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Research Article

ACUTE AND SUB-ACUTE TOXICITY STUDIES OF ETHYL ACETATE ROOT EXTRACT OF *GUIERA SENEGALENSIS* IN RATS

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ABSTRACT

The acute and sub-acute toxicity studies of ethyl acetate root extract of *Guiera senegalensis* was carried out in rats. Administration of 80,1600 and 3200 mg/kg body weight of the ethyl acetate root extract produced 20,80 and 100 % mortality in rats respectively. The calculated LD_{50} was 1160 mg/kg body weight. There was a statistically significant increase in the intestine body weight ratio with mean of(1.27±0.21 %) of the group given 400 mg/kg body weight of the extract relative to the control with mean of (0.75±0.13 %). There was an increase in the RBC count of the extract (400 mg /kg) treated group with mean value of 8.15 ± 0.45 x 10^6 /mm^3 when compared with the control group with mean value of 7.10 ± 0.05 x 10^6 /$mm^{3.}$ The WBC count also increased significantly when the control group with mean value of 10.55±50. 00 x 10^3 /mm^3 were compared with the extract treated group with mean values of 12.75 ± 50.00, 14.45 ± 50.00 and 17.65 ± 50.00 x 10^3/mm^3 respectively. The differential leucocyte count analysis showed that there was an increase in the lymphocyte, monocyte and neutrophil count which was statistically significant. There was a slight decrease (4.15 ± 0.47 mg/dl) in the urea concentration of the group given the dose of 400 mg/kg of the extract which was statistically significant ($p<0.05$). The histopathologic examination of the organs of rats harvested from the sub-acute studies (liver, kidney and intestine) of extract treated groups when compared with the control group revealed some histologic lesions which included congestion of central vein of liver, mild hydrophobic change in the tubular lumen and interstitial nuclear cell infiltration of the kidney and globlet cell hyperplasia of the intestine in rats. These lesions were observed to be pronounced in the in group treated with 400 mg/kg body weight of the ethyl acetate extract. In conclusion, the study established the safety of ethyl acetate root extract of *G. senegalensis* and thus could be used at lower doses as an antidiarrhoeal drug.

Key Words: Acute, sub-acute, LD_{50}, Ethyl acetate, haematology, LFT, KFT and histopathology.

INTRODUCTION

The plant *Guiera senegalensis* J. F. Gmel is a member of the family Combretaceae[1]. It is a small shrub with green leaves. It is called "sabara" in Hausa and "kashishi" in Kanuri. The plant is widely distributed in Nigeria, Senegal, Gambia, Mali, Niger and Burkina Faso[2,3] stated that the macerated leaves of the plant were used orally for the treatment of febrifuge as well as for hyperglycaemia and hypertension whereas the roots were used mainly as antileprosy[4] claimed that the plant is used by Fulani traditional healers to treat several disorders including veneral diseases. The root concoction is used to cure diarrhoea, dysentery and microbial infections. The plant continues to be one of the plants used by local livestock farmers, traditional healers and Fulani herdsmen in the treatment of snake bite in northern Nigeria[5]. Phytomedicine derived from plants have shown great promise in the treatment of intractable infectious disease including opportunistic acquired immuno deficiency syndrome (AIDS) infections[6]. About 80% of the rural population in developing countries, Nigeria in particular depends on it as an alternative to primary health care. This represents a potential pharmaceutical market and is an incentive for research into new drugs. Thus, in this study, the effect of ethyl acetate root extract of *Guiera senegalensis* on some haematological, biochemical and histopathological parameters was assessed.

MATERIALS AND METHODS

The plant material (root) of *Guiera senegalensis* was collected from Jere Local Government Area of Borno State, Nigeria. It was identified and authenticated by a plant taxonomist from the Department of Biological sciences, University of Maiduguri. A voucher specimen with number BCH GRI was deposited at the herbarium of the Biochemistry Department, University of Maiduguri, Nigeria. Fresh root of *G. senegalensis* was dried in the open air and ground to powder form and kept in cellophane bag at 4°C before extraction.

Plant Extraction

A 2000 g portion of the weighed, powdered dried root sample was extracted using ethyl acetate. The sample was put into 1 litre separating funnel, this was covered, shaken every 30 min for 6 h and then allowed to stand for about 48 h. The solution was subsequently shaken and filtered using Whatman filter paper NO.1[7]. The filtrate was evaporated to dryness using a rotary evaporator. The extract was then stored below ambient temperature.

Experimental Animals

White Wistar strain albino rats of both sexes weighing between 150–200g were acquired from the animal house of Department of Biochemistry, University of Maiduguri, Nigeria. All animals were used after 1 week of acclimatization; they had free access to water and food. The experiments reported here comply with ethical procedures with investigated animals.

Acute Toxicity Study

Determination of lethal dose (LD_{50}) of the ethyl acetate root extract of *Guiera senegalensis* using rats was determined by the arithmetic method of Karber as modified by[8].

A total number of 35 rats weighing between 150 – 200g were randomly divided into 7 groups (A, B, C, D, E, F and G) of 5 rats each. The rats in groups B, C, D, E, F and G were intraperitonealy treated with increasing doses of 100, 200, 400, 800, 1600 and 3200 mg/kg body weight i.e 200 g/100ml concentration of ethyl acetate root extract of *G. senegalensis.* The rats were observed over a 24 h period for clinical symptoms and death. The LD_{50} of the *G. senegalensis* was

then calculated using the[8]. In this method, the difference between the consecutive doses and the mean of the number of rats dying at the two dose levels were calculated and the sum of the products of the mean dead and the dose difference divided by the number of rats in each group. The resulting quotient was subtracted from the least dose that killed all the rats so as to obtain the LD_{50}.

Sub-Acute Toxicity Studies

Treatment and Experimental Design

A randomized block design with 5 rats in each group representing a block was used. The rats were divided into 4 groups (A – D) of 5 animals each. Rats in group A served as the control to obtain baseline data, while rats in groups B, C and D were treated orally with 100, 200 and 400 mg/kg body weight of the ethyl acetate root extract of *G. senegalensis* respectively, using a feeding tube (BIDI feed tube, size 8). The experiment lasted for a period of 28 days. Haematological, biochemical and histopathological parameters were used to assess the effects of the various doses of the ethyl acetate extract on rats. Blood samples were collected from the tail of each rat one day before commencement of extract administration and before being sacrificed for the determination of terminal haematological parameters.

Determination of Haematological Parameter

The red blood cells (RBC) count was carried out using the improved Neubauer counting chamber as described by[9].The white blood cells (WBC) were counted using a haemocytometer as described by[10]. The estimation of the packed cell volume (PCV) was by the Microhaematocrit method[9].The haemoglobin concentration was determined by the Cyanmethaemoglobin method using a colorimeter[11]. The differential leucocyte count (DLC) was carried out by the longitudinal method.

Collection of Blood Sample

The blood sample for the biochemical analysis was collected after the animals were humanely sacrificed by decapitation 24 h after the last treatment. The blood was centrifuged and the clear serum was used for analysis[12].

Determination or Assay of Biochemical Parameters

ASAT and ALAT were assayed using kits based on the method of [13]. ALP was assayed using the method of[14]. Serum albumin was assayed by the bromocresol green method of [15]. Serum urea and creatinine were assayed by the method of[16] using Randox laboratory kit. The electrolytes were determined using the flame photometric method of[11].Globulin and total bilirubin were analysed by colorimetric method.

Statistical Analysis

The data collected were summarized as mean ± SD and subjected to analysis of variance (ANOVA) and Turkey comparison test using INSTAT statistical software.

RESULTS

Acute Toxicity Study in Rats

There was weakness, depression, ruffled hair, transient anorexia observed some minutes after administration of *G. senegalensis* ethyl acetate root extract, while dehydration, coma and death appeared after 2 h at 800, 1600 and 3200 mg/kg.

Mortality Scores (LD_{50})

Administration of 800, 1600 and 3200 mg/kg of the extract produced 20, 80 and 100 % mortality in rats respectively. The calculated LD_{50} was 1160 mg/kg body weight. At doses of 100, 200 and 400 mg/kg, there was no mortality recorded. Table 1 shows the LD_{50} of ethyl acetate root extract of *G. senegalensis*.

Sub-acute Toxicity

Effect of Ethyl acetate Root Extract of *G. senegalensis* on Mean Body Weight of Rat

The result of the effect of the extract on the weekly body weight changes of rats is shown in (Table 2) did not show any change in weight ($p>0.05$)

Effect of Ethyl acetate Root Extract of *G. senegalensis* on Organ-body Weight Ratio in Rats

The result of the organ-body weight ratio is shown in (Table 3).There were no changes in the relative organ weight of all the groups treated with the extract relative to the control. However, there was a statistically significant increase in the intestine body weight ratio with mean of (1.27±0.21 %) of the group given 400 mg/kg body weight of the extract relative to the control with mean of (0.75±0.13%).

Effect of Ethyl acetate Root Extract of *G. senegalensis* on some Haematological parameters in Rats

The result of the analysis of the haematological parameter is shown in (Table 4). There was an increase in the RBC count of the extract (400 mg /kg) treated group with mean value of $8.15 \pm 0.45 \times 10^6$ /mm^3 when compared with the control group with mean value of $7.10 \pm 0.05 \times 10^6$ /mm^3 The WBC count also increased significantly when the control group with mean value of $10.55 \pm 50.00 \times 10^3$ /mm^3 were compared with the extract treated group with mean values of 12.75 ± 50.00, 14.45 ± 50.00 and $17.65 \pm 50.00 \times 10^3$/mm^3 respectively. The differential leucocyte count analysis showed that there was an increase in the lymphocyte, monocyte and neutrophil count which was statistically significant.

Effect of Ethyl acetate Root Extract of *G. senegalensis* on some Liver Function Parameter in Rats

Table 5 shows the result of the effect of ethyl acetate root extract of *G. senegalensis* on serum aspartate amino transferase, alanine amino transferase, alkaline phosphatase, albumin, total protein, globulin and total bilirubin.

The result showed that there was no significant difference ($p>0.05$) in the ASAT levels when the control group was compared with the extract treated groups at doses of 100, 200 and 400 mg/kg respectively. At the highest dose of 400 mg/kg and the lowest dose of 100 mg/kg there was increase in the serum ASAT levels (55.5 ± 4.04 and 43.5 ± 15.30 u/L) when compared with the control group (36.25 ± 15.43 u/L) though not statistically significant.

The increase in ALAT concentration was not statistically significant ($p>0.05$) when the control group (23 ± 2.3 u/L) was compared with the extract treated groups that were given 200 and 400 mg/kg (24 ±2.00 and 27±2.30u/L) respectively as against the one that was given 100 mg/kg (29.25 ± 3.68 u/L) which was statistically significant ($p<0.05$).

There was no statistically significant difference ($p>0.05$) in the ALP levels when the control group was compared with the extract treated groups.

There was a significant difference ($p<0.05$) in the serum albumin levels when the control group (31.5 ±0.57 g/dl) was compared with the extract treated group (27.25±1.70 g/dl) that was given 100 mg/kg while the groups given 200 and 400 mg/kg the difference is not statistically significant. There was no statistically significant difference in the globulin and

total bilirubin concentrations when the control group was compared with the extract treated group.

Effect of Ethyl acetate Root Extract of *G. senegalensis* on Some Kidney Function Parameter in Rats

There was a slight decrease (4.15 ± 0.47 mg/dl) in the urea concentration of the group given the dose of 400 mg/kg of the extract which was statistically significant ($p<0.05$). The increase in urea concentration observed in the groups given 100 and 200 mg/kg of the extract was not statistically significant ($p>0.05$) when the control group (5.25±1.21 mg/dl) was compared with the extract treated groups 2 and 3 (6.75±1.29 and 6.37±1.31 mg/dl) respectively (Table 6)

There was no statistically significant difference in the creatinine concentration of the control group and that of the extract treated groups.

There was no statistically significant difference in the concentration of electrolytes of the control group and that of the extract treated groups.

Histopathology

The histopathologic examination of the organs of rats harvested from the sub-acute studies (liver, kidney and intestine) of extract treated groups when compared with the control group revealed some histologic lesions which included congestion of central vein of liver, mild hydrophobic change in the tubular lumen and interstitial nuclear cell infiltration of the kidney and globlet cell hyperplasia of the intestine in rats. These lesions were observed to be pronounced in the in the group treated with 400 mg/kg body weight of the ethyl acetate extract (plates 1-5).

Table 1: LD_{50} of Ethyl acetate Root Extract of *G. senegalensis*

Group	Doses mg/kg	No of rats	No of death	Dose difference (Dd)	Mean death (Md)	Dose difference x mean death
A	Saline	5	0	0	0	0
B	150	5	0	0	0	0
C	200	5	0	100	0	0
D	400	5	0	200	0	0
E	800	5	1	400	0.5	200
F	1600	5	4	800	2.5	2000
G	3200	5	5	1600	5.0	8000
Total						**10,200**

$LD_{50} = LD_{3200} - \frac{Md \times Dd}{n}$ $\quad LD_{50}$ = 1160 mg/kg body weight

$LD_{3200} - \frac{10,200}{5}$

$LD_{3200} - 2040 - 1160$

Table 2: Effect of Ethyl acetate Root Extract of *G. senegalensis* on the Body Weight in Rats

Groups	Dosage Mg/kg	Body weight change Days of Treatment				
		0	7	14	21	28
1	control	139.05±8.70	170.37±20.89	187.12±23.85	185.47±50.78	210±44.74
2	100	201.62±32.55	234.07±38.01	247.25±37.72	260.75±30.78	271.87±27.49
3	200	188.05±555.87	208.45±37.27	212.87±34.31	224.62±44.91	232.92±53.09
4	400	174.17±34.48	184.57±42.71	193.50±35.21	195.25±48.79	218.85±27.58

Values are mean ± S.D of 5 replications, Mean ± S.D are not statistically significant ($p>0.05$).

Table 3: Effect of Ethyl acetate Root Extract of *G. senegalensis* on the Relative Organ Weights in Rats

Group	**Dosage**	**Organ body-weight ratio**	**Kidney-body**	**Intestine-body**
	Mg/kg body weight	**Liver-body weight ratio (%)**	**weight ratio (%)**	**weight ratio (%)**
1	**Control**	3.41 ± 0.38	0.78 ± 0.12	0.75 ± 0.13
2	100	3.10 ± 0.30	0.63 ± 0.03	0.64 ± 0.05^{ns}
3	200	3.25 ±0.40	0.66 ±0.08	0.71 ± 0.31^{ns}
4	400	3.11 ±0.19	0.17 ±0.03	$1.27 \pm 0.21^{*}$

Values are mean ±S.D of 5 replicates, Values with *- statistically significant ($p>0.05$), when compared with the control, NS: not significant ($p>0.05$).

Table 4: Effect of Ethyl acetate Root Extract of *G. senegalensis* on Some Haematological Parameters in Rats

Haematological Parameters	**Treatment**			
	Control	100mg/kg	200mg/kg	400mg/kg
RBC($x10^6$/)	7.10±0.05	7.75±0.05ns	6.75±0.05ns	8.15±0.45*
WBC($x10^3$)	10550±50.00	12750±50.00***	14450±50.00**	17650±50.00***
PCV (%)	45.5±0.95	44.25±1.10	45.5±1.84	47.75±0.25
Hb (g/dl)	16.21±0.21	15.7±0.25	16.45±0.73	17.05±0.78

Key: RBC- Red blood cells count, WBC- White blood cells count, PCV- Packed cell volume, Hb- Haemoglobin, Ns- Not significant, *** ($p<0.001$) significantly different relative to the control group, *($p<0.05$), Values are mean ± S.D of 5 replications

Table 5: Effect of Ethyl acetate Root Extract of *G. senegalensis* on Differential Leucocyte Count in Rats

Haematological Parameters	Treatment			
	Control	100mg/kg	200mg/kg	400mg/kg
Eosinophil (%)	9.5±0.28	10.00 ± 0.00^{ns}	9.75 ± 0.25^{ns}	9.50 ± 0.28^{ns}
Lymphocyte (%)	53.75±0.28	53.5 ± 0.28^{ns}	53.75 ± 0.25^{ns}	$57.75 \pm 0.25^{***}$
Monocyte (%)	8.50±0.28	$10.75 \pm 0.25^{**}$	9.00 ± 0.00^{ns}	9.00 ± 0.40^{ns}
Neutrophil (%)	28.25±0.25	$25 .25 \pm 0.25^{***}$	27.5 ± 0.28^{ns}	$21.75 \pm 0.25^{***}$

Key: Ns: Not significant, *** ($p<0.001$)-Highly significant, ** ($p<0.01$)-Moderately significant

Table 6: Effect of Ethyl acetate Root Extract of *G. senegalensis* on some Liver Function Parameters

Treatment	Dosage Mg/kg	ASAT U/L	ALAT U/L	ALP U/L	ALBUMIN U/L	T.PROTEIN g/L	GLOBULIN mmol/L	T.BILIRUBIN µmol/L
Group 1	Control	36.25±15.43	23.00±2.30	283±43.88	31.5±0.57	68.40±1.22	50.60±1.34	7.40±0.54
Group 2	100	43.5±15.30	29.25±3.68*	265±54.63	27.25±1.70*	67.20±1.34	49.70±1.67	7.32±0.07
Group 3	200	35.25±27.42	24.00±2.00ns	290.5±25.25	29.75±25.25ns	68.00±1.99	50.75±0.97	7.80±0.06
Group 4	400	55.5±4.04	27.00±2.30ns	239±49.14	31.25±1.25ns	67.84±1.99	50.50±1.31	7.83±0.02

Values are Mean ±S.D of 5 replications, Means with asterics are statistically significant ($p < 0.05$) when compared with the control.
Key: ASAT- Aapartate amino transferase, ALAT- Alanine amino transferase, ALP- Alkaline phosphatase

Table 7: Effect of Ethyl acetate Root Extract of *G. senegalensis* on some Kidney Function Parameters in Rats

Treatment	Dosage	Urea	Creatinine	Na^+	k^+	Cl^-	HCO_3^-
Group 1	Control	5.25±1.21	83.5±6.55	136±1.41	5.75±0.68	98±2.30	20.5±0.57
Group 2	100	6.75±1.29	97.75±17.63	136.25±1.25	5.7±0.83	99±2.00	21.25±0.95
Group 3	200	6.37±1.31	78.25±15.00	138±1.63	5.7±0.14	100±0.00	20.75±1.50
Group 4	400	4.15±0.47*	64±6.37*	135.5±4.12	5.67±0.23	95±5.77	20.5±0.57

Values are Mean± S.D of 5 replicates, Values with * (Group 2 Vs Group 4 of both urea and creatinine) are statistically significant within the group ($p > 0.05$).

Photomicrographs of Liver, Kidney and Intestine of Rats

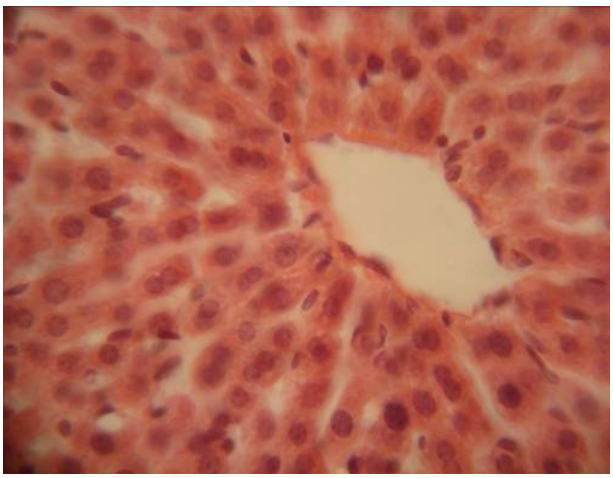

Plate 1: Photomicrograph of rat liver of control group showing hepatocytes (black arrows) radiating away from the central vein (CV) and clear sinusoids (white arrows) H&E x400

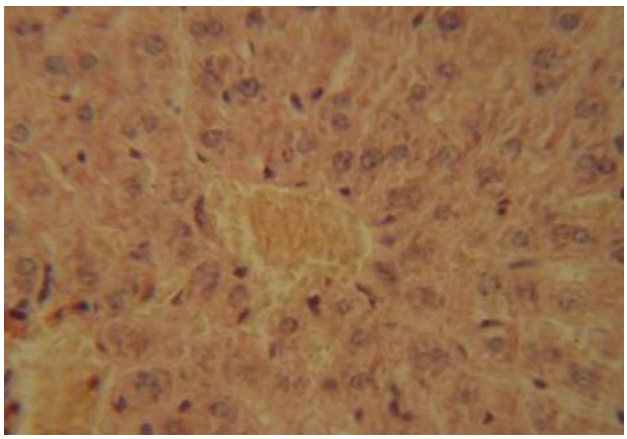

Plate 2: Photomicrograph of rat liver of group 2 showing congestion of the central vein (arrows) H&E x400

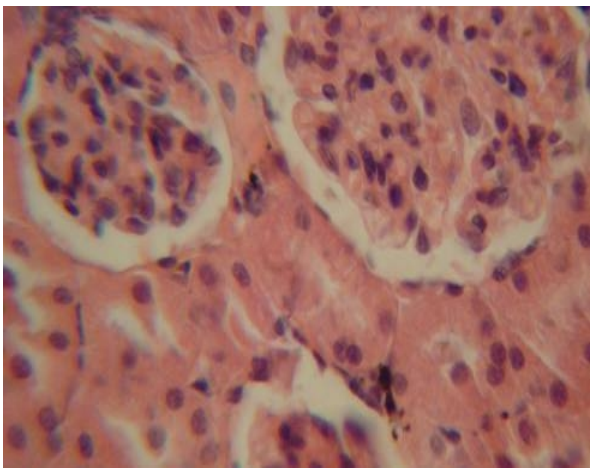

Plate 3: Photomicrograph of rat kidney of control showing normal features H&E x400

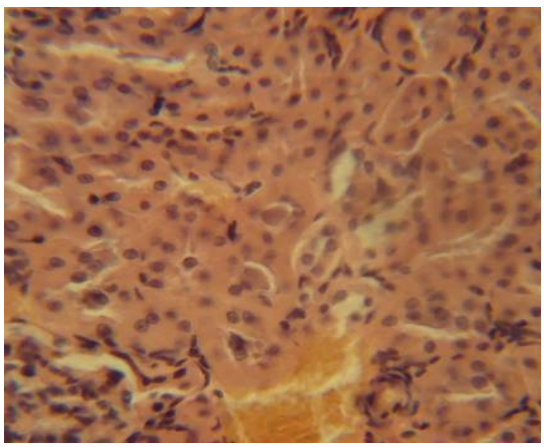

Plate 4: Photomicrograph of rat kidney of group 2 showing mild hydrophobic change (arrows) in the tubular lumen and focal haemorrhage (H) H&E x400.

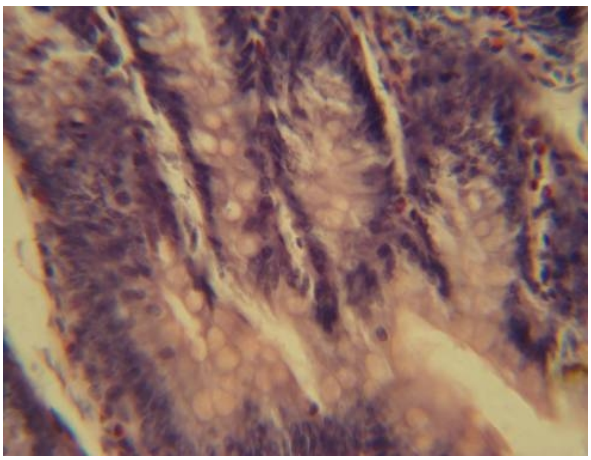

Plate 5: Photomicrograph of rat intestine of group 4 showing mild eosinophilic cells reaction and goblet cell hyperplasia (GB) H&E x400

DISCUSSION

Acute toxicity study of ethyl acetate root extract of *G. senegalensis* revealed that the intraperitoneal administration gave the LD_{50} of the extract as 1160 mg/kg body weight which suggest that the extract is moderately toxic to rats and may be associated with the content of tannins and alkaloids[17,18]. Clinical signs of the acute toxicity were dose dependent. At high doses there were weakness, depression and ruffled hair and transient anorexia followed by dehydration, coma and finally death at 3200 mg/kg body weight.

According to[17,19,20] reported that substances with LD_{50} of between 500 and 5000 mg/kg are moderately toxic and could be administered with some degree of caution especially through the oral route where the absorption might not be completed but gradual due to inherent factors limiting absorption in the gastrointestinal tract[21].

Liver and kidney are important organs of the body which play a vital role in metabolic processes of the body. Liver detoxifies substances that are harmful to the body. The kidney helps in maintaining homeostasis of the body by reabsorbing vital substances and excretion of waste products[10]. In the assessment of liver damage by drugs or any other chemical, determination of ALAT, ASAT and ALP levels is largely used[22]. In the present study, oral administration of the extract to the rats did not cause any significant increase in the serum AST, ALAT and ALP levels. The increase in the ALAT levels of the rats given 100 mg/kg was statistically significant ($p<0.05$). Ordinarily, liver cell damage is characterized by rise in plasma enzymes (ASAT and ALAT etc), from the findings, ASAT concentrations were consistently higher than ALAT levels which is expected since body cells contain more ASAT than ALAT[11], usually, about 80% of ASAT is found in the mitochondria whereas ALAT is a purely cytosolic enzyme. Therefore, ASAT appears in higher concentrations in a number of tissues (Liver, kidney, heart, pancreas) and is released slowly in comparison to ALAT. But since ALAT is localized primarily in the cytosol of hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage than ASAT and within limits can provide a quantitative assessment of the degree of damage sustained by the liver[23]. Therefore, the significant elevation in ALAT (29.25±3.68u/L) may be an indication of hepatocellualr damage.

A rise in plasma alkaline phosphatase (ALP) level is usually a characteristic finding in obstructive hepatobiliary disease as found in cholestatic liver disease [24]. Hence the reduction in ALP levels though not significant by the ethyl acetate extract of *G. senegalensis* shows that no possible cholestasis occurred at the tested doses. Similarly, the non-remarkable changes in urea and creatinine probably indicate that *G. senegalensis* extract did not interfere with the capacity of the kidney to excrete these metabolites. It may also be a reflection of the preserved renal integrity of treated rats [24].

Electrolytes are compounds that are ionizable in solution, they play an essential role in many biochemical processes of the body and cells create electrical energy as ions more from the intracellular solutions of the cells to the extracellular solutions of the cells. These changes in the concentration of one or more of these ions can occur during various acute and chronic disease states and can lead to serious consequences [25]. In this study, there was no significant difference ($p>0.05$) in the concentrations of the electrolytes (Na^+, K^+, Cl and HCO_3^{2-}) at the doses tested, thus indicating that the extract did not have any effect on the electrolyte concentrations.

The findings of the study showed that oral administration of the ethyl acetate root extract of *G. senegalensis* for 28 days did not induce any biochemical signs of toxicity, thus it can be defined as the no-observed-adverse-effect level (NOAEL) for Wistar strain rats under the experimental conditions used. The Histopathological results did not reveal any lesions when the extract was used at lower doses.

In conclusion, the study established the safety of ethyl acetate root extract of *G. senegalensis* and thus could be used at lower doses as an antidiarrhoeal drug.

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