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Biochemical and Toxicological Activity of *Guiera senegalensis* Leaves Extract

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Abstract

Guiera senegalensis ("Senegal *guiera"* in English; "Sabara" in Hausa) (*Combretaceae*) is traditionally used for various purposes in Africa and other parts of the world with little or no scientific basis. It is thus the aim of this research to evaluate its biochemical and toxicity effects for its medicinal properties, safety, efficacy of treatment, and the optimum dose. The study qualitatively screened for phytochemicals, acute toxicity (LD_{50}) and sub-chronic toxicity indices in the ethylacetate extract of the plant. The result of phytochemical screening revealed *G. senegalensis* ethylacetate leaf extract to contain some important phytochemical compounds that may attribute to the biochemical properties possessed by the plant. The result of acute toxicity study showed that the plant is practically non-toxic (oral LD_{50}>5000mg/kg) when used for a short period of time and some signs of toxicity on heart in sub-chronic study (on long term of use). Also, the Haematological results indicated that the plant may have some effects on the immune system, might have caused an increase in RBC and haemoglobin production, and may also enhance O_2 – transport capacity of the blood. *Guiera senegalensis* should therefore be used with care when used for a long period of time.

Keywords: *Guiera Senegalensis*, Phytochemicals, Acute Toxicity, Sub-Chronic Toxicity.

1. INTRODUCTION

Plants are sources of potential therapeutic agents against various diseases due to their biodiversity and presence of a wide array of bioactive phytochemicals (Farombi, 2003). The most important of these bioactive phytochemicals are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Many plant products are consumed in reasonable quantity as food in addition to their medicinal effects, whereas some are purely medicinal, few apparently quite safe and others more potent (e.g., containing cardioactive glycosides), which can only be consumed in small quantities and such dosage are appropriate for the treatments of disease (Mohammed, 2008). Thus, plant resources become potential targets for research and development of alternative drugs with novel modes of action (Muregi *et al*., 2003). However, little scientific data exist to validate the properties of most plants. It is therefore, important to investigate these claimed properties in order to establish the efficacy and determine the potential of these plants as sources of new drugs.

Guiera senegalensis (Division: Magnoliophyta; Order: Myrtales; Family: *Combretaceae*; Genus: *Guiera*), commonly known as Senegal *guiera* and "Sabara" in Hausa is a (Semi-) evergreen flowering shrub usually growing up to 1-3 m tall, all parts covered with black glandular dots. The bark is fibrous, more or less smooth to finely scaly and grey to brown in color with soft-hairy young branches. The wood is whitish or tinged red, coarse-grained, knotted and short, but very hard. The leaves are grey-green, opposite or sub-opposite, petiole of 2–5 mm long that is short and hairy, oblong-elliptical blade. The flowers are small and yellowish green in color with linear fruit 3-4 cm long, crowned by the persistent perianth, grouped and densely silky-velvety. *Guiera senegalensis* grow in low rainfall areas and light dry soils, widespread in Western Africa from Mauritania and Senegal, indigenous to Nigeria and Cameroun and in East Africa in Egypt and Sudan. The plant is a

pioneer species and is often found growing on very poor and degraded land.

2. METHODS

Collection and Identification of Plant Material

Fresh leaves of *Guiera senegalensis* leaves were collected from Dawakin Tofa, Kano State Nigeria, and identified at the Plant Biology Department, Bayero University, Kano.

Extraction of the Powdered Leaf of the Plant

The leaves of *Guiera senegalensis* were plucked from the stem, cleaned, air-dried under shade and ground into powder. The powder was stored in an airtight container and kept in a cool, dry place. Extraction was carried out by dispersing 2.20 kg of the powdered leaves in 7.5 L of 99.8% methanol (Sigma-Aldrich) in an air-tight plastic bucket. The bucket containing the powdered leaves was tightly closed, shaken and left to stand at room temperature. Every day for four days, the soaked leaves were filtered, the extract was then collected and concentrated by evaporation using a rotary evaporator at temperature of 37°C to give 277g of the crude extract. The recovered solvents were reused for soaking (Abosi and Raseroka, 2003). The extract was exhaustively partitioned with ethyl acetate to afford the ethylacetate fraction.

Qualitative Phytochemical Screening of the Ethylacetate Extract

The GS ethylacetate extract was screened qualitatively for the phytochemical constituents using standard methods of analysis (Trease and Evans, 2002; Sofowora, 1993) to test for alkaloids (Dragendorff's test), terpenoids (Soforowa, 1993), steroids (Liebermann-Burchard test), phenols (Ellagic acid test), flavonoids (Ferric chloride test), saponins (Foam test), tannins (Ferric chloride test), anthraquinones (Borntrager's test), quinones (Alcoholic KOH test) and cardiac glycosides (Kellar Killiani test)

Toxicity Study of the Ethyleacetate Extract

Acute Toxicity Study (LD_{50})

Acute toxicity of *Guiera senegalensis* ethyleacetate extract was carried out using modified Lorke's method (1983). The study was carried out in two phases. In phase one of the study, nine albino rats were randomized into three groups of three rats each and were given 10, 100 and 1,000 mg/kg body weight of the extract orally. The rats were observed for 24 hours for behavioral changes and mortality. In the second phase of the study another fresh set of nine rats were randomized into three groups of three rats each and were given 1600, 2900 and 5000 mg /kg body weight of the extract orally based on the result of the first phase. These were observed for 24 hours, and then 7 days for behavior as well as mortality.

Sub-Chronic Toxicity Study

A total of twenty (20) albino rats were divided into two groups: control and experimental groups. The control group consisted of five rats while the other group was further divided into three equal sub-groups each consisting of five rats. The three sub-groups were for Low dose (160mg/kg), Medium dose (290mg/kg) and High dose (500mg/kg). They were administered with the extract orally using syringe once daily for a period of four weeks. At four weeks, all the rats were sacrificed. Blood samples were taken in plain tubes for analysis of liver, heart and kidney functions. The three organs were taken for histopathological analysis. Also, blood samples were collected from each rat into EDTA tubes for haematological analyses using Haematological Auto-analyzer (SYSMEX XE-2100 Haematology Automated Analyzer).

Body Weight

The body weight of each rat was recorded once before and after the commencement of the experiment. The relative body weight (% change in weight) of each animal was calculated using the formula:

% change in weight = [absolute body weight of one-time interval (g) / body weight of rat on commencement of the experiment (g)] × 100 (Kuete *et al*., 2010).

3. RESULTS AND DISCUSSION

Extraction Yield

The plant gave an appreciable yield after extraction and also when partitioned with ethylacetate (table 1).

Table 1: Extraction Yields of *Guiera senegalensis*

Extract	Extraction Yield (%)
GS	12.59
GSetact	1.19

GS = *G. senegalensis* crude extract, GSetact = *G. senegalensis* ethylacetate fraction

Qualitative Phytochemical Analysis of Ethylacetate Crude Extract of the Plant

The fact that many of the conventional drugs are derived from plants directly or indirectly, may suggest that some plants contain compounds with pharmacological activity that could be used as medicinal agents to treat various diseases or basis of developing drugs. Most of these compounds are not serving the nutritional purpose in plants, hence they are called secondary metabolites, examples of which include: alkaloids, tannins, flavonoids, steroids, quinones, phenols etc. The present study showed the presence of different kinds of these phytochemicals whose biological activity can be of valuable therapeutic importance.

Gueira senegalensis was found to contain alkaloids, terpenoids, quinones, anthraquinones, flavonoids, tannins, phenols, steroids and cardiac glycosides (table 2). The presence of tannins in GS may suggest the use of the plant as anticarcinogenic, which could be attributed to the antioxidative properties of tannins that protect living cells from oxidative damage (Chung *et al.,* 1998) as well as antibacterial effects. Similarly, the observed antibacterial effects of the medicinal plants' extracts have been related to the presence of tannins, flavonoids and saponins (Osadebe *et al.,* 2004). In addition, tannins have been found by Lata and Dubey (2010) to have a wide range of antimicrobial and anti-inflammatory effects.

It is documented in this study that steroids were present in GS ethylacetate extract. These steroids were reported to have antibacterial effects (Raquel, 2007), and are important due to their relationship with steroid hormones such as sex hormones estrogen and testosterone (Okwu, 2001). One of the phytochemicals found in GS leaves extract are terpenoids, which are found to have a variety of pharmacological activities such anti-bacterial, anti-inflammatory and anti-malarial activities (Staden and Grobbelaar, 1995; Mahato and Sen, 1997). This may explain why GS extracts are credited for curing several diseases and infections in Africa.

Alkaloids found in medicinal plants are used as anaesthetic agents (Hérouart, *et al.,* 1988). The plant was also found to contain anthraquinones, a class of natural compounds that consists of several hundreds of compounds that differ in the nature and positions of substituent groups (Schripsema *et al.,* 1999). In the pharmaceutical industry, the natural and synthetic derivatives of 9, 10 anthraquinones includes many important drugs collectively called anthracenediones, among which are antimalarials (such as rufigallol) (Chan *et al.,* 2011). The presence of the anthraquinones in the leaf extract of Gueira senegalensis provides evidence to the reason why the plant is employed as antimalarials in traditional medicine**.**

Table 2: **Qualitative Phytochemical Analysis of Ethylacetate Crude Extract of *Guiera senegalensis* (GS)**

Bioactive agent	**Extract**
Saponins	-
Alkaloid	+
Tannins	+
Phenols	+
Flavonoids	+
Cardiac glycoside	+
Terpenoid	+
Steroid	+
Quinones	+
Anthraquinones	+

(+) = Present; (-) = Absent

Acute Toxicity Study

Toxicological study is carried out in various groups of experimental animals to determine the safety of drugs for human consumption. Consumption of herbal preparations has been recently questioned due to reports of illnesses and even mortalities (Park *et al*., 2010). Based on this, acute and sub-chronic toxicity studies were carried out to evaluate the safety of the leaf extract of GS in experimental animals. In the acute toxicity study, a maximum dose of 5000 mg/kg of ethylacetate leaves extract from GS did not cause any toxicity-no food and/or water refusal, changes in posture, convulsion, and mucous secretions or death up to 7 days, even though, immediately after the administration the rats became drowsy and weak but gradually became normal again, thus the oral LD_{50} was greater than 5000mg/kg body weight (table 3). The LD_{50} being greater than 5,000 mg/kg is thought to be practically non-toxic based on Hodge and Sterner Scale (Muhammad, 2015), which could be due to low levels of toxic phytochemicals (Aiyegoro and Okoh, 2010; Kuete *et al*., 2010; Pengelle, 2004) in the plant.

Acute toxicity study alone is not enough to judge a drug safe. Because of this, long-term (sub-chronic) toxicity study was conducted to further evaluate the toxicity risk of GS. Upon exposure to toxins, animals often consume less food and lose weight, and increase in organ weight may be due to a tumor, fluid, or triglyceride accumulation, enzyme induction or hypertrophy. These changes may be confirmed by biochemical, haematological or histopathological analyses.

Table 3: Acute toxicity profile of *G. senegalensis* ethylacetate extract towards experimental animals (Albino rats)

Doses (mg/kg)	**Survival rate (phase I)**
10	0/3
100	0/3
1000	0/3
Doses (mg/kg)	Survival rate (phase II)
1600	0/3
900	0/3
5000	0/3

LD_{50} of the ethylacetate extract of *G. senegalensis* is greater than 5000mg/kg body weight. The formula of calculating LD_{50} by determining the square root of the product of maximum value that did not cause death and lowest value that causes death (Lorke, 1983) cannot be used here, since no dose caused death.

Sub-Chronic Toxicity Study

The weight of the experimental animals was recorded before and after the study. The weights of the experimental animals were found to increase at the end of the experiment (Table 4). However, no mortality was recorded in any of the groups studied in the first twenty-four hours, seven days and up to four weeks after oral administration of various concentrations of the plant's crude extract.

Liver Function Tests

Liver plays a central role in metabolic homeostasis and xenobiotic transformations. Of all the liver marker enzymes only the GGT is liver specific. The GGT levels of all the treatment groups were found to be statistically not significant (P>0.05) with the control, suggesting no liver abnormality in all the treatment groups. To show the presence of bile duct obstruction, both ALP and GGT levels are expected to be high. A normal GGT level coupled to elevated ALP level has been shown to associate with bone diseases (Mauro *et al*., 2006; Rosalki and Mcintyre, 1999). Generally, the higher the GGT level the greater the extent of liver damage. In this research the levels of ALP in rats treated with the plant's extract at all the doses were found to show significant increase (P<0.05) when compared with the control.

Table 4: Weight of albino rats after subchronic toxicity study of *G. senegalensis* ethylacetate extract

Group	Mortality	Initial weight (g)	Final weight (g)	% change in weight
Low Dose (160mg/kg)	0	132.60±0.23^{a}	143.77±2.89^{c}	92.23
Medium Dose (290mg/kg)	0	135.67±0.83	155.67±0.32^{b}	87.15
High Dose (500mg/kg)	0	148.20±0.03^{a}	169.20±0.27^{b}	87.59
Control	0	135.83±0.05^{a}	140.83±5.00^{b}	96.45

Results are mean ± standard deviation, n=5. Values in the same column bearing similar super script are significantly different at P<0.01 and values bearing different super script are significantly not significant at P>0.05
Key: Substance administered = GS ethylacetate extract, Duration = once daily for 28 days, route of administration = oral

The aminotransferases (formerly transaminases) are the most frequently utilized and specific indicators of hepatocellular necrosis. ALT (formerly serum glutamic pyruvate transaminase-SGPT) is primarily found in the liver whereas the AST (formerly serum glutamate oxaloacetic transaminase-SGOT) is a sensitive but non-specific liver enzyme as it is also found in a wide variety of tissues like the heart, skeletal muscle and kidney in large amounts with small amounts present in the brain, liver, pancreas and lungs (Cheesbrough, 2005; Friedman, *et al*., 2003; Rosen and Keefe, 2000). The level of activity of AST and ALT in serum at any moment reflects the relative rate at which they enter and leave circulation. The basis of abnormal levels is leakage from damaged tissues (Crook, 2006). However, in this study, AST was found to increase which may be the reason of histopathological changes observed in the high dose group of GS. This implies that high dose of GS (used in this research) has some hepatotoxic effects.

Bilirubin is an endogenous anion derived from haemoglobin degradation of the aged RBC. It is helpful in testing the liver's capacity to transport organic anions and to metabolize drugs with decreased hepatic clearance regarded as a basis of abnormality. Bilirubin in body is a careful balance between production and removal of the pigment in body. The serum total bilirubin level was found to decrease from low to high dose in *G. senegalensis* (GS) with significant difference (P<0.05) between all the test groups and the control. This also suggests that the liver's capacity (in all the treatment groups of the plant) to transport organic anions and to metabolize drugs is normal.

The liver is the major source of most the serum proteins. The parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of the and b globulins. Albumin is quantitatively the most important protein in plasma synthesized by the liver and is a useful indicator of hepatic function. Because the half-life of albumin in serum is as long as 20 days, the serum albumin level is not a reliable indicator of hepatic protein synthesis in acute liver disease. Liver is the only site of synthesis of albumin. The serum levels at any time reflect its rate of synthesis, degradation and volume of distribution.

The results of histopathology showed unremarkable liver tissue for the low and medium doses of GS (plates 1b and 1c respectively), while a marked vascular congestion, inflammation and fibrosis were observed in the liver of rats treated with high dose (plate 1d), indicating that the plant at this concentration has some toxic effects. This may be as a result of the significant decrease in both ALT and GGT levels in the group administered with the high dose (500mg/kg).

Table 5: Effects of four weeks oral administration of *G. senegalensis* ethylacetate extract on liver function parameters in rats

Group	AST (u/l)	ALT (u/l)	ALP (u/l)	GGT (u/l)	Total Protein (g/dl)	Albumin (g/dl)	Total Bilirubin (mg/dl)
Control	85.1±21.96^{a}	50.93±5.55^{b}	57.12±5.36^{d}	3.76±1.23^{e}	5.07±0.18^{f}	3.43±0.01^{g}	0.50±0.04^{h}
Low Dose (160mg/kg)	239.43±10.06^{a}	66.33±0.96^{b}	263.55±14.21^{d}	2.03±1.23^{e}	6.25±0.84^{f}	2.73±0.15^{g}	30.40±4.45^{h}
Medium Dose (290mg/kg)	185.80±2.05^{a}	45.23±2.94^{c}	146.13±5.59^{d}	2.03±0.41^{e}	6.53±0.35^{f}	2.50±0.27^{g}	19.73±1.64^{h}
High Dose (500mg/kg)	156.77± 27.33^{a}	43.83±1.25^{b}	129.95±13.64^{d}	1.45±0.41^{e}	6.97±0.42^{f}	2.67±0.35^{g}	17.23±1.61^{h}

Results are mean ± standard deviation, n=5. Values in the same column bearing similar super script are significantly different at P<0.001 and values bearing different super script are significantly not significant at P>0.05
Key: Substance administered = GS ethylacetate extract, Duration = once daily for 28 days, Route of administration = oral; u/l = unit per litre; method of statistical analysis = 2-way ANOVA

Heart Function Indices

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. CVDs are the leading cause of death globally; it is estimated that approximately 30% of all deaths are caused by CVDs**.** The indices commonly used in Cardiotoxicity include; Lactate dehydrogenase (LDH), Creatine kinase (CK) and Cardiac Troponins (cTnI, cTnT) activities. Serum CK was developed and utilized as cardiac marker due to its early increase after injury (Amador *et al.*, 1963; Dunn and Siegel, 1965) and early clearance. Troponin T is present chiefly in two forms: bound to the contractile elements of the myocardial cells and free in the cytoplasm. Troponin T exhibits a dual release: initially of the cytoplasmic component and later of the bound component (Katus *et al.*, 1991). Troponin I is extremely specific for the cardiac muscle and has not been isolated from the skeletal muscle. This absolute specificity makes it an ideal marker of myocardial injury (Higgins and Higgins, 2003).

There were no significant changes in the cardiac parameters tested between the high dose group and the control group of the plant (table 6). Surprisingly, the photomicrographs of the heart cells of all the treatment groups showed a remarkable change (plates 3b-3d). These could be explained by considering the factors that determine the speed of release of a marker from injured myocardial tissue. Generally, small molecules are released at a faster rate than large molecules (Adams, 1999). The intracellular location of a marker also limits its rate of release, thus, molecules located in the cytoplasm will appear in the bloodstream sooner than structural proteins. For example, troponins are located intracellularly, and this may explain why its level in the treatment groups is significantly not significant with the control even though there may be a myocardial injury. Continuing breakdown of the myofibrillary-bound complex also explains the prolonged elevation of both serum troponins after myocardial injury (Maynard *et al.*, 2000). However, the non-significant difference in CK-MB and LDH between treatment groups and the control may be due to early clearance, so the levels might have returned to the baseline. The increased level of AST recorded in the liver function test may be originated from cardiac muscle, as it was reported that serum glutamate oxaloacetate transaminase (AST) increased in patients with acute myocardial infarction (AMI) (Karmen *et al.*, 1954; LaDue *et al.*, 1954).

Table 6: Effects of four weeks oral administration of *G. senegalensis* on heart function parameters in rats

Group	LDH (U/L)	CK-MB (U/L)	Troponin-I (U/L)
Control	1476.27±1.74^{a}	16.51±5.84^{b}	0.38±0.10^{f}
Low Dose (160mg/kg)	893.89±195.02^{a}	20.64±7.67^{c}	0.43±0.08^{g}
Medium Dose (290mg/kg)	897.58±203.73^{a}	12.38±0.00^{d}	0.61±0.41^{h}
High Dose (500mg/kg)	1511.98±20.90^{b}	12.38±5.84^{e}	0.34±0.09^{i}

Results are mean ± standard deviation, n=5. Values in the same column bearing similar super script are significantly different at P<0.001 and values bearing different super script are significantly not significant at P>0.05
Key: Substance administered = GS ethylacetate extract, Duration = once daily for 28 days, Route of administration = oral; u/l = unit per litre; method of statistical analysis = 2-way ANOVA

Kidney Function Tests

The kidney helps maintain electrolyte balance by filtering electrolytes and water from blood, returning some to the blood and excreting any excess into the urine. Kidney damage is normally associated with decrease in renal function which could lead to renal failure. Urea and creatinine are nitrogenous end products of metabolism. Urea is the primary metabolite derived from dietary protein and tissue protein turnover. Creatinine is the product of muscle creatine catabolism. The bulk of the urea is excreted by the kidney in a process that begins with glomerular filtration. At high urine flow rates (greater than 2 cm3/min), 40% of the filtered load is reabsorbed, and at flow rates lower than 2 cm3/min, reabsorption may increase to 60%. Low flow, as in urinary tract obstruction, allows more time for reabsorption. As kidney function declines, urinary excretion of urea and creatinine also declines and blood concentration of both increases. Potassium is often increased in renal failure (acute or chronic) in addition to bicarbonate deficiency which may lead to metabolic acidosis. Sodium is generally retained, but may appear normal, or hyponatremic, because of dilution from fluid retention.

The no significant increase in potassium ion (K^+), Na^+, HCO_3^- and creatinine levels in addition to decrease in urea observed in rats orally administered with low dose of GS as well as the non- significant increase in $K^{+,}$ Na^+, HCO_3^- and creatinine coupled to decrease in urea in rats orally administered with medium dose of GS (table 7) may suggest normal

kidney function as confirmed from the plates of histopathological analysis (Plates 2b and 2c respectively). However, rats treated with high dose of GS showed increased levels of $K^{+,}$ creatinine and/or urea coupled to decreased levels of HCO_3^-. These may suggest decrease in renal function evidenced by haemorrhage in the microphotograph (Plate 2d). So, the plant should be used with care.

Table 7: Effects of four weeks oral administration of *G. senegalensis* on kidney function parameters in rats

Group	K+ (mmol/l)	Na+ (mol/l)	Cl- (mmol/l)	HCO3- (mol/l)	Urea mmol/l	Creatinine (mmol/l)
Control	6.46±0.02[a]	144.23±0.26[a]	78.15±1.06[a]	144.13±0.26[a]	94.46±0.56[a]	0.72±0.01[a]
Low Dose (160mg/kg)	6.76±0.05[b]	145.01±23.62[b]	85.57±0.30[b]	144.91±23.61[b]	70.35±1.68[a]	0.86±0.26[b]
Medium Dose (290mg/kg)	6.81±0.37[a]	140.11±22.21[c]	80.52±6.54[c]	140.02±22.22[c]	82.80±19.84[b]	1.14±0.14[a]
High Dose (500mg/kg)	7.14±0.02[a]	116.36±2.01[d]	78.48±7.30[d]	116.26±2.01[d]	56.32±2.52[c]	1.27±0.08[a]

Results are mean ± standard deviation, n=5. Values in the same column bearing similar super script are significantly different at P<0.001 and values bearing different super script are significantly not significant at P>0.05

Key: Substance administered = GS ethylacetate extract, Duration = once daily for 28 days, Route of administration = oral; u/l = unit per litre; method of statistical analysis = 2-way ANOVA

Hematological Analysis

According to (Oslon *et al*., 2000), there is a correlation of toxicity in haematological, gas-trointestinal and cardiovascular adverse effects between animals and humans. Haematological indices in animals are important to determine the toxicity risk since the changes in the blood system have a higher predictive value for human toxicity. In this study, haematological indices were used to assess sub-chronic toxicity of GS in Wistar albino rats.

WBC and RBC showed no significant changes (P>0.05) between treatment groups (160, 290 and 500mg/kg) and the control group. Furthermore, there were significant changes (P<0.05) in HGB, HCT, MCH, MCHC and PDW between all the treatment groups and the control. Again, there was significant change (P<0.05) in MPV and P-LCR between two treatment groups (medium and high dose) when compared with the control. Significant change (P<0.05) in MPV and PCT was recorded between high and low dose groups respectively when compared with the con-trol group (table 8).

RBC_s are involved in the transport of oxygen (O_2) and carbon dioxide in the body. Similarly, HGB is an iron-containing O_2-transport metalloprotein in the RBC_s of all vertebrates (Maton *et al*., 1993) with the exception of the fish family, channichthyldae (Sidell and O' Brien, 2006) as well as tissues of invertebrates. HGB has the physiological function of transporting O_2 to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport CO_2 out of the body of animals (Ugwuene, 2011; Omiyale *et al*., 2012; Soetan *et al*., 2013; Isaac *et al*., 2013). The significant increase in RBC and HGB levels for the low and medium dose groups coupled with the relative increase for the high group are indications that the leaves extract of the plant might have caused an increase in RBC and HGB production. HCT is the % of RBC_s in blood (Purves *et al*., 2003). It is involved in the transport of O_2 and absorbed nutrients. HCT, HGB and MCH are major indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce RBC_s as in mammals (Awodi *et al*., 2005; Chineke *et al*., 2006). The marked increase in HCT values for low dose group coupled to the relative increases in other groups rule out the possibility of anaemia or disturbances in the erythrocyte or haemoglobin production and also suggests the increased rate of RBC_s production by the bone marrow. This therefore means that the leaves extract of GS enhances the oxygen-transport capacity of the blood. MCV, MCH and MCHC are indices for the basis of morphological anaemia classification (Moreno Chulilla *et al*., 2009). Though some of these parameters had significantly low values as well as relative increases and decreases, these cannot be used in anaemia classification since RBC and HGB levels in all treatment groups recorded significant and relative increases ruling out the incidence of anaemia. PLT and other indices such as PDW, MPV and P-LCR are implicated in blood clotting. The observed decrease in PLT level for the low dose group as well as the relative decreases for the rest of treatment groups may indicate thrombocytopenia. PDW is a measure of the variation in the circulating blood. Platelets recently released from bone marrow tend to be larger and to contain more RNA than older, small platelets, which discard their endoplasmic reticulum as they mature. It reflects how uniform the platelets are in size. MPV reflects the average size of platelets present in a person's sample of blood. A high MPV is usually a sign that there are more young platelets circulating in bloodstream. WBC usually show increase in activity in

response to toxic environment (Robins, 1974), it therefore fights infections, depend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. This therefore means that the insignificant increases in WBC_s values for all treatment groups give an indication that GS extract have some effects on the immune system of the experimental animals in the treatment groups.

Table 8: Effects of Four Weeks Oral Administration of *Gueira Senegalensis* Ethylacetate Extract on Haematological Parameters in Rats

Parameter	Low Dose	Medium Dose	High Dose	Control
WBC($X10^3$/µl)	10.93±0.44[b]	11.65±0.50[a]	11.87±2.72[a]	8.75±1.23[a]
RBC($X10^6$/µl)	7.65±0.71[a]	7.26±0.47[a]	6.96±1.04[a]	3.31±0.01[a]
HGB (g/dL)	13.05±0.76[a]	12.75±0.35[a]	12.10±1.30[a]	7.90±0.00[a]
HCT (%)	48.6±1.07[a]	47.55±3.47[a]	45.53±6.12[a]	22.95±0.07[a]
MCV (fL)	67.50 ±0.80[a]	65.50±0.57[a]	65.47±0.84[a]	69.30±0.14[a]
MCH (pg)	6.40±0.81[a]	7.55±0.64[a]	8.43±0.82[a]	23.90±0.00[a]
MCHC(g/dL)	28.60±0.80[a]	26.85±1.20[a]	28.20±0.87[a]	34.50±0.00[a]
PLT(µL)	33.50±4.95[a]	573.50±16.26[b]	497.67±203.36[c]	654.00±0.00[a]
PDW (fL)	9.20±0.28[a]	10.55±0.50[a]	9.57±0.21[a]	13.05±0.07[a]
MPV (fL)	9.00±0.71[b]	8.50±0.14[a]	7.80±0.17[a]	9.40±0.00[a]
P-LCR (%)	26.50±2.12[a]	15.85±1.49[a]	11.63±0.32[a]	24.05±0.07[a]
PCT (%)	0.02±0.01[a]	0.49±0.01[b]	0.39±0.17[a]	0.61±0.01[a]

Results are mean ± standard deviation, n=5. Values in the same column bearing similar super script are significantly different at P<0.001 and values bearing different super script are significantly not significant at P>0.05

Key: Substance administered = GS ethylacetate extract, Duration = once daily for 28 days, Route of administration = oral; u/l = unit per litre; method of statistical analysis = 2-way ANOVA

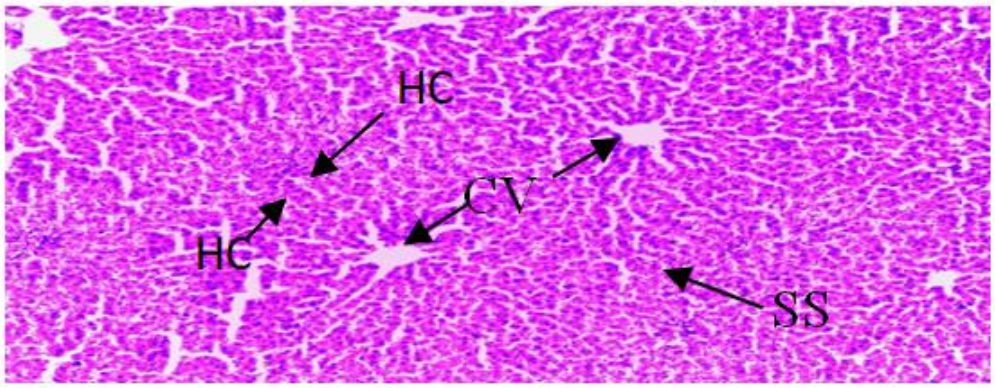

Plate 1a: Photomicrograph of a Section of Hepatocytes of normal albino rat (H and E, ×100) showing normal Hepatocytes (HC) arranged in polygonal units containing central venules (CV) and sinusoidal spaces (SS).

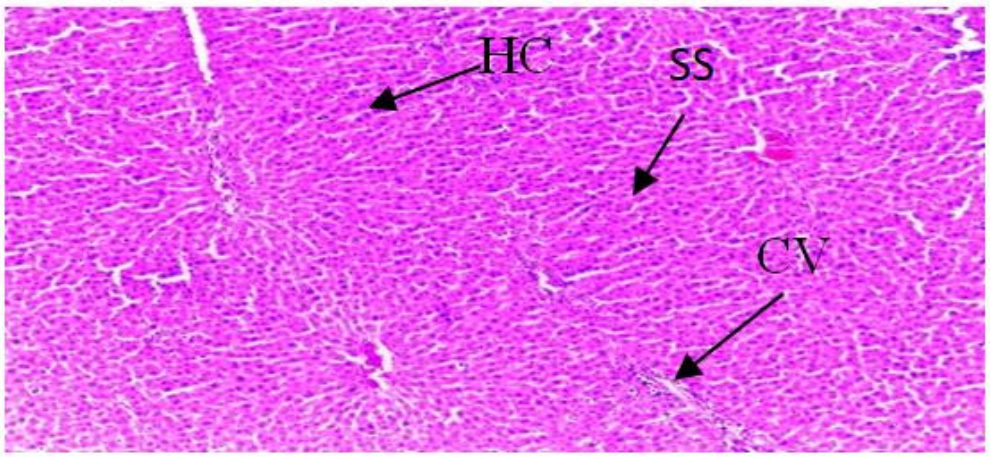

Plate 1b: Photomicrograph of a Section of Hepatocytes of Albino Rat administered with dose of 160mg/Kg of GS for a period of 28 days (H and E, ×100). Arrow Showing Normal Hepatocytes (HC) arranged in polygonal units containing central venules (CV) and sinusoidal spaces (SS).

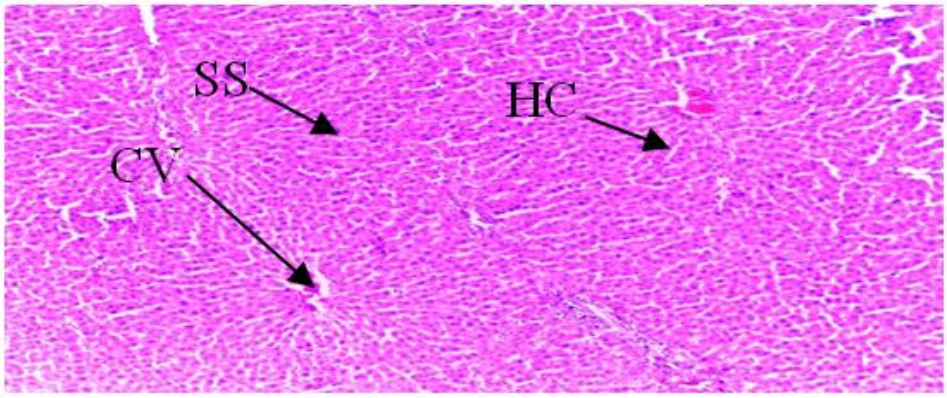

Plate 1c: Photomicrograph of a Section of Hepatocytes of Albino Rat administered with dose of 290mg/Kg of GS for a period of 28 days (H and E, ×100), Arrow Showing Normal Hepatocytes (HC) arranged in polygonal units containing central venules (CV) and sinusoidal spaces (SS).

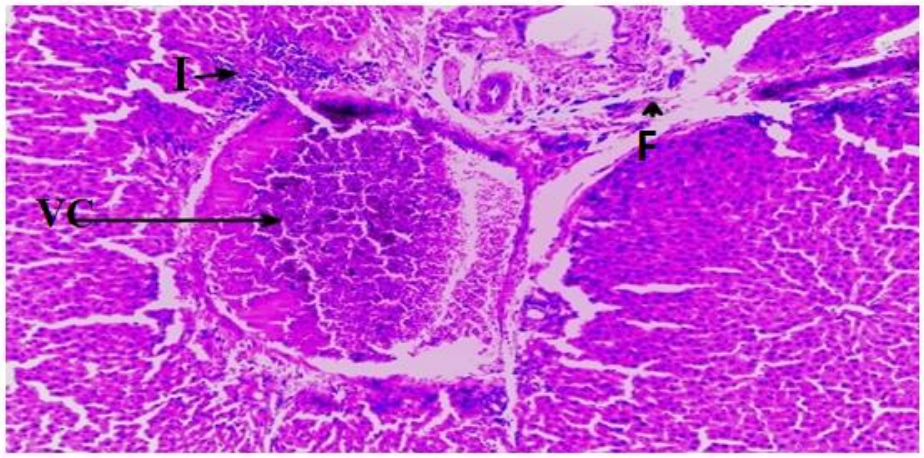

Plate 1d: Photomicrograph of a Section of Hepatocytes of Albino Rat administered with dose of 500mg/Kg of GS for a period of 28 days (H and E, ×100), Arrow Showing Inflammation (I), Fibrosis (F) and Vascular congestion (VC).

4. CONCLUSION

From the results of this research, it can be concluded that *G. senegalensis* plant contain some important phytochemical compounds that may attribute to the biochemical properties possessed by the plant. The results of acute toxicity study showed that the plant is practically non-toxic when used for a short period of time and on long term of use should be used with care. Also, the Haematological results indicated that the plant may have some effects on the immune system, might have caused an increase in RBC and haemoglobin production, and may also enhance O_2 – transport capacity of the blood.

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6. ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

7. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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