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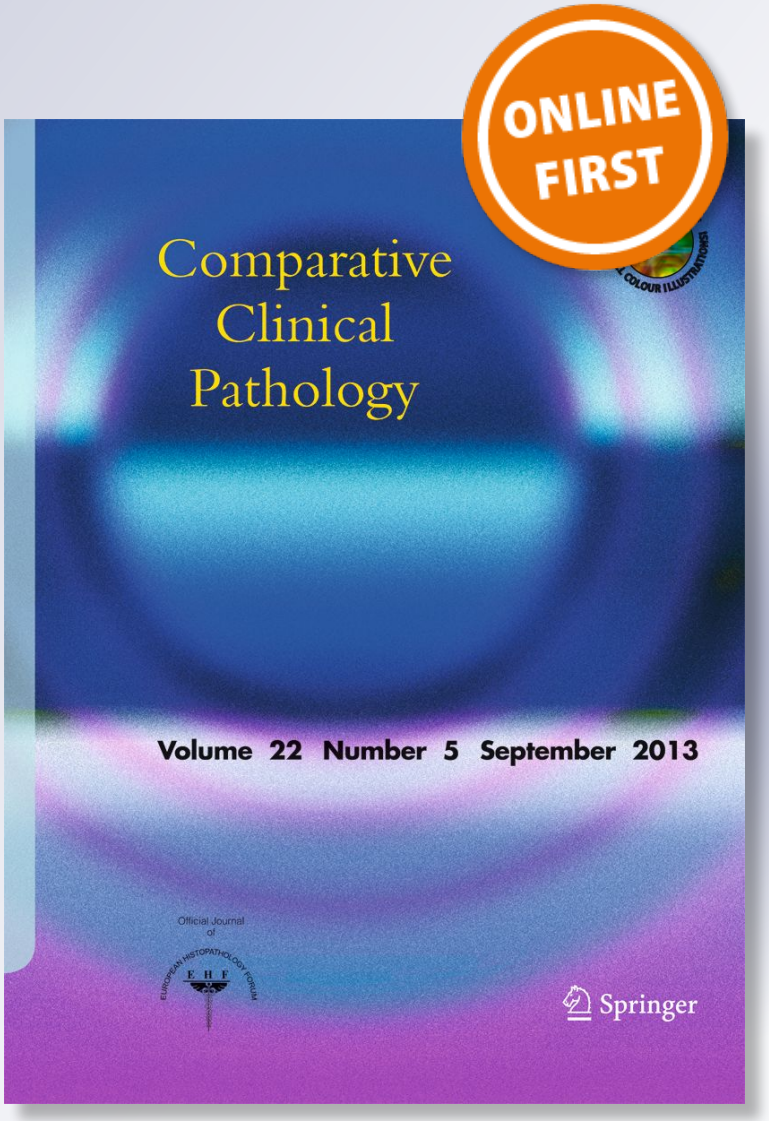

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ORIGINAL ARTICLE

Anti-plasmodial, Anti-inflammatory, anti-nociceptive and safety profile of Maytenus senegalensis root bark extract on hepato-renal integrity in experimental animals

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Abstract

Maytenus senegalensis is a plant with several medicinal claims in folk medicine. Methanol root extract of the plant was evaluated for antiplasmodial, anti-inflammatory, anti-nociceptive, and safety profile. Antiplasmodial, anti-inflammatory (egg albumin induced paw edema), and analgesic (hot plate test) experiments were set up, each consisting of four (4) groups (A-D) of five mice each. Groups A–C of each experiment were treated with 2.0 ml/kg bw normal saline, 400 and 800 mg/kg bw of the extract according to the procedures. Chloroquine, acetyl salicylic, and aspirin respectively were used as the reference drugs. Hematological and biochemical parameters were evaluated after 4-week administration of the extract to four groups of five rats each at oral daily doses of 0, 200, 400, and 800 mg/kg bw, respectively. At 400 and 800 mg/kg bw, the extract caused 58.88% and 58.49% inhibition of parasitemia, and 51.92% and 54.66% inhibition of paw edema, respectively. The reaction time to the thermal stimuli increased ($p < 0.05$) with increased extract concentrations. All the doses of the extract significantly ($p < 0.05$) increased the concentration urea, creatinine, alkaline phosphatase (ALP), and white blood cell (WBC) count, while aspartate transaminases (AST), alanine transaminase (ALT), sodium, potassium, chloride, packed cell volume (PCV), red blood cell (RBC), and hemoglobin (HGB) compared well ($p > 0.05$) with their reference values. The extract alters some functional integrity of kidney, and thus caution should be exercised when using the extract for the above pharmacological activities or other oral remedy.

Keywords Antiplasmodial · Anti-inflammatory · Analgesic · *Maytenus senegalensis*

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Introduction

Malaria is a parasitic disease that is transmitted by the bites of *Plasmodium* infected Anopheles mosquitoes. With about 3.2 billion people at risk, malaria has a greater morbidity and mortality than any other infectious diseases globally (WHO 2016). It accounts for about 219 million cases and 435,000 deaths in 2017, 90% of which occurred in sub-Saharan Africa, with 35% of the total death being in Nigeria and Congo (WHO 2018). Increased resistance of the *Plasmodium falciparum* parasite to many of the available synthetic drugs has made the treatment of malaria increasingly difficult particularly in children and pregnant women due to their vulnerability to malaria fatality (Lawal et al. 2018).

Pain and inflammation are increasingly gaining global scientific interest due to their implicative role in the etiology of various human diseases (Mohiuddin et al. 2018). A number of drugs including dexamethasone opioids, morphine, and aspirin have been developed for management of pain and inflammatory conditions. Though these drugs have considerable

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effect in inflammation inhibition, major side effects are also linked to their usage (Mostafa et al. 2010; Jigam et al. 2017). Thus, the search for new drugs alternative with little or no side effects is highly recommended.

African medicinal plants and other natural products represent ample sources of natural bioactive metabolite with therapeutic values against several diseases (Lawal et al. 2015a, 2017; Bashir et al. 2015a). They serve as divine alternatives to synthetic drugs which are besieged with undesirable side effect in addition to loss of efficacy and resistance. In comparison with synthetic drugs, medicinal plants have proven to be more effective, easily available, least expensive, and friendly to the body system Toiu et al. (2018). The therapeutic properties of these plants are, however, attributed to the quality and quantity of the secondary metabolites including alkaloids, phenolics, flavonoids, anthraquinones, saponins, and terpenes that they contain Yakubu et al., (2015). Primarily, these metabolites play defensive role in plants but exert different pharmacological effects such as anti-inflammatory, analgesic, antidiabetics, antimicrobial, anti-parasitic, and antioxidants effects when ingested by human/animals (Lawal et al. 2015a, 2017; Bashir et al. 2015a). Despite the diverse pharmacological activities of these metabolites, they also confer toxic attribute to plants, thus making it unsafe for prolong consumption or clinical application. It is therefore recommended that safety profile should be considered in the evaluation and use of medicinal plants for pharmacological activities.

Maytenus senegalensis (Lam.) is an African shrub of *celastraccae* family. It grows in the semi-desert regions of Asia and tropical regions of Africa. Its roots and bark are traditionally used in the folk medicine for the treatment of a number of ailments, including rheumatism, snakebites, diarrhea, eye infection, and dyspepsia (da Silva et al. 2011). An extract of the roots and barks of the plant is used for severe headaches, skin rashes, muscle spasms, fevers, and parasitic infections (Zangueu et al. 2018). It is also used for fertility problems, venereal diseases, pneumonia, epilepsy, and as a tonic (Jorge et al., 2004). Previous study has demonstrated that the leaf, root, and stem bark extracts of *M. senegalensis* possess in vitro anti-*plasmodial*, anti-leishmanial, and antibacterial activities (El Tahir et al. 2014). However, literature survey revealed dearth of scientific information on the pharmacological activities and safety/toxicity effect of the extract.

The leaves of *M. heterophylla* extract at 1200 mg/kg have shown to be non-toxic (da Silva et al. 2010). Previous studies have also reported safety of *M. senegalensis* leaves by demonstrating LD_{50} of above 5000 mg/kg bw (Murjanatu et al. 2015; Malebo et al. 2015). Aqueous extract of *M. senegalensis* administrated orally in mice caused no any toxicity to mice (Sanogo et al. 2006). Previous findings also suggest that *M. senegalensis* is practically non-cytotoxic as it exhibited IC_{50} values of 87.82 ± 3.02 and > 90.00 against mammalian cell lines viz.: Vero cell lines and rat skeletal myoblast (L-6) cells, respectively (Malebo et al. 2009; Ahmed et al. 2013). Therefore, the present study was set out to evaluate the antimalarial, anti-inflamatory, analgesic activities, and the effect of the extract on some kidney and liver function indices in rats.

Materials and methods

Plant materials

The *Maytenus senegalensis* root bark was collected from Bida, Niger State. The plant was authenticated by a botanist, from the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria.

Extraction of plant materials

The root bark sample was washed and dried for 2 weeks (37 °C) and finally grounded using a grinder mill. A 200 g of the plant material was extracted with 200 mL of methanol using Soxhlet apparatus, and the resulting extract was concentrated using rotary evaporator. The resulting extract was placed in air-tight container and refrigerated until when required.

Phytochemical study

Evaluation of qualitative phytochemical composition of the *Maytenus senegalensis* root bark methanol extract was carried out using standard methods of Trease and Evans 1989 and Sofowora (1993).

Experimental animals

A total of sixty (60) adult Swiss albino mice and forty one (Shittu et al., 2015) male albino rats (*Rattus norvegicus*) weighing 25.34 ± 0.98 g and 150.65 ± 5.89 g, respectively, were obtained from National Veterinary Research Institute (NVRI), Vom, Plateau State of Nigeria. The mice were used for antiplasmodial, analgesic, and anti-inflammatory study, while the rats were used for toxicity study. The animal handling and experimentation were in concordance with the guidelines for laboratory animal use and care as contained in the European Convention on Animal Care Guidelines and Protocol.

Parasite

Plasmodium berghei NK65 chloroquine-sensitive strain was obtained from the National Institute of Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria, and maintained in the laboratory by serial passage in mice.

Antiplasmodial study

Four days (Mohiuddin et al., 2018) suppressive test was used to evaluate the antimalarial properties of the crude, methanol extract of *Maytenus senegalensis* as described by Jigam et al. (2011). One milliliter of the parasitized erythrocytes was obtained from a donor-infected mouse through the jugular vein into an EDTA-sample bottle and diluted with physiological saline (5 ml). A total of 20 mice were inoculated with infected blood suspension (0.2 ml) and were observed for 72 h, after which thin blood smears were prepared from tail of each mouse onto slides. The slides were allowed to dry, fixed with methanol and stained with Giemsa's solution for 30 min and examined microscopically with × 100 magnification to check for the parasites.

The infected mice were randomly grouped into four (A–D) of five mice each. Groups A and B mice were treated with 400 and 800 mg/kg bodyweight crude extract, respectively. Groups C and D received normal saline (2 ml/kg bw) and chloroquine (5 mg/kg bodyweight) to serve as negative and positive controls, respectively. All the treatments were done orally for four consecutive days. Percentage growth inhibition of the parasite was calculated using the formula:

$$\%\text{inhibition} = \frac{\text{Mean parasitemia in negative control} - \text{Mean parasitemia in treated}}{\text{Mean parasitemia in negative control}} \times 100$$

Anti-inflammatory study

Anti-inflammatory activity of the extract was tested using egg albumin-induced paw edema in mice according to the methods of Winter et al. (1962). A total of 20 mice were randomly grouped into four (A–D) of five mice each and were administered a single dose of 400 and 800 mg/kg bw of the extract, 150 mg/kg bw acetyl salicylic acid, and 2 ml/kg bw normal saline, respectively, 30 min before the injection of the albumin into the right hind limb. The percentage inhibition of edema was calculated for each dose using the formula:

$$\%\text{inhibition} = \frac{\text{Mean increase in paw in negative control} - \text{Mean increase in paw in treated}}{\text{Mean increase in paw in negative control}} \times 100$$

Analgesic study

The hot plate test (Thermal stimuli) was used to measure analgesic activity of the extract according to the method described by Mohiuddin et al. (2018). A total of 20 mice were randomly grouped into four (A–D) of five mice each and were administered a single dose of 400 and 800 mg/kg bw of the extract, 150 mg/kg bw aspirin, and 2 ml/kg bw normal saline, respectively. The time (seconds) taken by the mice to react to the thermal pain by licking their paw or jumping were recorded at 0, 15, 30, 45, and 60 min of extract treatment.

Toxicity studies

Acute toxicity of the extract was determined in two phases according to Lorke's (1983) as described by Tsado et al. (2015). In phase 1, a total of nine rats were grouped into three of three rats each and were given a single dose of 10, 100, and 1000 mg/kg bw of the extract, respectively. A control group was also set up comprising of three rats and was given 2 ml/kg bw normal saline. The absence of death after 24 h of extract administration led to the initiation of phase II which was set up with another three groups of three rats each and were given a single dose of 1600, 2900, and 5000 mg/kg bw of the extract, respectively. All extract were administered orally using esophageal cannula. The animals were observed for any adverse effect and mortality within 24 h of treatment and after a week.

Sub-chronic toxicity study was carried out according to the methods of Yusuf et al. (2018a). Four groups (A–D) of five rats each were given extract at doses of 0, 200, 400, and 800 mg/kg bw, respectively, for 4 weeks. Extract was administered orally once daily at exactly 10:00 a.m. using esophageal cannula. After 28 days of extract administration, the animals were sacrificed under ether anesthesia and blood was collected in EDTA bottle for hematological analysis. Another set of blood was collected in EDTA free sample bottle, and the blood was allowed to cloth and centrifuge at 3000 rpm for 10 min. The serum was extracted and stored in the refrigerator (− 4 °c) before been used for biochemical studies (Bashir et al. 2015b).

Evaluation of hematology and biochemical parameters

Assay kits for aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and urea were products of Randox Laboratories, Co-Antrim, UK. All

Table 1 Phytochemical composition of *Maytenus senegalensis* root extract

Phytochemicals	Results
Alkaloids	+
Flavonoids	+
Phenols	+
Tannins	+
Glycosides	+
Terpenes	+
Steroids	–
Saponins	+
Anthraquinones	+

Table 3 Anti-inflammatory activity of crude methanol root extract of *M. senegalensis* against egg albumin-induced paw edema

Treatment	Dose (mg/kg bw)	Mean paw edema	Inhibition (%)
M. senegalensis	400	3.51 ± 0.35 b	51.92
M. senegalensis	800	3.31 ± 0.27 b	54.66
Acetyl salicylic Acid	150	2.12 ± 0.16 a	70.96
Control	2.0 ml/kg bw	7.30 ± 0.60 c	–

Values are mean ± SEM of five determinations. Values along the same column with different lowercase letters are significantly different ($p < 0.05$)

other kits used were product of Agape Diagnostic. The serum activities of ALT, AST, and ALP were determined using standard procedures (Reitman and Frankel 1957; Wright et al. 1972). The concentrations of serum urea and creatinine were determined using standard procedures (Veniamin and Varkirtzi 1970; Bartels and Bohmer 1972), while the concentrations of sodium, potassium, and chloride ions were determined as described by Tietz (1995).

The hematological components including hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), and platelet count (PLT) were determined using the automated hematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by Dacie and Lewis (1991).

Data analysis

Data analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan multiple range test (DMRT). Data were expressed as mean ± SEM of triplicate determinations. Significance was considered at $p < 0.05$.

Results

Phytochemical compositions of *Maytenus senegalensis*

Crude methanol root bark extract of *Maytenus senegalensis* contains alkaloids, flavonoids, phenols, tannins, glucosides, terpenes, saponins, and anthraquinones. However, steroid was not detected (Table 1).

Antimalarial, anti-inflammatory, and analgesic effect

Treatment with the crude methanol root extract of the *Maytenus senegalensis* showed a significant reduction in parasitemia, with 58.88% and 58.49% inhibition of parasitemia at 400 and 800 mg/kg bw, respectively (Table 2). Crude methanol root extract of *M. senegalensis* at 400 and 800 mg/kg bw showed a dose-dependent reduction of paw edema induced by egg albumin. The extract exhibited 51.92% and 54.66% inhibition of paw edema, compared to inhibition of 70.96 recorded for acetyl salicylic acid (Table 3). The higher dose of the extract demonstrated the highest and significant ($p < 0.05$) nociceptive inhibition of thermal stimulus (Table 4).

Toxicity study

No mortality was recorded in any of the experimental animals during the phase I (10, 100, 1000 mg/kg bw of the extract) and phase II (1600, 2900, and 5000 mg/kg bw of the extract) study (Table 5). The LD_{50} is thus set to be > 5000 mg/kg bw. In subchronic toxicity study, all extract doses tested ($p < 0.05$) significantly increased the serum concentration of alkaline phosphatase (ALP), urea, and creatinine when compared with the control rats. The extract did not cause any significant ($P > 0.05$) alteration to the concentration of alanine transaminases (ALT), aspartate transaminases (AST), sodium

Table 2 Antiplasmodial activity of crude methanol root extract of *M. senegalensis*

Treatment	Dose (mg/kg bw)	Average parasitemia	Inhibition (%)
M. senegalensis	400	21.33 ± 1.64	58.88
M. senegalensis	800	21.53 ± 2.66	58.49
CQR	5	13.13 ± 4.26	74.69
NS	2.0 ml/kg bw	51.87 ± 10.27	–

Values are mean ± SEM of five determinations

NS normal saline, *CQR* chloroquine

Table 4 Analgesic effects of the methanol root extract of *M. senegalensis* in the hot plate (Thermal stimuli) test

	Time (s)				
	0 min	15 min	30 min	45 min	60 min
400 mg/kg bw extract	3.20 ± 0.12 a	4.20 ± 0.46 b	4.60 ± 0.27 bc	6.40 ± 0.22 b	7.50 ± 0.30 b
800 mg/kg bw extract	3.17 ± 1.57 a	6.10 ± 0.49 ab	6.40 ± 0.45 b	7.20 ± 0.64 b	9.70 ± 0.90 ab
150 mg/kg bw ASA	3.60 ± 0.21 a	7.00 ± 0.75 a	7.30 ± 0.58 a	9.60 ± 1.56 a	11.60 ± 1.21 a
2.0 ml/kg bw NS	3.00 ± 0.90 a	2.90 ± 0.21 c	3.00 ± 0.39 c	2.70 ± 0.33 c	2.30 ± 0.49 c

Values are mean ± SEM of three determinations. Values along the same column with different superscript letters are significantly different ($p < 0.05$)
ASA acetyl salicylic acid, *NS* normal saline

potassium, and chloride in the serum of rats when compared with their reference values (Table 6).

The WBC count in group of rat administered 400 and 800 mg/kg bw extract was significantly ($p < 0.05$) higher when compared with the control. Platelet count was significantly ($p < 0.05$) higher in rats dosed 800 mg/kg bw when compared with the control. However, no significant difference exist in PCV, RBC, and HGB among the groups of rats treated with 200, 400, and 800 mg/kg bw of *M. senegalensis* root extract when compared with the control group (Table 7).

Discussion

Phytochemical constituents are an integral part of medicinal plants and are responsible for their numerous bioactivities. The phytochemical screening of *M. senegalensis* showed the presence of alkaloids, flavonoids, phenols, tannins, glycosides, terpenes, saponins, and anthraquinones. This agrees with the previous studies that reported the presence of alkaloids, flavonoids, tannins, and phenols in *Maytenus senegalensis* (Amom and Vershima 2013). The presence of terpenoids, saponins, flavonoids, alkaloids, and tannins indicates that the plant extract might possess some medicinal qualities such as antimalarial, anti-inflammatory, analgesic, anti-diabetic, and anti-cholesterolemia effect (Aquino et al. 2001). However, the absence of steroids in *M. senegalensis* agrees with the previous study that reported that not all phytochemicals are present in medicinal plants and those present differs with their extraction solvent (Lawal et al. 2014).

The traditional use of *M. senegalensis* for the treatment of malaria could be attributed to the presence of certain phytochemicals that constitute the bioactive principles in the plant. Some of the secondary metabolites detected in this study have been implicated in anti-*plasmodium* activities. Preliminary antiplasmodial activities of the extract show that *Maytenus senegalensis* produce significant reduction in the degree of *P. bergei* load. This shows that the plant possesses antimalarial activity; this is in agreement with the work done by Malebo et al. (2015), who reported a 71% inhibition of parasitemia in mice treated with crude ethanolic extract of *M. senegalensis*. It has been established that crude extracts from plants tend to be better at inhibiting parasite multiplication (plasmodistatic) rather than being destructive to the parasite (plasmodicidal), as unpurified bioactive principles require initial conversions which time lag allows for parasite proliferation (Jigam et al. 2011, 2012). The anti-*plasmodium* activity of *Maytenus senegalensis* could be attributed to the presence of alkaloids, flavonoids, and terpenes, all of which are contained in this plant. The anti-oxidant flavonoid and phenolic compounds have been shown to exert anti-*plasmodium* activity by counteracting the plasmodium parasite-induced oxidative damage (Lawal et al. 2016, 2017).

The crude extract of *M. senegalensis* was found to possess a significant anti-inflammatory activity; this is in conformity with the findings of other researchers which indicated crude

Table 5 LD_{50} of crude extract of *M. senegalensis*

	Doses (mg/kg bw)	Mortality observation	
Phase 1	10	0/3	Animals were calm, no observable physical changes
	100	0/3	
	1000	0/3	
Phase 2	1600	0/3	
	2900	0/3	Weakness
	5000	0/3	Weakness, erythremia which disappear before 24 h
Normal saline (control)	2.0 ml/kg bw	0/3	Animals were calm, no observable physical changes

Table 6 Biochemical parameters in serum of rats administered methanol root extract of *M. senegalensis*

	Control	200 mg/kg bw	400 mg/kg bw	800 mg/kg bw
ALT (U/L)	23.00 ± 2.58 a	21.99 ± 1.98 a	20.87 ± 2.48 a	20.17 ± 2.32 a
AST (U/L)	87.92 ± 7.78 a	81.78 ± 4.89 a	80.87 ± 2.09 a	80.30 ± 2.69 a
ALP (U/L)	76.18 ± 10.85 a	139.64 ± 4.74 b	152.92 ± 23.84 b	167.17 ± 2.17 b
Creatinine (mmol/L)	1.68 ± 0.81 a	2.01 ± 0.56 b	2.05 ± 0.19 b	2.25 ± 0.17 b
Urea (mmol/L)	6.50 ± 0.78 a	7.16 ± 0.78 b	7.30 ± 1.74 b	6.90 ± 0.71 b
Na^+ (mEq/L)	144.88 ± 3.35 a	139.78 ± 2.56 a	149.30 ± 2.15 a	146.58 ± 1.82 a
Cl^- (mmol/L)	110.49 ± 3.43 a	108.98 ± 3.21 a	118.68 ± 2.90 a	112.21 ± 7.86 a
K^+ (mEq/L)	12.57 ± 0.38 a	11.89 ± 0.78 a	11.20 ± 0.49 a	10.80 ± 0.90 a

Values are mean ± SEM of five determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$)

M. senegalensis as having 51% inhibition of paw edema in carrageenan-induced paw edema in rats (Sosa et al. 2007). In this study, significant increase was observed in pain threshold of animals treated with the crude methanolic extract of *M. senegalensis.* The extract of *M. senegalensis* root significantly delayed the reaction time of thermally induced pain. This model is selective for centrally acting analgesics and indicates narcotic involvement (Mohiuddin et al. 2018) with opioid receptors, suggesting that is a centrally acting analgesic. The observed effect is in conformity with the previous studies carried out on *M. senegalensis* (Murjanatu et al. 2015; Sosa et al. 2007).

The absence of death in the acute toxicity test for crude extract of *M. senegalensis* even at dose 5000 mg/kg bw suggests that the extract may be safe for treatment. This finding is in agreement with work done by Murjanatu et al. (2015), on the methanolic leaf extract of *M. senegalensis*, where no mortality or toxic effect was observed up until 5000 mg/kg bw of extract.

It has been established that oral ingestion of medicinal plants or drugs can alter the normal values of hematological indices (Lawal et al. 2015b). As such, the hematopoietic system is an important index of physiological and pathological status in man and animal. In the present study, administration of methanolic extract of *M. senegalensis* at various doses significantly increased ($p < 0.05$) white blood cell count (WBC). WBC and its differentials are known for their defensive role against foreign body and infectious agents through the production, transportation, and distribution of antibodies in immune response (Berinyuy et al. 2015). The significantly increased WBC counts observed in the extract treated rats reflect leucopoetin-release and possible immune-modulatory effects of the extract. It has been reported that plants with immune-modulatory effects enhanced the production of more WBC in order to overcome the stress induced by the plant (Lawal et al. 2015c). This will increase the animal's ability of generating antibodies in the process of phagocytosis which have high degree of resistance to diseases and enhance adaptability to local environmental and disease prevalent conditions (Borsuk et al. 2011).

Packed cell volume (PCV), red blood cell counts (RBC), and hemoglobin all compared well with values of the control rats at all doses of the methanolic extract of *M. senegalensis* tested. This is an indication that the extract did not cause destruction of existing RBC and also did not inhibit or stimulate the erythropoietin release in the kidney, which is the humoral regulator of RBC production (Shittu et al. 2015). Hemoglobin and RBC are very essential in transferring respiratory gases (Nwaka et al. 2015). This finding also indicates that the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues was not compromised by extract of *M. senegalensis*. There was no significant difference

Table 7 Hematological parameters in rats administered methanol root extract of *M. senegalensis*

	Control	200 mg/kg bw	400 mg/kg bw	800 mg/kg bw
PCV (%)	40.50 ± 0.29 a	40.66 ± 0.83 a	43.50 ± 0.86 a	40.50 ± 0.86 a
RBC (× 109/L)	7.65 ± 0.08 a	7.67 ± 0.56 a	7.85 ± 0.08 a	7.80 ± 0.73 a
PLT (× 109/L)	682.50 ± 38.9 a	645.83 ± 34.48 a	657.50 ± 65.52 a	777.50 ± 46.47 b
WBC (× 109/L)	6.25 ± 0.43 a	6.13 ± 0.32 a	7.10 ± 0.52 b	8.35 ± 0.43 b
HB (g/dl)	12.50 ± 0.15 a	12.32 ± 0.49 a	12.75 ± 0.31 a	12.27 ± 0.16 a

Values are mean ± SEM of five determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$)

PCV packed cell volume, *HB* hemoglobin, *RBC* red blood cells, *WBC* white blood cells, *PLT* platelet count

in platelet count of test animals and the control group. Platelets are fragments of large bone marrow cells that function to aid blood clotting and also initiate the repair of walls of blood vessels (Barrett and Ganong 2012).

Evaluation of serum biochemical indices in animals has become the most valuable tools for assessing the integrity and functionality of organs as well as risk assessment, pathological condition, and general health status of the body (Yusuf et al. 2018b). AST and ALT are biomarkers of hepatic integrity and to a certain level can be used to assess the extent of hepatocellular damage. In this study, serum ALT and AST activity was not significantly altered in test animals when compared with those in the control group. This is an indication that the integrity of liver has not been compromised.

Previous studies have used the levels of activities of serum alkaline phosphatases as an indicator of the integrity of plasma membrane (Lawal et al. 2016; Shittu et al. 2017). The observed significant increase from the control values in the activities of ALP suggests that the integrity and functionality of endoplasmic reticulum and plasma membrane might have been comprised. This increase ALP activities could be a threat to cells that are dependent on a variety of phosphate group for their vital process, e.g., synthesis of major membrane phospholipids (phosphatidylethanolamine and phosphatidylcholine) since there may be over production of the phosphate group which consequently affect membrane fluidity and altered the permeability of the epithelial cells (Yakubu et al. 2015).

The levels of electrolytes, creatinine, and urea play important roles in determining the synthetic and excretory roles of the kidney (Ikanone et al. 2017). The significant increase in serum urea and creatinine following the administration of the extract may be due to the increased protein catabolism or may be an indication of renal impairment, especially with regard to the glomerular filtration rate which directly affects the rate of clearance of waste substance by the kidney. The extract might have either interfered with creatinine metabolism leading to increased synthesis, or the tissue might have compromised all or part of its functional capacity of tubular excretion (Zilva et al. 1991). Increases in serum urea levels as observed might be the effect of nephrotoxicity of *M. senegalensis* extract which is also indicative of impaired kidney function (Badole and Kotwal 2015). Ikanone et al. (2017) stated that renal failure leads to retention of creatinine and other non-protein nitrogenous constituents of the blood which may be responsible for the increases observed with the test groups in this study. The non-significant effects in the level of Na^+, K^-, and Cl^- concentrations following administrations of *M. senegalensis* extract at all doses tested suggest that normal functioning of liver and kidney tubules as regards to these electrolytes was preserved.

Conclusion

The methanol crude extract of *M. senegalensis* root bark exhibits promising antimalarial, anti-inflammatory, and analgesic activity. The extract alters some functional integrity of the kidney, and thus caution should be exercised when using the extract for the above pharmacological activities or other oral remedy.

Author's contributions This work is a collaboration of all the authors. All authors read and approved the final manuscript.

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Data availability All relevant data are presented in the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna, Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

Consent for publication Not applicable.

Abbreviations ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate amino transferase; CRT, creatinine

References

Ahmed AS, McGaw LJ, Eloff JN (2013) Evaluation of pharmacological activities, cytotoxicity and phenolic composition of four Maytenus species used in southern African traditional medicine to treat intestinal infections and diarrhoeal diseases. BMC Complement Altern Med 13:100

Amom TT, Vershima AJ (2013) Phytochemical evaluation and antibacterial activity: a comparison of various extracts from some Nigerian trees. Peak J Med Plant Res1 2:13–18

Aquino R, Morelli S, Lauro MR, Abdo S, Saija A, Tomaino A (2001) Phenolic constituents and antioxidant activity of an extract of *Anthurium versicolor* leaves. J Nat Prod 64:1019–1023

Badole S, Kotwal S (2015) Biochemical, hematological and histological changes in response to graded dose of extract of *Equisetum Arvense* in adult female Wistar rats. Int J Pharm Sci Res 6(8):3321–3326

Barrett KE, Ganong WF (2012) Ganong's review of medical physiology. McGraw-Hill Medical, New York

Bartels H, Bohmer M (1972) Kinetic determination of creatinine concentration. Clinical Chimica Acta 37:193–197

Bashir L, Shittu OK, Sani S, Busari MB, Adeniyi KA (2015a) African natural products with potential Antitrypanosoma properties: a review. Inter J Biochem Res Rev 7(2):45–79

Bashir L, Shittu OK, Busari MB, Sani S, Aisha MI (2015b) Safety evaluation of Giant African land snails *(Archachatina*marginata) Haemolymph on hematological and biochemical parameters of albino rats. J Adva med Pharm Sci 3(3):122–130

Berinyuy EB, Lawal B, Olalekan AA, Olalekan AA, Yusuf AA, Sakpe S, Ossai PC (2015) Hematological status and organs/body-weight parameters in Wister rats during chronic administration of Cassia occidentalis. Inter Blood Res Rev 4(3):1–7

Borsuk OS, Masnaya NV, Sherstoboev EY, Isaykina NV, Kalinkina GI, Reihart DV (2011) Effects of drugs of plant origin on the development of the immune response. Bull Exp Biol Med 151(2):194–196

Dacie JV, Lewis SM (1991) Practical hematology, 7th edition Edingburgh edn. Churchill Livingston Inc, pp 34–55

El Tahir A, Ibrahim AM, Satti GM, Theander TG, Kharazmi A, Khalid SA (2014) The potential antileishmanial activity of some Sudanese medicinal plants. Phytother Res 12:576–579

Ikanone CEO, Akinloye OA, Augbaja RN, Omotainse SO, Ajayi OL, shopein TM (2017) Effect of sub-acute exposure to bonny light crude oil on plasma biochemistry and liver histopathology of albino rat. Animal Res Inter 14(1):2652–2659

Jigam AA, Akanya HO, Dauda BEN Ogbadoyi EO (2011) Antiplasmodial, analgesic and anti-inflammatory effects of crude *Guiera senegalensis* Gmel (Combretaceae) leaf extracts in mice infected with *Plasmodium berghei*. J Pharmacog and Phyto 3(10): 150–154

Jigam AA, Samuel T, Oibiokpa FI (2012) Plasmodistatic, Anti-Inflammatory & Analgesic Effects of different fractions of *Mormodica balsamina* extracts in rodents. Asian J Biochem Pharma Res 4:2231–2560

Jigam AA, Mahmood F, Lawal B (2017) Protective effects of crude and alkaloidal extracts of *Tamarindus indica* against acute inflammation andnociception in rats. J Acute Dis 6(2):78–81

Jorge RM, Leite JP, Oliveira AB, Tagliati CA (2004) Evaluation of antinociceptive, anti-inflammatory and ulcerogenic activities of the *Maytenus* family. J Ethnopharmacol 94:93–100

Lawal B, Ossai PC, Shittu OK, Abubakar AN (2014) Evaluation of phytochemicals, proximate, minerals and anti-nutritional compositions of yam Peel, maize chaff and bean coat. Inter J Appl Biol Res 6(2): 01–17

Lawal B, Shittu OK, Kabiru AY, Jigam AA, Umar MB, Berinyuy EB, Alozieuwa BU (2015a) Potential antimalarials from African natural products: a review. J Intercult Ethnopharmacol 4(4):318–343

Lawal B, Shittu OK, Abubakar AN, Haruna GM, Sani S, Ossai PC (2015b) Haematopoetic effect of methanol extract of Nigerian honey bee (*Apis mellifera*) Propolis in mice. J Coast Life Med 3(8):648–651

Lawal B, Shittu OK, Rotimi AA, Olalekan AA, Kamooru AA, Ossai PC (2015c) Effect of methanol extract of *Telfairia occcidentalis* on Haematological parameters in Wister rats. J Med Sci 15(5):246–250

Lawal B, Shittu OK, Oibiokpa IF, Mohammed H, Umar SI, Haruna GM (2016) Antimicrobial evaluation, acute and sub-acute toxicity studies of *Allium sativum*. J Acute Dis 5(4):296–301

Lawal B, Shittu OK, Inje, OF, Berinyuy EB, Muhammed H (2017) Potential antioxidants and Hepatoprotectives from African natural products: a review. Clin Phytosci 2(23):1–66

Lawal B, Shittu OK, Abubakar A, Kabiru AY (2018) Human genetic markers and structural prediction of *Plasmodium Falciparum* multi-drug resistance gene (*Pfmdr1*) for ligand binding in pregnant women attending general hospital Minna. J Envir public health 2018:1–13

Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 54:275–287

Malebo HM, Tanja W, Cal M, Swaleh SM, Omolo MO, Hassanali A (2009) Antiplasmodial, anti-trypanosomal, anti-leishmanial and cytotoxicity activity of selected Tanzanian medicinal plants. Tanzan J Health Res 11:226–234

Malebo HM, Wiketye SJK, Nteghenjwa AK, Vitus AN, Calister PI, John WO, Richard S, Paulo PM, Julius JM, Mammuya B et al (2015) *In vivo* antiplasmodial and toxicological effect of Maytenus senegalensis traditionally used in the treatment of malaria in Tanzania. Mal J 14:79

Mohiuddin M, Dewan SMD, Das A, Sarwar SM (2018) Anti-nociceptive, anti-inflammatory and antipyretic activities of Ethanolic extract of *Atylosia scarabaeoides* (L.) Benth (family: Fabaceae) leaves in experimental animal. J Appl Life Scie Inter 17(4):1–12

Mostafa M, Appidi JR, Yakubu MT, Afolayan AJ (2010) Anti-inflammatory, antinociceptive and antipyretic properties of the aqueous extract of *Clematis brachiata* leaf in male rats. Pharm Biol 48:6–11

Murjanatu AA, Musa KY, Ibrahim G, Magaji MG (2015) Toxicity and analgesic studies of leaf methanolic extract of *Maytenus senegalensis* (lam.) Exell (Celastraceae). Int J Med Invest 4(2):241–244

Nwaka AC, Ikechi-Agba MC, Okechukwu PCU, Igwenyi IO, Agbafor KN, Orji OU (2015) The effects of ethanol extracts of *Jatropha curcas* on some hematological parameters of chloroform intoxicated rats. Amer-Eur J Sci Res 10(1):45–49

Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–63

Sanogo R, Maiga A, Diallo D (2006) Activités analgesique et anti-inflammatoire des extraits de Maytenus senegalensis, Stereospermum kuntrianum et Trichilia emetica utilisés dans le traitement traditionnel des dysmenorrhées au Mali. Pharm Med Trad Afr 14:123–136

Shittu OK, Lawal B, Blessing Uchenna AB, Haruna GM, Abubakar AN, Berinyuy EB (2015) Alteration in biochemical indices following chronic Administration of Methanolic Extract of Nigeria bee Propolis in Wister rats. Asian Pac J trop dis 5(8):654–657

Shittu OK, Lawal B, Adeniyi AK, Kilani LT, Saka RB (2017) Effect of methanol extract of *Musca domestica* larva on some enzymes and Haematological parameters in *Trypanosoma brucei brucei* - infected rats. Nig J Basic Appl Sc 25(2):66–74

da Silva G, Taniça M, Rocha J, Serrano R, Gomes ET, Sepodes B, Silva O (2010) *In vivo* anti-inflammatory effect and toxicological screening of *Maytenus heterophylla and Maytenus senegalensis* extracts. Hum exp tox 23(7):693–700

da Silva G, Serrano R, Silva O (2011) Maytenus heterophylla and Maytenus senegalensis, two traditional herbal medicines. J Nat Sc Biol Med 2:59–65

Sofowora A (1993) Medical plants and traditional medicine in Africa, (2nd ed.) edn. John Wiley and Sons Ltd, New York, pp 97–145

Sosa S, Morelli C, Tubaro A, Cairoli P, Speranza G, Manitto P (2007) Anti-inflammatory activity of Maytenus senegalensis root extracts and of Maytenoic acid. Phytomedicine 14:109–114

Tietz NW (1995) Clinical guide to laboratory tests, 3rd edn. WB Saunders Company, Philadelphia, PA, pp 286–288

Toiu A, Mocan A, Vlase L, Pârvu AE, Vodnar AC, Gheldiu AM, Moldovan C, Oniga I (2018) Phytochemical composition, antioxidant, antimicrobial and in vivo anti-inflammatory activity of traditionally used Romanian Ajuga laxmannii (Murray) Benth. ("Nobleman's beard" – Barba Împa˘ ratului). Front Pharmacol 9(7):1–16

Trease GE, Evans WC (1989) Pharmacology, 12th edn. Bailliere Tindal, New York, pp 683–685

Tsado NA, Lawal B, Santali ES, Mohammed AS, Balarabe MM, Ibrahim HA, George JJ (2015) Phytochemicals and acute toxicity profile of aqueous and Methanolic extracts of Crateva adansonii leaves in Swiss albino rats. Asian Journal of Biochemistry, Asian J Bioch 10(4):173–179

Veniamin MP, Varkirtzi C (1970) Chemical basis of the carbamidodiacetyl micro-method for estimation of urea, citrulline and carbamyl derivatives. Clin Chem 16:3–6

Winter CA, Risley EA, Nuss GV (1962) Carrageenin induced oedema in hindpaw of rats as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 3:544–547

World Health Organization (2016) Global technical Strategy for malaria elimination in the in endemic countries (2016–2030). World Health Organization Malaria Report, Manila

World Health Organization (2018) This year's malaria report at glance. World Malaria Report. https://www.who.int/malaria/publications/world-malaria-report-2018/en/. Accessed 19 Nov 2018

Wright P, Leathwood P, Plummer D (1972) Enzymes in rat urine: alkaline phosphatase. Enzymologia 42(4):317–327

Yakubu MT, Akanji MA, Oladiji AT (2015) Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (schweinf. Ex. hiern) stem in male albino rats. Asian J of Androl 7:399–404

Yusuf AA, Lawal B, Yusuf MA, Omonije YO, Adejoke AA, Raji FH, Wenawo DL (2018a) Free radical scavenging, antimicrobial activities and effect of sub-acute exposure to Nigerian *Xylopia Aethiopica* seed extract on liver and kidney functional indices of albino rat. Iran J toxicol 12(3):51–58

Yusuf AA, Lawal B, Abubakar AN, Berinyuy EB, Omonije YO, Umar SI (2018b) In-vitro antioxidants, antimicrobial and toxicological evaluation of Nigerian Zingiber officinale. Clin Phyto 4(12):1–8

Zangueu CB, Olounlade AP, Ossokomack M, Djouatsa YND, Alowanou GG, Azebaze AGB (2018) In vitro effects of aqueous extract from Maytenus senegalensis (lam.) Exell stem bark on egg hatching, larval migration and adult worms of Haemonchus contortus. BMC Vet Res 14:147

Zilva JF, Panmall PR, Mayne PD (1991) clinical chemistry in diagnosis and treatment, 5th edn. England Clays Ltd. St. Ives Plc, England