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ORIGINAL RESEARCH

Evaluation of Aqueous Stem Bark Extract of *Guiera senegalensis* on Wistar Rats

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Abstract

Objective: Global herbal products however have enormous potential as natural drugs and are of vast commercial significance, are often processed and procured without being scientifically evaluated for their toxicity. This study evaluated the toxicological effects of *Guiera senegalensis* on wistar rats.

Material-Method: An acute toxicity evaluation was carried out to determine the LD_{50} of *Guiera senegalensis* stem bark extract where eleven (11) rats were used. Sub-acute toxicity was carried out to determine the effect of the plant extract on some liver and kidney function parameters and haematological parameters. For sub-acute toxicity studies, twenty (20) rats were randomly placed into 4 groups of 5 rats each. Group 2, 3 and 4 were orally treated with aqueous stem bark extract of *Guiera senegalensis* at a daily dose of 200, 400 and 800 mg/kg body weight (b.wt.) respectively for 28 days while group 1 served as a control group.

Results: Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin levels increased significantly as dose increased. No significant increase in K^+ and urea was observed in group 2 and 3 treated with 200 and 400 mg/kg respectively. However, significant increase was observed in group 4 treated with 800 mg/kg. Na^+ and Creatinine showed significant increase when compared with control group. Red blood cells, packed cell volume, and haemoglobin concentrations decreased significantly whereas a significant increase was observed in white blood cells with increase in dose respectively.

Conclusion: The aqueous stem bark extract of *Guiera senegalensis* have a dose-dependent toxic effect on liver, kidney and haematology of Wistar rats.

Keywords: Toxicological Evaluation, *Guiera senegalensis*, Liver, Kidney, Haematological Parameters

INTRODUCTION

Nearly all cultures use plants as a source of medicine from ancient times to present day, hence, a considerable percentage of people in both developing and developed countries use medicinal plants to remediate, alleviate or treat both human and animal diseases[1]. As a consequence, medicinal plants are now of substantial significance due to the special characteristics they possess as a large source of therapeutic phytochemicals, that may lead to the discovery and development of novel drugs as most of the phytochemicals from natural sources such as phenolics and flavonoids have been reported to have positive impact on health and disease prevention[2]. Ethnobotanical studies confirmed that indigenous plants are the main sources of traditional medicines[3-4]. Thus, there has been increase in herbal drugs usage globally with about 70% - 80% of the African population relying on nonconventional drugs which are predominantly of herbal sources both locally manufactured and imported[4-5]. However, most of these herbal drugs are often processed and procured without been scientifically evaluated for their safety.

The plant *Guiera senegalensis* a member of the family combretaceae is a tropical shrub widely distributed around the globe with high diversity in Africa and Asia growing on leached soils, fallows, and mostly on sandy soil and on very dry stations[6] to a height of 3 to 5 m depending on the habitat[7]. *G. senegalensis* has been used to treat various illness where the same plant part is used to treat a number of different diseases and at the same time different parts of the plant used to treat the same disease[7]. It has been used to relief aches and pains and for treating fever and malaria[8-9]. It has also been reported to possess healing properties against respiratory congestion and cough[10], to ease breathe and treat bronchial disorder, severe diarrhea and

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dysentery[9, 11]. It's safety has not been well documented. Hence this study evaluated the effects of aqueous stem bark extract of *G. senegalensis* on Wistar rats by determining the effects of the extract on haematological parameters, liver and kidney functions.

MATERIALS AND METHODS

Plant material

Fresh stems of *G. senegalensis* were collected from Song Local Government Area, Adamawa state. The plant material was taxonomically identified by a Botanist in Botany Department of Adamawa State University, Mubi, Nigeria where the voucher specimen was deposited.

Experimental animals

Thirty-one (31) mature and healthy Wistar rats (both sexes) weighing about 180 g to 200 g obtained from the Animal Resource Unit, National Veterinary Research Institute (NVRI) VOM, Plateau State, Nigeria were used for the study. The animals were housed at room temperature in wired cages for one week acclimatization before the initiation of the experiment where a 12 h light/dark cycle was maintained. An ethical clearance for conducting the experiment on research animals was secured from the University Ethical Committee prior to the initiation of the experiment with an approval number of IACEC/ANP-A045/2021

Extraction of plant material

G. senegalensis stem bark was washed, peeled and dried for four weeks under shade and was then pulverized to powder form using mortar and pestle. Maceration method of extraction described by Abdullahi and Mainul[12] was used to extract the powdered *G. senegalensis* stem bark. The powdered plant material was soaked in distilled water and allowed to stand at room temperature where it was stirred every 24 h for a period of three (3) days in order to soften and break the cell wall of the plant to release the soluble phytoconstituents. After three days, the mixture was pressed and strained by filtration, and the solvent was evaporated using a water bath and crucible at 40^0c until the extract was dried.

Phytochemical analysis

The qualitative phytochemical method described by Sofowara[13] and Banu and Catherine[14] was used to analyze the phytochemical constituents of aqueous *G. senegalensis* stem bark extract.

Acute toxicity

After one week of acclimatization according to criteria, the method described by Chinedu *et al.*[15] was used for acute toxicity determination. The method is categorized into 3 separate stages with the result of each stage determining whether to further proceed to the next stage or end the process. The result of the final test was validated using a confidence (confirmatory) test. Eleven rats were used in total. At stage 1 four rats were divided into 4 groups of 1 rat each and were treated with 50 mg/kg, 200 mg/kg b.wt., 400 mg/kg b.wt., and 800 mg/kg of *G. senegalensis* stem bark extract. The rats were closely observed for 1 h post-administration and 10 min every 2 h space interval for 24 h. The testing proceeded to stage 2 if mortality was not recorded. In stage 2, three rats were divided into 3 groups of 1 rat each and were treated with 1000 mg/kg b.wt., 1500 mg/kg b.wt., and 2000 mg/kg b.wt. of the stem bark extract of *G. senegalensis* which were closely observed for 1 h post-administration and 10 min every 2 h space interval for 24 h. The testing proceeded to stage 3 if no mortality was recorded. In stage 3, three rats were divided into 3 groups of 1 rat each and were treated with 3000 mg/kg b.wt., 4000 mg/kg b.wt., and 5000 mg/kg b.wt. of *G. senegalensis* stem bark extract which were also observed closely for 1 h post-administration and 10 min every 2 h space interval for 24 h. Finally, 1 rat was used for the confirmatory stage and was treated with 5000 mg/kg b.wt. of the *G. senegalensis* stem bark extract and was observed closely for 1 h post-administration and 10 min every 2 hours space interval for 24 h.

Table 1. Doses for acute toxicity determination of aqueous *G. senegalensis* stem bark extract

Stages	Group 1	Group 2	Group 3	Group 4
1	50 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
2	1000 mg/kg	1500 mg/kg	2000 mg/kg	
3	3000 mg/kg	4000 mg/kg	5000 mg/kg	

Sub-acute toxicity

Three (3) doses were administered to rats for 28 days for sub-acute toxicity determination. The doses administered for sub-acute toxicity study were chosen based on the result acquired from the acute toxicity study. Twenty (20) rats were grouped into 4 groups of 5 rats each. The rats were grouped accordingly: group 1 received normal saline only, group 2 received 200 mg/kg b.wt. extract, group 3 received 400 mg/kg b.wt. extract and group 4 received 800 mg/kg b.wt. extract.

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Sample collection

After 28 days of daily administration, the rats were weighed and sacrificed by decapitation 24 h after the last dose was administered. The blood samples were collected and analyzed for haematological parameters based on the method described by Dacie and Lewis[16], liver function parameters (ALT and AST) based on the method described by Rietman and frankel[17], ALP based on the method described by Wright *et al.*[18], while bilirubin based on the method described by Penhaker *et al.*[19] and kidney function based on the method described by Rifai[20]. Cypress diagnostic kits were used for the analysis.

Statistical analysis

All data collected from the study were statistically recorded as mean ± standard error of mean. Statistical differences between the mean values were determined using Analysis of Variance Test (ANOVA). Duncan's Multiple Range Test was used for comparison. IBM SPSS statistics software version 27 was used to analyze all data collected.

RESULTS

Phytochemical analysis

Phytochemical components of aqueous *G. senegalensis* stem bark extract are presented in Table 2. Phytochemicals tested included alkaloids, flavonoids, saponins, tannins, steroids and terpenoids. The result disclosed the presence of all the phytochemicals tested for.

Table 2. Phytochemical constituent of aqueous *G. senegalensis* stem bark extract.

Phytochemical	Inference
Alkaloid	+
Flavonoid	+
Saponin	+
Tannins	+
Steroid	+
Terpenoid	+

Key: + = present

Acute toxicity

Oral administration of aqueous *G. senegalensis* stem bark extract did not produce mortality up to a dose level of 5000 mg/kg b.wt. The bodyweight, food and water consumption of the rats did not show any significant difference when compared with the control group. Signs of toxicity such as aggression, depression, writhing, diarrhea and hypermotility were not recorded in rats used for oral LD_{50} determination when compared with control.

Sub-acute toxicity

Table 3 shows the serum liver function parameters alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin of rats treated with aqueous stem bark extract of *G. senegalensis* for 28 days. The result showed that the levels of liver parameters significantly ($p<0.05$) increased with an increase in dose of the extract when compared with the control.

Table 3. Effect of aqueous stem bark extract of *G. senegalensis* on liver function.

Parameters Groups	Alanine aminotranferase (IU/L)	Aspartate aminotransferase (IU/L)	Alkaline phosphatase (IU/L)	Total bilirubin (mmol/L)	Conjugated bilirubin (mmol/L)
1. Control	25.66 ± 1.20^{a}	94.33 ± 0.88^{a}	37.33 ± 0.33^{a}	7.63 ± 0.15^{a}	2.03 ± 0.03^{a}
2. 200 mg/kg b.wt	35.00 ± 0.58^{b}	103.33 ± 0.33^{b}	51.00 ± 1.00^{b}	8.63 ± 0.15^{b}	2.60 ± 0.12^{b}
3. 400 mg/kg b.wt	39.00 ± 0.88^{c}	121.66 ± 1.20^{c}	76.00 ± 1.16^{c}	12.33 ± 0.26^{c}	4.000 ± 0.58^{c}
4. 800 mg/kg b.wt	41.00 ± 1.15^{c}	141.33 ± 0.88^{d}	80.33 ± 1.45^{d}	13.50 ± 0.15^{d}	5.97 ± 0.09^{d}

All values are presented as mean ± SEM. Different superscripts down the column indicates that they are significantly different at ($p<0.05$), n=5

Values for AST, ALP and bilirubin of all the groups vary significantly with each other.Table 4 shows the serum kidney function parameters (urea, creatinine, Na^+ and K^+) of rats treated with aqueous *G. senegalensis* stem bark extract. Values of Na^+ and creatinine showed a significant ($p<0.05$) increase when compared with the control group whereas K^+ and urea values for group 2 and 3 did not differ significantly ($p<0.05$) when compared with the control group. However, group 4 that received 800 mg/kg b.wt. showed a significant increase when compared with the control.

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Table 4. Effect of aqueous stem bark extract of *G. senegalensis* on kidney function

Parameters Groups	Na^+ (mmol/L)	K^+ (mmol/L)	Creatinine (mmol/L)	Urea (mmol/L)
1. Control	137.67 ± 0.33^{a}	4.17 ± 0.07^{a}	25.00 ± 0.58^{a}	7.20 ± 0.12^{a}
2. 200 mg/kg b.wt	148.33 ± 0.67^{ab}	4.40 ± 0.06^{a}	36.33 ± 0.33^{b}	7.43 ± 0.09^{a}
3. 400 mg/kg b.wt	148.67 ± 0.33^{ab}	4.20 ± 0.12^{a}	45.66 ± 0.20^{c}	7.60 ± 0.12^{a}
4. 800 mg/kg b.wt	149.33 ± 0.33^{b}	5.07 ± 0.03^{b}	54.00 ± 1.16^{d}	8.67 ± 0.15^{b}

All values are presented as mean ± SEM. Different superscripts down the column indicates that they are significantly different at ($p<0.05$), n=5

Table 5 shows the haematological parameters, packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC) and white blood cells (WBC) of rats treated with aqueous *G. senegalensis* stem bark extract. PCV, Hb and RBC significantly decreased with an increase in dose of the extract while WBC significantly increased with an increase in dose when compared with control.

Table 5. Effect of aqueous stem bark extract of *G. senegalensis* on haematological indices

Parameters Groups	Packed cell volume (%)	Haemoglobin (g/dL)	White blood cells ($\times 10^9$/L)	Red blood cells ($\times 10^2$/L)
1. Control	36.00 ± 1.15^{c}	12.43 ± 0.80^{c}	9.80 ± 1.17^{a}	6.37 ± 0.26^{c}
2. 200 mg/kg b.wt	32.66 ± 2.03^{ab}	11.43 ± 0.38^{bc}	12.93 ± 0.49^{b}	5.87 ± 0.26^{b}
3. 400 mg/kg b.wt	32.33 ± 1.20^{ab}	10.53 ± 0.30^{ab}	13.77 ± 0.19^{bc}	4.90 ± 0.46^{ab}
4. 800 mg/kg b.wt	30.33 ± 0.33^{a}	9.57 ± 0.37^{a}	14.47 ± 0.32^{c}	4.12 ± 0.28^{a}

All values are presented as mean ± SEM. Different superscripts down the column indicates that they are significantly different at ($p<0.05$), n=5

DISCUSSION

Acute toxicity

The determination of LD_{50} is usually the first step in evaluating and screening novel drugs. It is an initial assessment and evaluation of toxic characteristics and manifestation of test substance[21]. Oral LD_{50} of aqueous stem bark extract of *G. senegalensis* was indeterminable up to a dose level of 5000 mg/ kg, thus the LD_{50} is greater than 5000 mg/kg. This may indicate that the aqueous *G. senegalensis* stem bark extract of is safe via the oral route. This is similar to the report of Moo *et al*.[22] in which no mortality was recorded up to a dose level of 5000 mg/kg of N-hexane extract of *Leptadenia hastata*.

Effect of aqueous stem bark extract of *G. senegalensis* on liver function

Liver is an important organ that performs different varieties of biochemical activities such as synthetic and excretory functions. Therefore, no single biochemical test is capable of identifying or detecting the general function of the liver[23]. The serum ALT and AST reflect the hepatocellular injury, serum ALP reflects the impaired bile excretion and bile flow, while the serum total and conjugated bilirubin represent the metabolic functions of the liver[24].

The observed significant increase in the liver function indices of the rats treated with aqueous *G. senegalensis* stem bark extract may indicate that the aqueous stem bark extract of *G. senegalensis* is dose dependently toxic to the liver. The increased levels of ALT may indicate damage to hepatocytes that results to the release of the enzymes into the circulation, increased ALP may indicate the effect of the stem bark extract that leads to both

intrahepatic and extrahepatic obstruction of bile flow and the increased bilirubin level may indicate haemolysis and overproduction of bilirubin induced by the stem bark extract of *G. senegalensis*. This result is consistent with the report of Ashafa[25] in which the aqueous leaves extract of *Felicia muricata* Thunb. affected the liver function parameters of Wistar rats.

Effect of aqueous stem bark extract of *G. senegalensis* on kidney

Serum creatinine concentration has been used for assessment and evaluation of kidney function which begins to rise only when the glomerular filtration rate (GFR) has by one-half diminished and thereafter the rise of creatinine is exponential to decline in GFR[26]. Urea, the by-product of protein and amino acid breakdown produced by the liver which is distributed throughout the intracellular and extracellular fluid is filtered out from the blood by the glomeruli in the kidney and is partially being reabsorbed with water. It is another frequent biochemical parameter for estimating kidney function which is useful in the differential diagnosis of acute and pre-renal conditions[27]. Electrolytes are negatively and positively charged ions that are found within extracellular fluids and cells, blood and plasma. Test for serum electrolytes such as measurement of sodium, and potassium is used to evaluate renal functions and comprehensive metabolic biochemistry profiles.

The increased Na^+ may indicate that the aqueous *G. senegalensis* stem bark extract causes alteration in osmotic pressure that leads to dehydration and a consequent increase in Na^+. The increased K^+ may indicate the poor function of the kidney thus may lead to abnormal or sometimes fatal cardiac arrhythmias[28]. The increase in the K^+ level may indicate that the membrane channels were affected by the stem bark extract or the plant stem bark may have a hyperkalemic effect. Hyperkalemia occurs most often in renal failures leading to declined potassium excretion[29-30]. The increased serum creatinine and urea concentration may indicate that there is decreased GFR by the kidney due to the effect of the aqueous *G. senegalensis* stem bark extract. This result is not in agreement with the reports of Unuofin[31] in which the whole-plant aqueous extract of *Vernonia mespilifolia* Less. did not affect the biochemical parameters of Wistar rats.

Effect of aqueous stem bark extract of *G. senegalensis* on haematological parameters

Haematological parameters are valuable in observing and monitoring the toxicity of substances. RBCs are involved in the transport and distribution of oxygen in the body and transports CO_2 to the lungs; WBCs functions to defend the body by phagocytosis against foreign invaders, fight infections and to produce, transport and distribute antibodies throughout the body during immune response[32].

The decrease in PCV and Red blood cells count may indicate that the stem bark extract of the plant may have haemolytic effect as reflected by the increased total bilirubin. This may lead to hyperkalemia and consequently disturbances in acid-base balance when administered at higher doses. The decrease in the level of haemoglobin may indicate anaemia as a result of erythrocyte haemolysis caused by the aqueous *G. senegalensis* stem bark extract which may lead to decreased oxygen transport to tissues as well as transport of CO_2 back to the lungs. The increased WBC may indicate that the plant may have some toxic compounds which trigger the immune response leading to the production of more immune cells. This study supports the report of Ilham *et al*.[33] in which the leaves of *Ambrosia maritima* affected the haematological parameters of Nubian goats.

This study is limited to four functional parameters of liver and kidney and haematological parameters.

CONCLUSION

In conclusion, considering the serum level of biochemical parameters and the haematological parameters of the experimental rats treated with different oral doses of aqueous *Guiera senegalensis* stem bark extract, it suggests that the stem bark extract of the plant may be toxic especially at higher doses and longtime exposure.

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