Toxicity studies of aqueous, methanolic and hexane leaf extracts of Guiera senegalensis in rats

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Abstract-Toxicity of aqueous, methanolic and hexane leaf extracts of Guiera senegalensis in rats was investigated. An acute toxicity study was carried out to determine the LD50 of the plant's extract. Sub-acute toxicity study was carried to determine the effects of the extracts on serum concentrations of liver function indicators, heart enzymes and electrolytes. The extracts were administered orally up to a dose of 5000mg/kg for the LD50 determination. In the sub-acute toxicity, 40 rats grouped into 10 of 4 rats each, were orally administered with the aqueous, methanolic and hexane extracts at a daily dose of 250mg/kg, 500mg/kg and 1000mg/kg of each extract respectively for 4 weeks. No death was recorded during the acute toxicity test which may imply that the plant is practically non toxic. No significant difference was observed in liver function indices but levels of potassium and bicarbonate were found to be significantly higher ($P<0.001$) at 500 and 1000mg/kg following the administration of the methanolic extract. Levels of bicarbonate at 1000mg/kg, potassium at 250mg/kg and bilirubin at 250,500 and 1000mg/kg were found to be significantly higher ($P<0.001$) as well as that of alanine aminotransferase at 1000mg/kg and potassium at 500 and 1000mg/kg ($P<0.05$) after the administration of the hexane extract for 4 weeks. Administration of the aqueous leaf extract of the plant showed that levels of bilirubin and potassium at 250 and 500mg/kg respectively and bicarbonate levels at all 3 doses administered were significantly higher ($P<0.001$), while bilirubin Level at 500 and 1000mg/kg as well as potassium level at 1000mg/kg were significantly higher ($P<0.05$) when compared to the normal control. However the methanolic and hexane extract at 1000mg/kg and the aqueous extract at 250, 500 and 1000mg/kg led to a significant decrease in chloride levels ($P<0.001$). The above finding may indicate that the leaf extract may have erythrocyte haemolytic effect which may lead to hyperkalemia and could lead to a disturbance in acid- base balance when administered at higher doses.

Index Terms- Acute toxicity, Aqueous extract, Electrolytes, Guiera senegalensis, Heart enzymes, Hexane extract, Liver function indicators , Methanolic extract, Sub-acute toxicity.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities [9]. It is estimated that there are about 700,000 species of tropical flowering plants that have medicinal properties [5]. Their actions include: antibacterial, antifungal, antiviral, antihelminthic and anticarcinogenic among others. These medicinal values lie in some chemical substances they contain [26]. In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs. When adopted outside of its traditional culture, traditional medicine is often called complementary and alternative medicine. Herbal medicines can be very lucrative, generating billions of dollars in sales, but adulteration or counterfeit herbs can also be a health hazard [38]. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. Traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material [19]. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population, most of these medicines are taken without a clear knowledge of appropriate dosage.

The plant *Guiera senegalensis* is a tropical shrub of the family Combrataceae [17], and is known locally as *Sabara* in Hausa, *olofun* in Yoruba. It is found especially in the sahelo-sudanese zone, on sandy, leached or exhausted soils, fallow or arid lands. It is a colonizing plant dispersed by cattle in fallow land [4]. Its distribution is from Senegal to Cameroon including Nigeria and as far as Sudan, it is widely distributed in these areas, common, locally

gregarious and very abundant [4]. There are several recorded uses for *G. senegalensis* in traditional medicine to treat various illnesses [11]. *Guiera senegalensis* is a widely used plant in traditional medicine; it is one of the most important West African medicinal plants, often used to treat a variety of microbial infections. But there is a paucity of research work on the toxicity associated with the administration of the aqueous leaf extract of the plant. There is, therefore the need to comprehensively study the possible toxicity associated with the administration and the safe dose limit.

MATERIALS AND METHOD

MATERIALS

The under listed items were the equipment and apparatus used in the course of this study: Weighing balance (Gallenhamp), Centrifuge, 80-1 (Techmel and Techmel USA), NV 203 Spectrophotometre (B Bran Scientific & Instrument Company England), Water bath TT42D (Techmel & Techmel, USA), Freezer (Thermocool-superdeluxe), Hand gloves, Test tubes, Test tube racks, Centrifuge tubes, Micropipette (Switzerland, SOCOREX), Measuring cylinder (Pyrex, England) and What man No.1 filter paper.

CHEMICALS AND REAGENTS

The chemicals and reagents used for this study were manufactured by; Fidsons Scientific Equipment (England), Aldrich Chemical Co Ltd (Gillingham Dorset England), May and Baker Ltd (Dagenham England), BDH Laboratory Suppliers (England) and includes the following; Methanol and hexane. Others are: Alanine aminotransferase Kit, Bilirubin Kit, Electrolytes (Na, K, Cl and HCO) Kit, Aspartate aminotransferase Kit, Alkaline phosphatase Kit, Creatine- kinase Kit, Lactate dehydrogenase Kit. All from Randox laboratories Ltd United Kingdom.

COLLECTION AND PREPARATION OF PLANT EXTRACT

The leaves of *Guiera senegalensis* were collected at Potiskum Local Government Area of Yobe state and it was authenticated by a Botanist at Biological Science Department, Bayero University, Kano. The leaves were washed air dried and ground into powder and then percolated in methanol and hexane for 4 days each, it was then sieved in order to separate the extract from the residue and the resulting extract was concentrated using a rotary evaporator maintained at 60°C. The dried methanol extract was stored in a glass sample bottle and kept in a refrigerator until required.

EXPERIMENTAL ANIMALS

The animals used in this study were Wister albino rats obtained from the National Veterinary Research Institute, Vom, Plateau state. The experimental rats were housed in cages and kept in a room where a 12 hour light/dark cycle was maintained in the Department of Biological Sciences, Bayero University, Kano. They were fed with standard pellet diet and were allowed free access to feed and water throughout the period of the experiment.

Acute Toxicity

LD_{50} was determined using the method [23].

In the initial phase 18 rats were divided into 6 groups of 3 rats each. The rats were treated by administering orally the doses of the extracts as given below:

Group 1: was administered 10mg/kg methanolic extract
Group 2: was administered 100mg/kg methanolic extract
Group 3: was administered 1000mg/kg methanolic extract
Group 4: was administered 10 mg/kg hexane extract
Group 5: was administered 100 mg/kg hexane extract
Group 6: was administered 1000 mg/kg hexane extract

The rats were monitored for 24 hrs for mortality and general behavior.

In phase two 10 rats were used and grouped into 10 of 1 rat each. They were treated with doses based on the findings of phase 1 (1250 mg/kg, 2000 mg/kg 2750 mg/kg, 3750 mg/kg and 5000 mg/kg) and monitored again for 24 hrs.

Sub Acute Toxicity

Forty rats were grouped into 10 groups of 4 rats each. The rats were treated by administering orally the doses of the extracts as given below:

Group A: Control
Group B: was administered 250mg/kg methanolic extract
Group C: was administered500mg/kg methanolic extract
Group D: was administered 1000mg/kg methanolic extract
Group E: was administered 250mg/kg hexane extract
Group F: was administered 500mg/kg hexane extract
Group G: was administered 1000mg/kg hexane extract
Group H: was administered 250mg/kg aqueous extract
Group I: was administered 500mg/kg aqueous extract

Group J: was administered 1000mg/kg aqueous extract

The extract was administered for 4 weeks. Twenty four hours after the last dose, the rats were weighed and then sacrificed by decapitation. Individual serum enzymes (ALT, AST, ALP, LDH and CK), electrolytes and bilirubin of the rats were determined and the mean and SD of each parameter was calculated.

Statistical Analysis

The results were analysed using analysis of variance (ANOVA). Post Hoc Tests Multiple Comparisons using LSD was utilized to identify differences in means. The data was statistically analysed using GraphPad Instat3 Software (2000) version 3.05 by GraphPad Inc.

RESULTS

Acute Toxicity

Oral administration of methanolic and hexane extracts did not produce any mortality in rats up to a dose level of 5000mg/kg. The body weight, food and water consumption of the animals treated with the extracts did not show any significant change when compared with the control group. The oral LD_{50} was indeterminable up to a dose level of 5000mg/kg.

Sub- acute toxicity

Tables 3-5 show the result of Sub- Acute toxicity parameters for groups of rats treated for 4 weeks with different concentration of methanolic, hexane and aqueous extracts respectively. This was carried out in order to determine the toxic effect of the extract on liver function indices (AST, ALT, ALP and T.BIL.), Heart enzymes (CK and LDH) and serum electrolytes (Cl^-, Na^+, K^+ and HCO_3^-).

Liver Indices

The ALT level in the rats administered with 1000mg/kg hexane extract for 4 weeks (Group G) was significantly higher ($P<0.001$) than control ($P<0.05$), also the value of total bilirubin was significantly higher in rats administered with 250, 500 and 1000mg/kg hexane extract for 4 weeks (Groups E, F and G) than the control Group (Table 4), in rats administered with 250 and 500 aqueous extract for 4 weeks (Group H) ($P<0.001$) and (Groups I) ($P<0.05$). However significant decrease ($P<0.05$) was observed in the total bilirubin of rats administered with 1000mg/kg of the aqueous extract (Group J) when compared to the control Group (Table 5).

Heart Indices

No significant difference was observed in CK and LDH activities in all the groups administered with the methanolic, hexane and aqueous leaf extracts of *Guiera senegalensis* compared to the control Group.

Electrolytes

The level of K^+ in rats administered with 500 and 1000mg/kg of the methanolic extract for 4 weeks (Groups C and D) in Table 3, 250mg/kg (Group E) in Table 4 and 500mg/kg (Group I) in Table 5 was significantly higher ($P<0.001$) than the control and also in groups administered with 500 and 1000mg/kg of hexane extract for 4 weeks (Groups F and G) in Table 4 and 1000mg/kg (Group J) of the aqueous leaf extract at $P<0.05$ in Table 5. There was significant decrease ($P<0.001$) observed in Cl^- level in groups administered with 1000mg/kg of the methanolic extract (Group D) in Table 3, 1000mg/kg of the hexane extract (Group G) in Table 4 and 250, 500 and 1000mg/kg of the aqueous leaf extract of *Guiera senegalensis* for 4 weeks (Groups H, I and J) in Table 5 compared to the control Group. A significant increase ($P<0.001$) was also observed for HCO_3^- levels in rats administered with 500 and 1000mg/kg of the methanolic extract (Groups C and D) in Table 3, 1000mg/kg of the hexane extract (Group G) in Table 4 and 250, 500 and 1000mg/kg of the aqueous extract for 4 weeks (Groups H, I and J) in Table 5 compared to the control Group.

Table 1: Dose and mortality recorded following a 24hr administration of Guiera senegalensis leaf extract.

Phase 1(Groups)	No. of animals	Dose(mg/kg)	Mortality recorded
1	3	10 (methanolic extract)	0/3
2	3	100 (methanolic extract)	0/3
3	3	1000 (methanolic extract)	0/3
4	3	10 (hexane extract)	0/3
5	3	100 (hexane extract)	0/3
6	3	1000 (hexane extract)	0/3

Table 2: Dose and mortality recorded following a 24hr administration of Guiera senegalensis leaf extract.

Phase II (Groups)	No. of animals	Dose(mg/kg)	Mortality recorded
1	1	1250 (methanolic extract)	0/1
2	1	2000 (methanolic extract)	0/1
3	1	2750 (methanolic extract)	0/1
4	1	3750 (methanolic extract)	0/1
5	1	5000 (methanolic extract)	0/1
6	1	1250 (hexane extract)	0/1
7	1	2000 (hexane extract)	0/1
8	1	2750 (hexane extract)	0/1
9	1	3750 (hexane extract)	0/1
10	1	5000 (hexane extract)	0/1

Table 3: Serum liver (AST, ALP, ALT in IU, Bil. In µmol/l), electrolytes (Na^+, K^+ and Cl^- in mEq/L, HCO_3^- in mmol/L), and heart (Ck in U/I, LDH in IU/L) indices of rats treated with different doses of methanolic leaf extract of *Guiera senegalensis* for 4 weeks

Parameters Groups	AST	ALP	ALT	LDH	CK	T. BIL	HCO_3	Cl^-	K^+	Na^+
A 0mg/kg	59.75 ± 22.82	70.94 ± 11.50	12.75 ± 1.26	190.57 ± 9.77	128.20 ± 36.50	9.22 ± 0.52	17.43 ± 5.24	105.69 ± 7.10	1.91 ± 0.90	133.48 ± 4.58
B 250 mg/kg	55.00 ± 22.88	48.40 ± 5.70	13.75 ± 0.96	133.63 ± 23.20	189.87 ± 21.31	16.98 ± 1.02	19.52 ± 1.50	103.98 ± 1.00	4.29 ± 2.01	138.18 ± 2.44
C 500 mg/Kg	50.25 ± 8.42	50.68 ± 10.81	13.00 ± 1.83	145.23 ± 13.21	136.30 ± 16.66	4.44 ± 0.40	27.98[a] ± 0.28	99.24 ± 5.98	8.13[a] ± 1.98	136.03 ± 4.87
D 1000 mg/Kg	50.50 ± 12.56	76.33 ± 8.89	15.75 ± 2.63	174.39 ± 10.95	111.70 ± 8.45	3.69 ± 1.42	28.39[a] ± 0.42	81.24[a] ± 2.35	7.74[a] ± 2.12	138.83 ± 4.32

All values are presented as mean ± standard deviation. Values with superscripts [a] mean significant difference at $P<0.001$ when compared to control, n = 4 in each case.

Table 4: Serum liver (AST, ALP, ALT in IU, Bil. In µmol/l), electrolytes (Na^+, K^+ and Cl^- in mEq/L, HCO_3^- in mmol/L), and heart (CK in U/I , LDH in IU/L) indices of rats treated with different doses of hexane leaf extract of *Guiera senegalensis* for 4 weeks.

Parameters Groups	AST	ALP	ALT	LDH	CK	T. BIL	HCO_3	Cl^-	K^+	Na^+
A 0mg/kg	59.75 ± 22.82	70.94 ± 11.50	12.75 ± 1.26	190.57 ± 9.77	128.20 ± 36.50	9.22 ± 0.52	17.43 ± 5.24	105.69 ± 7.10	1.91 ± 0.90	133.48 ± 4.58
E 250 mg/kg	57.75 ± 10.72	48.28 ± 7.35	16.25 ± 4.19	145.27 ± 22.35	171.09 ± 26.24	46.62b ± 9.96	24.24 ± 5.16	100.76 ± 4.03	7.42[b] ± 1.72	135.15 ± 8.13
F 500 mg/Kg	45.00 ± 13.52	53.18 ± 14.03	8.75 ± 0.96	170.03 ± 27.91	136.21 ± 15.81	36.91[b] ± 10.45	26.22 ± 1.53	100.95 ± 5.08	6.66[a] ± 0.98	134.03 ± 4.03
G 1000 mg/Kg	58.50 ± 13.08	74.10 ± 15.97	19.25[a] ± 2.21	306.43 ± 67.82	94.41 ± 8.18	56.80[b] ± 7.45	27.70[b] ± 0.85	78.79[b] ± 5.20	6.66[a] ± 1.43	133.74 ± 4.23

All values are presented as mean ± standard deviation. Values with superscripts [a] and [b] mean significant difference at P<0.05 and P<0.001 respectively when compared to control, n = 4 in each case.

Table 5: Serum liver (AST, ALP, ALT in IU, Bil. In µmol/l), electrolytes (Na^+, K^+ and Cl^- in mEq/L, HCO_3^- in mmol/L), and heart (Ck in U/I , LDH in IU/L) indices of rats treated with different doses of aqueous leaf extract of *Guiera senegalensis* For 4 weeks.

Parameters Groups	AST	ALP	ALT	LDH	CK	T. BIL	HCO_3	Cl^-	K^+	Na^+
A 0mg/kg	59.75 ± 22.82	70.94 ± 11.50	12.75 ± 1.26	190.57 ± 9.77	128.20 ± 36.50	9.22 ± 0.52	17.43 ± 5.24	105.69 ± 7.10	1.91 ± 0.90	133.48 ± 4.58
H 250 mg/kg	65.00 ± 13.12	67.28 ± 8.30	17.25 ± 2.22	103.13 ± 6.32	135.70 ± 8.64	43.39[b] ± 8.75	27.48[b] ± 3.76	80.68[b] ± 3.87	5.46 ± 1.30	138.35 ± 4.99
I 500 mg/Kg	68.50 ± 15.86	76.18 ± 6.70	14.50 ± 4.51	223.97 ± 88.57	160.97 ± 28.20	11.48[a] ± 1.33	28.74[b] ± 0.28	81.44[b] ± 5.78	7.33[b] ± 0.46	140.45 ± 5.68
J 1000 mg/Kg	53.50 ± 19.28	82.55 ± 6.61	13.00 ± 2.16	357.53 ± 39.01	184.84 ± 53.29	5.51[a] ± 2.20	27.98[b] ± 1.28	87.42[b] ± 2.00	6.49[a] ± 1.14	130.07 ± 2.35

All values are presented as mean ± standard deviation. Values with superscripts [a] and [b] mean significant difference at P<0.05 and P<0.001 respectively when compared to control, n = 4 in each case.

DISCUSSION

Acute toxicity

In screening drugs, determination of LD_{50} [the dose which has proved to be lethal (causing death) to 50% of the tested group of animals] is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. It is an initial assessment of toxic manifestations (provides information on health hazards likely to arise from short-term exposure to drugs) and is one of the initial screening experiments performed with all compounds [2]. Oral administration of methanolic and hexane extracts did not produce any mortality in rats up to a dose level of 5000mg/kg. The animals used for the oral LD_{50} determination did not exhibit any toxicological signs such as depression, writhing, diarrhoea, hypermotility and aggression compared to the control.

Based on Hodge and Sterner scale [7], a test drug administered orally is considered extremely toxic at≤ 1 mg kg^{-1}, highly toxic at 1-50 mg kg^{-1}, moderately toxic at 50-500 mg kg^{-1}, slightly toxic at 500-5000 mg kg^{-1}, practically non toxic at 5000-15,000 mg kg^{-1} and relatively harmless at ≥15,00 mg kg^{-1}. In the current study, the crude extract could be declared practically non-toxic. Methanolic extract of the plant was found to be fatal to male albino rats at a daily concentration of 1000 and 500 mg/kg/day after one week when given intramuscularly and aqueous extract was found to have some toxicity but no mortality was recorded [6]. These may be due to endotheliotoxic effect of the plant as reflected by the hemorrhage seen in the internal organs [6]. The LD_{50} of *Boerhavia diffusa* aqueous leaf extract has been reported to be >2000 mg kg^{-1} body weight in both mice and rats [27]. The LD_{50} of *Albizzia chevalieri* Harms (Leguminosae) aqueous leaf extract has been reported to be > 3000 mg kg^{-1}body weight in rats. The LD_{50} of *Aspilia Africana* Leaves has been reported to be 6.1 g/kg and 7.5 g/kg body weight for males and females respectively with an average of 6.6 g/kg body weight in mice [22].

Sub- Acute Toxicity

Effect on Liver Indices

The liver function indices (ALT, AST, ALP and TBIL.) in groups of rats treated with different doses of methanolic extracts for 4 weeks did not show any significant difference when compared to the normal control. This may indicate that the methanolic extract does not have any toxic effects on the liver.

There was no significant difference observed in AST and ALP activities in animals administered the hexane extract, however there was significant increase observed in ALT levels of rats treated with 1000mg/kg (Group G) of the hexane extract ($P<0.05$). Also the value of total bilirubin was significantly higher in the rats administered with 250, 500 and 1000mg/kg hexane extract for 4 weeks (Groups E, F and G) than the control.

Also, there were no significant changes in ALT, AST and ALP activities in all the groups administered the aqueous extract compared to the control group, but there was a very significant increase observed in TBIL levels in rats administered with 250mg/kg (Group H) and 500mg/kg (Groups I) aqueous extract for 4 weeks at $P<0.001$ and $P<0.05$ respectively than the control. However significant decrease ($P<0.05$) was observed in rats administered with 1000mg/kg of the aqueous extract compared to the control.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are useful indices for identifying inflammation and necrosis of the liver [32]. ALT has its highest concentration in the liver and kidney, with skeletal muscles having lesser activity of the enzyme. The activity of AST is located in the microsomal and mitochondrial portions of the liver cells as well as in the skin, skeletal and cardiac muscles, pancreas and kidney. ALT measurements are more liver specific than AST and its activity is usually greater than AST activity at early or acute hepatocellular disease [36]. AST on the other hand tend to be released more than the ALT in chronic liver diseases such as cirrhosis [36]. In this study, the increase in ALT in rats administered with 1000mg/kg of the hexane extract (Group G) was dose dependent, as the values obtained for the animals on the highest dose of the extract was significantly ($p<0.05$) higher than the value obtained for the control. It may be inferred therefore, that these changes may be due to the solvent used (hexane) which could have been able to extract more toxic constituents of the leaf than other solvents used. These toxic constituents might have caused mild hepatotoxicity, which was not indicated in the activities of other enzymes (i.e. AST and ALP). Elevated levels of AST and ALT are often diagnostic of underlying cellular injuries [20],[35].

The activity of Alkaline phosphatase (ALP) is increased in many clinical states; the most important being bone and liver diseases. Accordingly, serum ALP is a useful diagnostic, screening and follow-up tools of cholestatic hepatobiliary lesions and osteoblastic bone diseases [37]. Cholestasis is the main, if not the only, liver disease responsible for increased plasma alkaline phosphatase activity. Thus, a normal alkaline phosphatase activity, in the presence of abnormal levels of other liver function parameters, may be suggestive of liver pathology other than obstruction [32]. In the present study, the alkaline phosphatase activities of animals treated with oral doses of the different extract were not significantly different from the normal control. This means oral administration of the leaf extract of *Guiera senegalensis* at up to a daily dose of 1000mg/kg for 4 weeks could not lead to cholestasis. In another study, it was demonstrated that animals treated with aqueous leaf extract of *Ocinum gratissium*, used for the treatment of rheumatism and paralysis, did not affect the activities of ALT, AST and ALP [10].

Bilirubin is a useful index of the excretory function of the liver, in addition to its being a useful tool in the assessment of haemolytic anaemia. In this study no significant difference was observed in the groups administered with the methanolic extract. There was however significant increase observed in groups treated with hexane (Group G; $P<0.05$) and aqueous extracts (Group H; $P<0.001$ and Groups I; $P<0.05$). This elevation may be attributed to haemolytic anaemia caused as a result of the solvents used which might have extracted some toxic components of the plant that were not extracted by methanol. The decrease observed in the group administered with 1000mg/kg of the aqueous leaf extract (Group J) may be because the aqueous extract of *Guiera senegalensis* at a higher dose could be curative as it has been observed that the decrease in serum bilirubin is a bimodal fashion when biliary obstruction is resolved [3].

Heart Indices

Creatine Kinase (MB) is usually present in serum at low concentration; it increases after an acute infarct of myocardium and later descends at normal levels, it also increases rarely in skeletal muscle damage [31]. LDH is one of the most clinically important protein markers in serum because its level changes in response to a number of health-related states. In addition, this enzyme can be used to detect cytotoxicity and cell number in *in vitro* cell culture systems. Therefore, monitoring serum levels of LDH has become a routine and fundamental means to monitor organ toxicity [15],[31]. In this study, there was no significant differences observed in CK and LDH activities in all the groups administered with the methanolic, hexane and aqueous extracts compared to the control group, which may indicate that the extracts may not have any toxic effect on the heart. Aqueous leaf extract of *Piper methylsticum* did not affect significantly the serum lactate dehydrogenase activity [30].

Electrolytes

The levels of electrolytes in the blood are the outcome of fine regulatory mechanism of ionic charges and the osmotic balance. This homeostasis is achieved by an interplay involving the kidney, the lungs and endocrine system [32]. Sodium is the major cation of the extracellular fluid where it regulates acid-base equilibrium and protects the body against excessive fluid loss. Potassium is the major intracellular cation with similar role to those of sodium. Hyperkalaemia is usually encountered frequently in renal failure, improper use of K^+ sparing diuretics, hypoaldosteronism, insulin deficiency associated hyperglycaemia, Addison's disease and massive tissue destruction [8],[32]. Plasma bicarbonate ion concentration is increased in respiratory acidosis and metabolic alkalosis but decreased in respiratory alkalosis and metabolic acidosis [8],[32],[36],[16].

In this study there was a significant increase ($P<0.001$) observed in the levels of K^+ in rats administered with 500 and 1000mg/kg of the methanolic extract for 4 weeks (Groups C and D) following the administration of the methanolic extract. A significant increase was also observed for rats administered with 250mg/kg ($P<0.001$), 500 and 1000mg/kg ($P<0.05$) of the hexane extract for 4 weeks (Groups E, F and G) when compared to the control group in the levels of K^+ when compared to the control group. Administration of the aqueous extract also resulted in significant increase in the levels of K^+ for Group I rats ($P<0.001$) which were administered with 500mg/kg and in Group J rats ($P<0.05$) which were administered with

1000mg/kg compared to the control group. Increase in serum potassium is seen in states characterized by excessive destruction of cells. The increase in the serum K^+ may also be an indication that membrane channels may possibly be affected by the leaf extract or that the plant may have hyperkalaemic effect. Hyperkalaemia, or excess potassium in the blood, occurs in cases of renal failure because the kidneys loose the ability to excrete the mineral. Severe dehydration will also produce Hyperkalaemia [21]. In another study, it was reported that aqueous extract of *C. occidentalis* seeds could have hyperkalaemic as well as hyponatremic effect when administered orally at a daily dose of 60 mg/kg for > one week [1].

The administration of the methanolic extract produced a significant decrease ($P<0.001$) in Cl^- levels in rats administered 1000mg/kg (Group D) compared to the control group. The administration of the hexane extract also produced a significant decrease ($P<0.001$) in Cl^- levels in rats administered 1000mg/kg (Group G) compared to the control group. The administration of the aqueous extract produced a significant decrease ($P<0.001$) in Cl^- levels in all the rats (Groups E, F and G) compared to the control group. A significant increase ($P<0.001$) was also observed in levels of HCO_3^- in rats administered with 500 and 1000mg/kg of the methanolic extract (Groups C and D) after 4 weeks of administration of the methanolic extract. The administration of the hexane extract produced a significant increase ($P<0.001$) in HCO_3^- levels in rats treated with 1000mg/kg (Group G) and there was significant increase ($P<0.001$) in HCO_3^- levels observed among all the groups administered with the aqueous extract at 250, 500 and 1000mg/kg (Groups H, I and J) when compared to the control group. This type of finding is often associated with total chloride depletion is metabolic alkalosis (blood pH greater than 7.45). The reabsorption of sodium bicarbonate ($NaHCO_3$) in the proximal and distal tubule is augmented because total body chloride depletion results in both ECF volume contraction (which stimulates HCO_3^- reabsorption) and decreased quantities of filtered chloride available to the tubules for reabsorption with sodium [25]. Specific acid–base abnormalities may also be associated with hypochloremia. Conditions associated with a respiratory acidosis cause the proximal tubule to increase its secretion of hydrogen ion. This results in sodium being retained prefentially as sodium bicarbonate and not sodium chloride. Although this is a compensatory mechanism to help ameliorate the acidemia, the end result is increased concentrations of serum bicarbonate and decreased serum chloride concentrations [25]. Increase in serum chloride is also seen in dehydration, renal tubular acidosis, acute renal failure, diabetes insipidus, prolonged diarrhoea [13]. Increase in the levels of HCO_3^- and decrease in Cl^- levels in this study may suggest that the extract induced some kind of metabolic alkalosis.

There was no significant changes in Na^+ level in all the groups administered the methanolic, hexane and aqueous extracts compared to the control group. This work is similar to the one reported by [12] in which normal serum level of Na^+ was found in animals treated with extract of *M. balsamina*.

CONCLUSION

In conclusion, considering the levels of the serum biochemical parameters of the animals treated with both the acute and sub-chronic doses of the oral aqueous leaf extract of *Guiera senegalensis*, it may be apparent to suggest that the plant extract may be safe, especially at the therapeutic dose which is far lower than the tested doses. . The leaf extract can be said to be practically non toxic as far as acute toxicity is concerned.

The little or no toxicity observed in this study may not be unconnected with the fact that the secondary plant metabolites that may likely cause toxicity effects may be either absent from the extract or present in minute quantity. The above finding may indicate that the leaf extract may have erythrocyte hemolytic effect which may lead to hyperkalemia and could lead to a disturbance in acid- base balance when administered at higher doses.

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