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RESEARCH ARTICLE

ACUTE AND SUBACUTE TOXICITY STUDY OF WATER EXTRACT OF LEAVES OF *GUIERA SENEGALENSIS* J.F.GMEL (COMBRETACEAE) IN WISTAR RATS

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ABSTRACT

The plant was investigated for possible toxicity (both acute and subacute toxicity) in wistar rats. Twenty five female rats were divided into five groups of five rats each. The first group was orally dosed with 2000 mg/kg body weight of the extract for acute toxicity testing. The remaining four groups were used for subacute testing and were dosed as follows; group A received orally administered distilled water, group B received 50mg/kg, and groups C and D received 100 and 200 mg/kg respectively of the extract orally. All animals survived the acute and subacute toxicity testing but histopathological changes were seen in the liver, kidney, spleen, and lungs of all the treatment groups. Haematological and biochemical parameters were also altered in all treated groups. This experiment suggests that the leaves of *Guiera senegalensis* do have toxic effects despite its wide use in African traditional medicine.

INTRODUCTION

Guiera senegalensis known as '*sabara*' in Hausa is widely used in African traditional medicine and is used in many traditional preparations for many different ailments. It has been shown to have an encouraging antiviral effect against herpes simplex virus (Silva *et al*., 1997). Oral administration of the macerated leaves of the plant was used by El-Gazali *et al* in 1994 against hyperglycaemia, hypertension and antileprosy. Aniagu et al. in 2005 demonstrated the ulcer protective effect of the aqueous root extract of the plant against ethanol induced ulcerations in rats. They also recorded a decrease in enteropooling activity induced using castor oil. These findings gave some support to the traditional use of the plant in treating diarrhoea and ulcers. Antioxidant and anti inflammatory activities of galls from the plant have been demonstrated by Sombie *et al.* 2010. Their results further justifies the use by traditional practitioners to treat a large number of metabolic diseases. Sombie *et al.* in 2011 demonstrated the neuroprotective and antioxidant property of *Guiera senegalensis* in rats by showing the galls capable of being anti acetylcholinestereases, anti lipid peroxidation, and prevention of red blood cells haemolytic activities. This study further compliments the traditional use of the plant for neurological and haemolytic crisis. Herbal medicines and their derivatives are said to have fewer side effects than the synthetic orthodox medicines (Gamaniel, 2000), but this does not rule out the fact that their toxicity profile should not be investigated, since the difference between a drug and a poison is in the dose. Practitioners of herbal medicine have no knowledge of procedures of investigating the toxicity profiles of their remedies, and they claim absolute lack of toxicity of their remedies, but in science, such claims can only be accepted when put to the test. This research was carried out to asses the acute and subacute toxicity of the leave extract in Wistar rats by looking at differences in blood chemistry, hematology parameters and effect on some organs histopathologically after repeated dosing.

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MATERIALS AND METHODS

Fresh leaves of *Guiera senegalensis* were collected from Majiya village of Dange Shuni Local Government Area of Sokoto state. The plant was identified in the taxonomy unit, Department Botany of Usmanu Danfodiyo University, Sokoto. The leaves were air dried until the weight was constant and the dried leaves were pulverized mechanically into dried powder. Five hundred grams of the powdered materials was soaked, mixed, and stirred for 10 minutes in 5 litres of distilled water. It was left to soak overnight before filtering according to the methods of Ajagbonna, 2000. The filtrate was evaporated in an oven at a temperature of 50^0C. It was weighed after drying and the percentage yield was calculated. The extract was then refrigerated at 4^0C for subsequent pharmacological evaluation.
Experimental Animals. A total of 25 female wistar rats weighing between 200 – 230 grams were obtained from Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The animals were housed in groups of five and kept in clean cages under. They were allowed free access to food and water *Acute Toxicity Study*.

Table 1. Mean ± SEM body weight before and after 28 days treatment with whole plant extract of *Guiera senegalensis*

Group	Dose	Day 1	Day 29
A	Distilled water	170.0 ± 8.80	182.40 ± 7.72
B	50mg	180.0 ± 8.41	190.42 ± 8.74
C	100mg	180.4 ± 10.78	190.74 ± 10.28
D	200mg	183.4 ± 5.88	188.80 ± 8.63

There was no statistical significance difference at P>0.05 in body weights of rats between day 1 and day 28 in all the groups

Table 2. Mean ± SEM organ weight after 28 days treatment with whole extract of *Guiera senegalensis*

Group	Treatment	Heart	Lungs	Liver	Kidney	Spleen
A	Distilled water	0.68±0.037	1.94±0.248	6.82±0.291	1.22±0.049	0.90±0.100
B	50 mg/kg	0.68±0.049	1.42±0.124	6.54±0.35	1.04±0.240	0.72±0.102
C	100 mg/kg	0.82±0.038	1.44±0.090	7.76±0.914	1.41±0.029	1.00±0.109
D	200 mg/kg	0.86±0.037	1.39±0.122	6.44±0.584	1.19±0.045	0.74±0.047

There was no significant difference at P>0.05 between the organ weights of all groups after 28 days of treatment at P<0.05.

Table 3. Mean ± SEM Hematological parameters of rats after 28 days of treatment with whole extract *Guiera senegalensis*

	Dose (mg/kg)	RBC (x 10^6U/l)	PCV (%)	WBC (x10^3U/l)	Neutrophil (%)	Eosinophil (%)	Monocyte (%)	Lympho. (%)	Basophil (%)
A	Distilled water	7.1±0.21	3.2±6.02	6.7±1.47	12±1.30	5.0±0.57	4.0±0.31	79±0.91	0.00
B	50	5.3±0.05	4.8±1.50	12.5±0.12	12±2.13	4.0±1.46	4.0±4.41	80±1.44	0.00
C	100	6.6±0.002	3.6±2.1	16.2±2.09	12±1.65	2.0±0.87	4.0±3.02	82±0.98	1.00
D	200	5.8±0.015	4.3±4.31	18.2±0.76	12.3±0.12	2.3±2.11	4.0±5.00	82±1.02	1.00

There was an increase in WBC count, and Packed Cell Volume between the control and the treatment groups (*Guiera senegalensis* treated groups) with statistical significance difference at P<0.05.

Table 4. Mean ± SEM values of proteins, bilirubins and cholesterol after 28 days treatment with whole extract *Guiera senegalensis*

	Total bilirubin g/dl	Conjugated bilirubin g/dl	Total cholesterol mg/dl	Total protein mg/dl	Albumin mg/dl
A	1.80±0.10	0.70±0.07	233.40±0.05	0.10±0.00	0.05±0.01
B	0.65±0.05	0.10±0.00	108.40±41.65	0.10±0.01	0.45±0.01
C	0.80±0.27	0.20±0.07	91.70±8.35	0.10±0.01	0.04±0.00
D	0.50±0.41	0.60±0.22	150.00±50.00	0.10±0.01	0.04±0.00

Table 5. Mean ± SEM values of blood electrolytes, urea, creatinine and alkaline phosphate)

	Urea	Creatinin	HCO_3	Na^{2+}	K^+	AlP IU/l	Ph
A	0.40±0.10	1.40±0.60	0.24±0.03	79.00±1.00	5.75±1.55	0.20±0.19	0.10±0.05
B	0.50±0.04	0.60±0.20	0.23±0.02	81.50±1.50	3.40±0.00	0.40±0.01	0.10±0.00
C	0.40±0.06	0.80±0.00	0.25±0.01	83.50±1.50	3.80±0.50	0.40±0.07	0.40±0.02
D	0.40±0.04	1.00±0.20	0.24±0.010	84.00±0.00	3.60±0.00	0.50±0.14	0.10±0.00

The limit dose test for acute oral toxicity testing was used according to OECD, 2006 guideline on the testing of animals with a limit dose of 2000 mg/kg. Five (5) rats were selected out of the twenty five females from a computer assisted method of selection of experimental animals. All rats were numbered from 1 to 25 and the computer was used to select the random numbers. The first animal was dosed and monitored for 48 hours, if the animal survived the next animal was dosed and so on until all five were dosed. The animals were left for 14 days and the weight checked weekly along with any other changes regarding the vital parameters of the rats under testing. *Subacute Toxicity Study*. Subacute toxicity study was carried out using the remaining 20 rats that were divided into 4 groups of 5 rats each. Group A was the placebo group and was orally administered distilled water. Group B, C, and D received 50mg, 100mg, and 200mg respectively of the plant extract (*Guiera senegalensis*) for a period of 4 weeks. Clinical manifestations of toxicity, body weights, hematological and biochemical parameters, and histopathological examinations were carried out following standard methods (Bergmeyer, 1980). At the end of the 4 weeks period the rats were sacrificed and spleen, liver, lungs, and kidneys were harvested and put into 10% formalin for histopathology. Body weights were taken using an open topped plastic container. The container was initially weighed empty and then with the rat. The weight of the container was subtracted from the weight with the rat inside (container + weight) to get the weight of the rat alone. The same method was applied when weighing the organs.

RESULTS

There were no deaths in rats administered 2000 mg/kg body weight of the extract in the acute toxicity testing. In the subacute testing also no mortality was observed when 50, 100 and 200 mg/kg were administered orally for a period of 28 days. The total bilirubin, creatinine and cholesterol levels concentrations decreased in all extract treated groups with group D, B and C having the lowest concentrations respectively, however the levels of alkaline phosphate increased. There was a statistically significant difference between the control and all the treated groups at P<0.05.

Histopathology

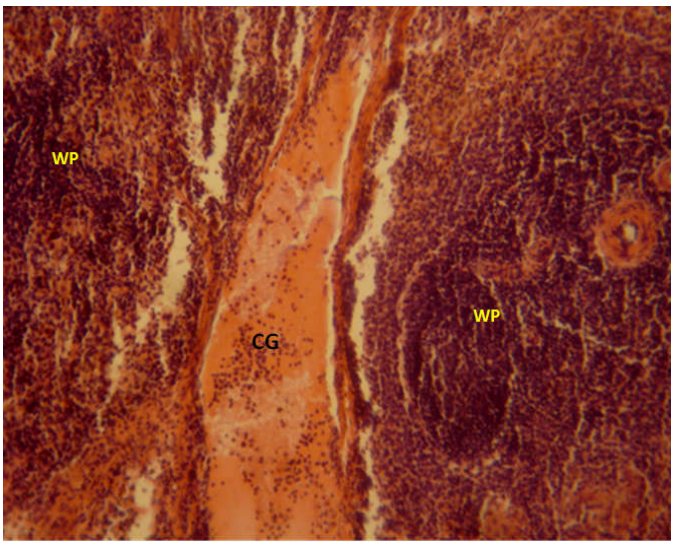

Figure 1. Photomicrograph of (Group B) rat spleen treated with extract showing focal congestion (CG) and white pulp hyperplasia (WP) H&E x400

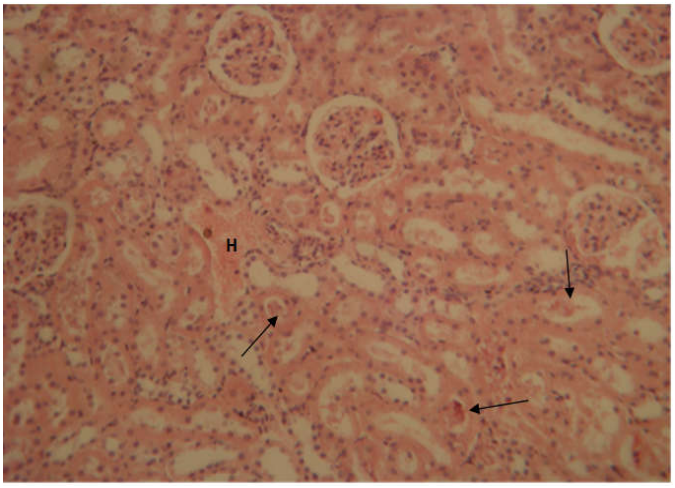

Figure 2. Photomicrograph of rat kidney of (Group B) showing moderate interstitial haemorrhage (H), hydrophobic change within the lumen of the tubules in the cortex (arrows) H&E x200.

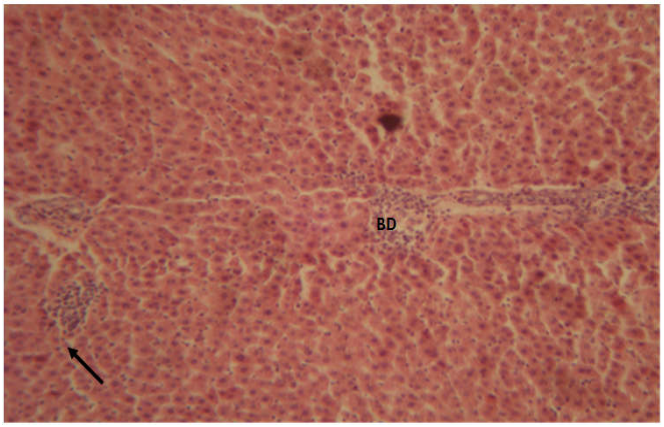

Figure 3. Photomicrograph of rat liver of (Group B) showing moderate bile duct hyperplasia and focal areas of mononuclear cell infiltration X200

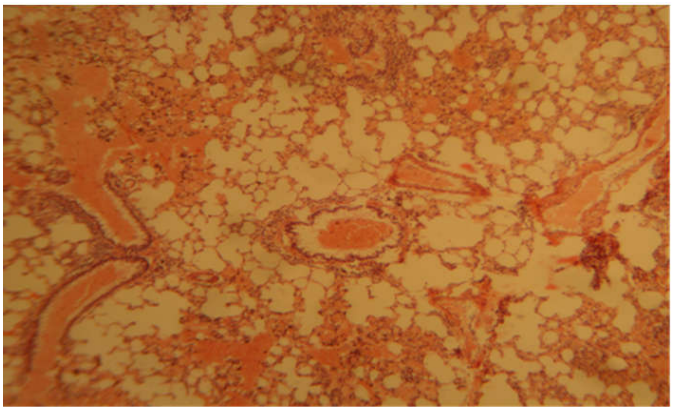

Figure 4. Photomicrograph of rat lungs of (Group B) showing moderate areas of vascular congestion and interstitial haemorrhage H&E x200

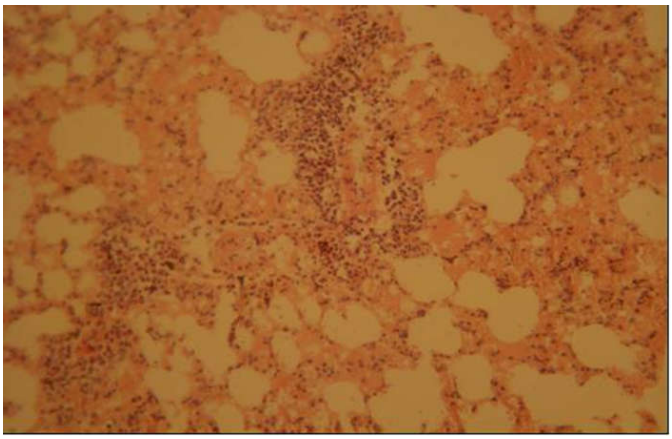

Figure 5. Photomicrograph of rat lungs of (Group C) showing moderate interstitial haemorrhage and mild lymphocytic infiltration H&E x200

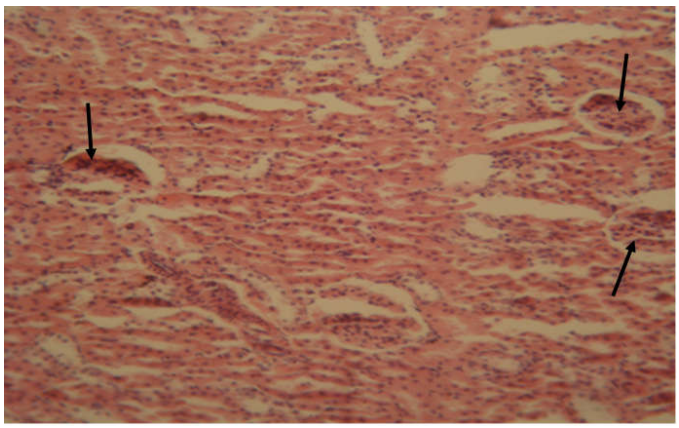

Figure 6. Photomicrograph of rat kidney of (Group C) showing moderate glomerular degeneration in the cortex (arrows) H&E x200

DISCUSSION

Although the repeated dose toxicity of 50, 100, and 200 mg/kg of the extract given to groups B, C and D respectively showed no mortality, there were pathological changes seen in the liver, kidney, spleen, and the lungs of all the treated groups. Mild inflammatory cells were seen only in the heart of the 100 mg/kg treated group.

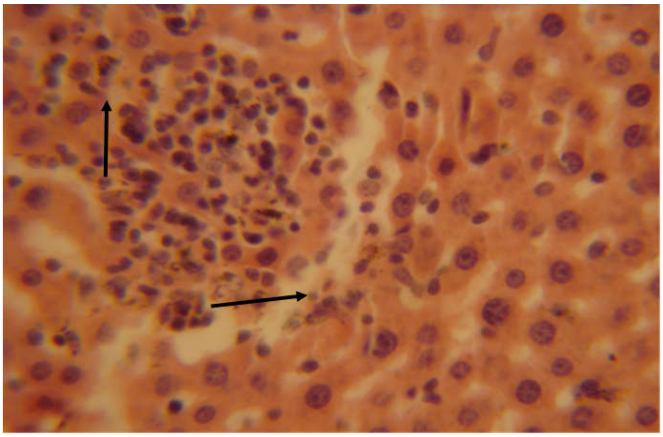

Figure 7. Photomicrograph of (Group C) rat liver treated with extract showing focal area of mononuclear cell infiltration H&E x400

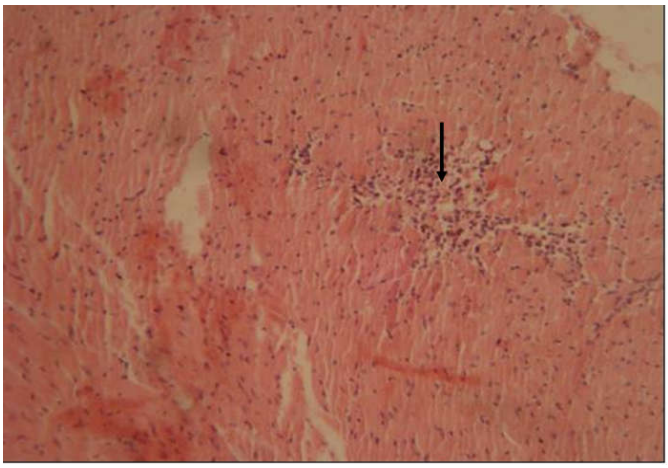

Figure 8. Photomicrograph of rat heart of (Group C) showing mild inflammatory cell (arrow) H&E x200

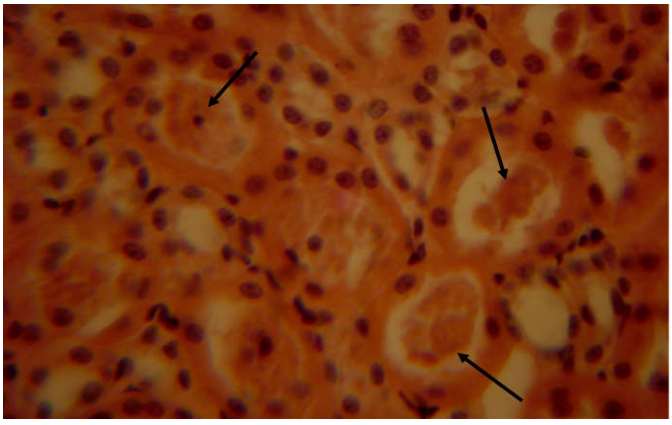

Figure 9. Photomicrograph (Group D) rat kidney treated with extract showing multifocal hydrophobic change in the tubular lumen (arrows) H&E x400

These cytotoxic findings are in line with the report of Azza *et al.* 2007 where the effect of water extract of *G. senegalensis* resulted in endothelial toxicity, hepatonephropathy, and pancreatic hyperplasia. They also observed alterations in hematological and biochemical parameters. There was an increase in WBC count in this study. This may be due to stimulation of the immune response mechanism since some degree of cytotoxicity was observed in this study after 28 days of sub acute treatment with the extract.

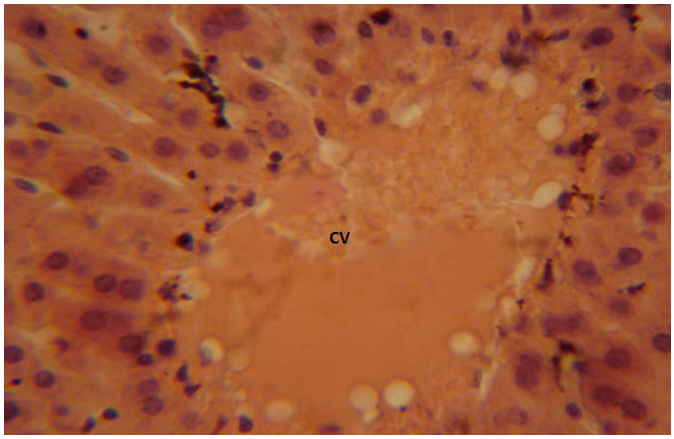

Figure 10. Photomicrograph of (Group D) rat liver treated with extract showing congestion of the central vein (CV) and black patches of iron pigment H&E x400

The cytotoxic effect of the plant extract could be due to the presence of guieranone A which is a cytotoxic component of the plant as reported by Julien *et al.* 2006. The increase in hemopoietic values regarding the PCV in this work could be due to the presence of flavonoids that are found in plants. Flavonoids are known to posses antioxidative effects that protect the hemopoietic system and formed blood cells from being attacked by reactive free radicals in the body thus stimulating the hemopoietic growth factor as reported by (Friday *et al.,* 2010). The levels of cholesterol dropped in the subacute toxicity study groups compared to the control with significant differences and this may be linked to high presence of saponnins which have been found to have hypocholesterolemic activity (Friday *et al*., 2010).

Conclusion

Based on the hematological, biochemical, and histopathology in this study, the water extract of the leaves of this plant do have certain level of toxicity. The toxicity profile is recommended for further analysis and correction measures be put in place to tackle the toxic effect following prolonged use of the extract if recommendations are to be made for its use.

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