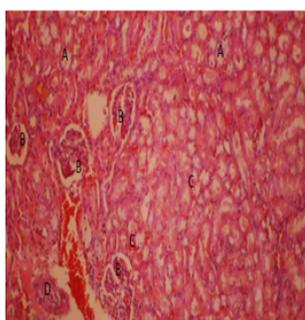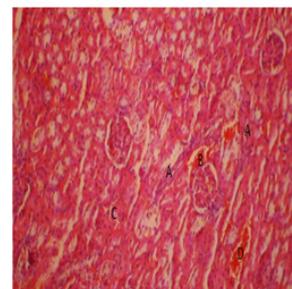

Figure 2. Kidney section of rat given SA only for 14 days (Group 2) showing: (A) Severe vascular stenosis (B) Ulceration (C) Patchy tubular necrosis and (D), Moderate intestinal congestion (H&E × 100).

Figure 1. Kideny section of contril (Group 1) composed of : (A) Tubules, (B) Glomeruli, (C) interstitial space (D) Arcuate artery (H&E × 100).
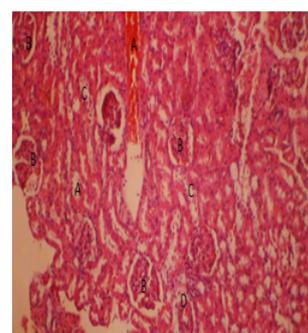

Figure 3. Kidney section of rat given SA followed by 100 mg/kg leaf extract for 14 days (Group 3) showing (A) Mild active interstitial congestion (B) Glomeruli and (C) Tubules and (D) Normal vascular architecture (H&E × 100).
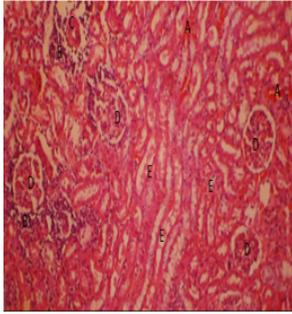
Figure 4. *Kidney section of rat given SA followed by 200 mg/kg (Group 4) leaf extract for 14 days showing: (A) Moderate active interstitial congestion, (B), Heavy infiltrates of lymphocytes, (C) Normal vascular architecture and normal, (D) Glomeruli and (E), Tubules (H&E × 100).*
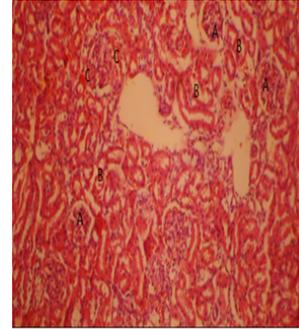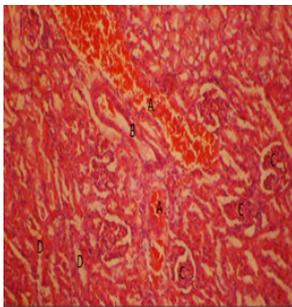

Figure 5. *Kidney section of rat given SA followed by 400 mg/kg leaf extract for 14 days (Group 5) showing; (A), Moderate active interstitial congestion (B), Mild vascular stenosis and (C), Normal glomeruli and (D), Tubules (H&E × 100).*
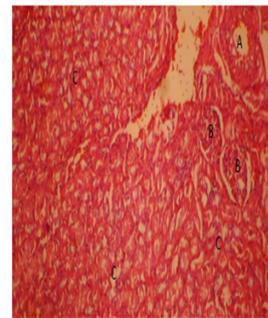

Figure 6. *Kidney section of rat co-administrated with SA and 100 mg/kg leaf extract for 14 days (Group 6) showing: (A) Normal glomeruli and (B), Tubules and (C), Mild active interstitial congestion (H&E × 100).*

Figure 7. *Kidney section of rat co-administerated with SA and 200 mg/kg leaf extract for 14 days (Group 7) showing: (A) Normal glomeruli and (B) Tubules and (C) Mild active interstitial congestion (H&E × 100).*

Figure 8. *Kidney section of rat co-administerated with SA and 400 mg/kg leaf extract for 14 days (Group 8) showing: (A), Normal vascular architecture, (B), Glomeruli and (C), Tubules (H&E × 100).*

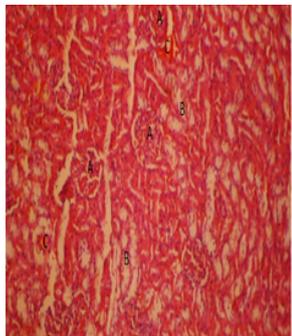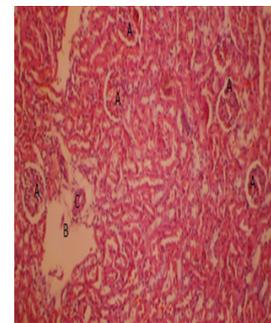***Figure 9.*** *Kidney section of rat given 100 mg/kg leaf extract only for 14 days (Group 9) showing: (A), Normal glomeruli, (B), Tubules and (C), Arcuate artery (H&E × 100).*
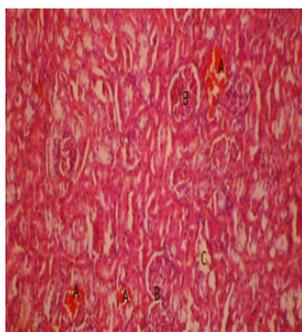
Figure 10. Kidney section of rat given 200 mg/kg leaf extract only for 14 days (Group 10) showing : (A), Moderate active interstitial congestion, (B), Normal glomeruli and (C), Tubules (H&E × 100).

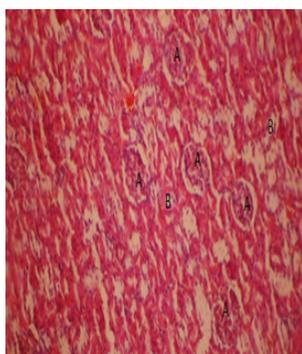*Figure 11: Kidney section of rat given 400 mg/kg leaf extract only for 14 days (Group 11) showing; (A), Normal Glomeruli and (B), tubules (H&E × 100).*

Discussion

The kidney is a major site of arsenic uptake and accumulation because a large percentage of arsenic absorbed is filtered in the kidney [31]. Exposure to arsenic has been reported to cause renal injury. Acute arsenic-induced renal intoxication may culminate in tubulointerstitial nephritis and acute tubular necrosis [13]. In addition, exposure to arsenic compounds has been reported to lead to pro-neoplastic changes in renal cells which may mark early steps in the progression of some forms of cancers [31,32]. Furthermore, consumption of arsenic-contaminated drinking water has been reported to enhance the development of hypertension and renal injury [14-15].

Urea is a non-protein nitrogenous (NPN) waste product of protein metabolism. Blood concentration of urea is determined by the excretion rate of urea from the renal tubules [33]. Thus, high blood urea nitrogen may be due to impairment in kidney function [34]. Creatinine is a non-protein nitrogenous (NPN) waste product that is produced from the breakdown of creatine and phosphocreatine [35]. Excretion of creatinine is also carried out by the renal system. Thus, it also serves as a biomarker of renal function [35]. It is a more suitable indicator of kidney function because it is less affected by diet. Endogenously synthesized creatinine is filtered off by the glomerulus. Hence, plasma/serum concentration of creatinine is a function of the glomerular filtration rate (GFR). In kidney dysfunction, GFR is decreased, which compromises creatinine clearance. The decreased or compromised creatinine clearance therefore leads to an elevation in plasma/serum concentration of creatinine [34].

In the present study, SA nephrotoxicity was confirmed by the fact that administration of SA alone produced significant increases in blood urea and serum creatinine concentrations, when compared with the control group. This suggests the inability of the kidney to excrete these waste products as a result of impairment in kidney function [36]. Sodium arsenite-induced renal toxicity has also been confirmed from findings of previous studies [37,38]. Treatment with ethanol leaf extract of *I. gabonensis* O'Rorke baill both simultaneously and two weeks after (post-treatment) produced significant decreases in blood urea and serum creatinine concentrations when, compared with rats administered SA only. The significant decrease in blood urea concentration on post-treatment with the ethanol leaf extract was in a dose-dependent manner. Administration of ethanol leaf extract of *I. gabonensis* O'Rorke baill only at different doses produced significant decreases in blood urea concentrations and non-significant differences in serum creatinine concentrations when compared with control. This connotes that the ethanol leaf extract could be ameliorative and curative. It is therefore possible that the ethanol leaf extract mitigated SA-induced toxicity/oxidative stress by enhancing the antioxidant defense systems in the treated rats thereby speeding up the mopping up of the reactive oxygen species generated. This may be due to the antioxidant phytochemicals present in the extract [39]. This is because arsenic has been reported to induce oxidative stress by generating free radicals that culminate in cellular damage via depletion of enzyme activities through lipid peroxidation and reaction with nuclear proteins and DNA [40]. Arsenic has also been reported to induce oxidative stress by producing ROS such as superoxide (O_2^-), peroxyl radical (ROO.), singlet oxygen ($^1O_2$), nitric oxide (NO·), hydrogen peroxide (H_2O_2) and dimethylarsinic peroxyl radicals ($(CH_3)_2AsOO·$) [41-43].

The kidney plays a very vital and pivotal role in the regulation of electrolyte and acid-base balance in the body system [44,45]. Sodium (Na^+), potassium (K^+), calcium (Ca_2^+) and magnesium (Mg^{2+}) are the principal cations in the body fluids while chloride (Cl^-), bicarbonate (HCO_3^-) and phosphate ($PO4_2^-$) are the most important anions. Certain disease conditions and xenobiotics may cause electrolyte imbalance [46]. Electrolyte levels are kept constant by the kidneys and hormones. Assessment of tubular integrity of the nephron may therefore be done by estimating the serum level of electrolytes [47]. A progressive loss of kidney function causes derangements in blood electrolytes levels and acid-base balance [45].

In the present study, administration of SA alone produced significant increases in serum levels of K^+ and Cl^-. It also produced significant increases and non-significant decreases in serum levels of Na^+ and HCO_3^- when compared with group 2, administered SA only. An elevated serum potassium level causes a condition called hyperkalemia, which is most commonly caused by kidney failure [45,48,49]. One major

feature of renal failure is decreased glomerular filtration rate that can also cause a decrease in distal tubular flow rate, a vital factor in the retention of potassium in chronic kidney failure [50]. Hypernatremia is a condition that results from high serum sodium concentration [44,51]. Renal failure has been reported as one of the factors that can cause hypernatremia [52]. Too high a concentration of serum chloride results in a condition called hyperchloremia which can be caused by several factors including kidney disease [53,54]. Metabolic alkalosis (too much alkali in the blood) is a condition that arises from excess or too high bicarbonate in the blood. This has also been reported to be caused by some kidney diseases [55]. A decline in kidney function may also result in a decrease in serum bicarbonate [56]. From the foregoing, it can therefore be inferred that administration of SA to the experimental animals caused derangements in serum sodium, potassium, chloride and bicarbonate levels which may have stemmed from its impairment of kidney function.

Treatment with ethanol leaf extract of *I. gabonensis* O'Rorke baill both simultaneously and two weeks after (post-treatment) reversed the derangements in serum electrolyte levels induced by SA. In addition, administration of ethanol leaf extract of *I. gabonensis* O'Rorke baill alone at various doses produced significant decreases in serum K^+ concentrations and non-significant differences in serum Na^+, HCO_3^- and Cl^- concentrations when compared with control. This confirms the nephroprotective effect of ethanol leaf extract of *I. gabonensis* O'Rorke baill.

Histopathological assessment of kidney tissues showed that there were no visible lesions in the kidneys of the control group. In contrast, exposure to SA alone produced acute tubular necrosis, vascular lesions of ulceration and stenosis. Treatment with graded doses of ethanol leaf extract both simultaneously and 14 days after SA intoxication achieved dose-dependent ameliorative and therapeutic effects, with the low dose having the most potent effect and the therapeutic treatment giving a better result than the ameliorative (simultaneous) treatment. Thus, the ethanol leaf extract was effective in attenuating acute tubular necrosis induced in the kidneys of the experimental animals by SA.

Conclusion

The results obtained in this study suggest that ethanol leaf extract of *I. gabonensis* O'Rorke baill possesses potent curative and ameliorative properties against SA-induced renal toxicity in Wistar rats. This may be due to the antioxidant phytochemicals present in the leaf extract.

Acknowledgements

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