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Anti-inflammatory activity and toxicological effect of *Securidaca longepedunculata* (Fresen.) root bark extract in albino rats

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ABSTRACT

Aim: This study investigated the anti-inflammatory potential and toxicological effect of *Securidaca longepedunculata* root bark methanol extract in formalin-induced paw edema in albino rats. **Materials and Methods:** A total of 35 male albino rats divided into seven groups of five rats each (Groups 1-7) were used for the study. Paw edema was induced in Groups 1-5 by injection of 0.1 ml of 2% formalin in the sub-planter region of the left hind paw. Three of the induced Groups (1-3) were treated with 100, 200, and 300 mg/kg of the 70% methanol extract of *S. longepedunculata,* respectively. The fourth group was treated with indomethacin while the fifth group was the positive control. Groups 6 and 7 served as the extract and normal control, respectively. **Results:** Treatment with various doses of *S. longepedunculata* resulted in marked decrease of paw edema after the 6 days of treatment. Rats treated with 100 and 300 mg/kg of the extract had the highest (93.01%) and least (77.25%) percentage inhibition, respectively. A dose-dependent increase in levels of aspartate aminotransferase, alanine aminotransferase and gamma-glutamyltransferase in rats treated with the extract coupled with mild hepatocellular vacuolations observed in the histology of the liver was indicative of liver impairment. An elevated level of white blood cells, eosinophils, and lymphocytes in the treated groups are suggestive of their roles in combating inflammation. **Conclusion:** The root extract of *S. longepedunculata* showed anti-inflammatory activity in rats and could be a good alternative drug in the management of inflammatory diseases.

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INTRODUCTION

Inflammation is the first response of the immune system to injury or infection characterized by swelling, heat, redness, and pain [1,2]. It is also diverse, ranging from the acute inflammation associated with *Staphylococcus aureus* infection of the skin (boil), through to chronic inflammatory processes resulting in remodeling of the artery wall in atherosclerosis, the bronchial wall in asthma and chronic bronchitis, and the debilitating destruction of the joints associated with rheumatoid arthritis. Acute inflammation is a short-term process, usually appearing within a few minutes or hours, and ceasing on the removal of the injurious stimulus [3]. Chronic inflammation may begin with a relatively rapid onset or in a slow and unnoticed manner that may persist for weeks, months, or years with excessive production of macrophage-derived mediators which may lead to collateral damage to normal cells, which results in diseases, including atherosclerosis, bowel disease, rheumatoid arthritis glomerulonephritis (several kidney diseases), and septic shock [4].

There are many drugs used in the treatment of inflammatory conditions which predate our current understanding of the biochemical processes involved in the disease. Medically, the standard treatment for inflammatory conditions has been to use non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are medicines that relieve pain, swelling, stiffness, and inflammation and are prescribed for a variety of painful conditions, including arthritis, bursitis, tendonitis, gout, menstrual cramps, sprains, strains, and other injuries according to the U.S. Food and Drug Administration [5]. Chizzolini [4] reported that NSAIDs can only alleviate symptoms without, however, altering the course of the disease. It was reported by Xiao *et al.* [6] that many of the commonly used anti-inflammatory agents are becoming less acceptable due to serious adverse reactions such as gastric intolerance, bone marrow depression, and water and salt retention, resulting from prolonged use. Thus, there is a clear unmet medical need for a drug that provides relief from the symptoms of inflammation but can be given systemically.

Drug therapy, such as paracetamol, NSAIDs, topical analgesics, opioid analgesics, and intra-articular steroid injection, may prove ineffective in some patients, and long-term therapy with NSAIDs often have been associated with serious adverse effects [7,8]. NSAIDs such as ibuprofen, indomethacin, ketoprofen, ketorolac, meclofenmate, naproxen, and tolmetin are known to cause minor side effects such as stomach pain, nausea, indigestion, heartburn, and headache, while serious side effects such as swelling of the face, hand, feet, rapid weight gain, fainting, breathing problem, and tightness of the chest have been reported by U.S. Food and Drug Administration [5]. Furthermore, side effects of NSAIDs also include heart attack, stroke, high blood pressure, heart failure from body swelling (fluid retention), kidney problems including kidney failure, bleeding and ulcers in the stomach and intestine, low red blood cells (RBC) (anemia), life-threatening skin reactions, life-threatening allergic reactions, liver problems including liver failure, asthma attacks in people who have asthma. The aforementioned side effects could lead to the death of infants and children, the aged and patients with heart problem [9].

Therefore, there appears to be a need for drugs with good efficacy and low toxicity in the treatment of inflammation, specifically for patients who do not respond well to conventional medical therapy. Such patients are turning increasingly to herbal medicines. The fact that a large number of patients with severe chronic inflammatory disease fail to respond to conventional systemic or topical therapy resulting in a huge clinical and socio-economic burden underlie the need to develop novel herbal therapies.

In literature, there are reports on the anti-inflammatory activity of *Persicaria stagnina* [10]; *Scoparia dulcis* [11]; *Polygonum viscosum* [12]; and *Sida cordifolia* [13]. In addition, Rashid and Mosaddek [14] reported the anti-inflammatory activity of two Bangladeshi medicinal plants namely *Mesua nagassarium* and *Kigelia pinnata*. Other authors also reported anti-inflammatory effect of phytocompounds such as sesquiterpenes, diterpenes, flavonoids, glycosides, and alkaloids [15-17].

Securidaca longepedunculata Fresen. (*Polygalaceae*) is a tree or shrub commonly found in parts of Africa and also in various parts of Western, Northern, and Eastern Nigeria where it is widely used for medicinal purposes [18]. The roots and bark are taken orally either powdered or as infusions for treating chest complaints, headache, inflammation, abortion, ritual suicide, tuberculosis, infertility problems, venereal diseases, and for constipation. Toothache can also be relieved by chewing the roots [19]. Mixed roots of the violet tree and dwarf custard apple are used to treat gonorrhea [20]. Ethnobotanical enquiry revealed that *S. longepedunculata* root is valuable in the management of inflammatory diseases in Ibadan, Nigeria. There is, however, no scientific study to elucidate the anti-inflammatory activities of the root of this plant using the formalin-induced model. This study, therefore, examined the anti-inflammatory activity and toxicological effects of *S. longepedunculata* root extract in albino rats.

MATERIALS AND METHODS

Identification and Preparation of Plant Material

The plant material used for this study was collected from Ajibode village, Ibadan, Nigeria. It was identified at the University of Ibadan Herbarium. The root bark was washed, dried, and powdered. The powdered sample was kept in an air tight bottle at 4°C for further experimental use.

Analyses of Powdered Plant Sample for Phytochemical, Proximate, and Mineral Components

The powdered sample was analyzed for the presence of phytochemical constituents using standard protocols [21-23]. The proximate analysis of the sample was done using AOAC methods (2005) in the laboratory of the Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria for mineral analysis. The wet digestion method was used for the analysis [24] at the Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria. After the digestion of sample, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), and iron (Fe) were analyzed using Atomic Absorption Spectrophotometer (FC 210/211 VGP Bausch scientific AAS). The phosphorous content was determined using vanadomolybdate (yellow method) [23]. The percentage transmittance was determined at 400 nm using Spectronic 20 (Bausch and Lomb) colorimeter.

Preparation of Extract

The powdered sample 750 g was extracted in 2 L of 70% methanol for 72 h using cold maceration method, with occasional stirring. The liquid extract was filtered with Whatman filter paper No. 1 and the filtrate was concentrated using a rotary evaporator. The extract was stored at 4°C for further experimental use.

Experimental Animals

The adult Swiss albino male rats (weighing 150-200 g) were obtained from the animal house of the Department of Pharmacology, University of Ibadan and transferred to the animal house of the Department of Zoology, University of Ibadan, Nigeria. The animals were acclimatized in steel cages under standard conditions for 3 weeks and fed with standard animal pellets and water *ad libitum* before the experiment. All experiments were performed in accordance with the National Institute of Health guidelines of care and use of laboratory animals.

Determination of Anti-inflammatory Activity of Extract

Inflammation was induced in rats according to the method described by Winter *et al*. [25] and Turner [26]. A volume of 0.1 ml of 2% formalin was injected into the subplanter region of the left hind paw of each rat. Mean increase in paw size was measured at 1 and 3 h after formalin injection in each rat. Paw thickness before induction was measured and recorded while

increase in paw thickness and percentage inhibition of paw edema was estimated through the 6 days of treatment using this formula: Anti-inflammatory activity (%) = (1−D/C) 100, where D = the change in paw diameter in treated group and C = is the change in paw diameter in control group. The rats were grouped into seven, with each group having 5 rats each and treated as follows:

- Group 1: Induced with inflammation; treated with 100 mg/kg of extract
- Group 2: Induced with inflammation; treated with 200 mg/kg of extract
- Group 3: Induced with inflammation; treated with 300 mg/kg of extract
- Group 4: Induced with inflammation; treated with indomethacin (10 mg/kg)
- Group 5: Induced with inflammation but was not treated
- Group 6: Treated with 200 mg/kg of extract only
- Group 7: Normal (control).

Hematological Analysis of Samples

The packed cell volume (PCV), total hemoglobin (Hb) concentration, white blood cell count (WBC) and differentials, RBC count, mean cell Hb (MCH), mean cell volume, and the MCH concentration were determined using standard methods.

Estimation of Liver Function

The levels of total and direct bilirubin, alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin were determined in the serum using assay kits from Roche Diagnostics on Roche modular (model P800) Mannheim, Germany.

Histological Studies

Livers of the rats were collected and fixed in 10% formalin and were routinely processed for histopathological evaluation.

Statistical Analysis

Data obtained from the study were expressed as mean ± standard error. Significant differences of mean between test and control group were carried out using least significant difference fishers for the paw edema and student test for hematology and liver function test.

RESULTS

Phytochemical, Proximate, and Mineral Constituents of *S. longepedunculata*

S. longepedunculata contained alkaloids, saponins, tannins, phlobatannins, phenols, glycosides, and flavonoids [Table 1] whereas steroids, terpenes, and chalcones were absent. The proximate analysis [Table 2] showed that the plant has high fiber content (34.29%), moisture content (11.24%), and fat (1.05%).

Table 1: Phytochemical constituents of *Securidaca longepedunculata* rootbark

Chemical	Quality
Alkaloids	+++
Saponins	+++
Tannins	+
Phlobatannins	+
Phenols	++
Anthraquinones	-
Terpenes	-
Cardenolides	-
Steroids	-
Glycosides	++
Chalcones	-
Flavonoids	++

+++: Present in appreciable amount, ++: Present in moderate amount, +: Present in trace amount, -: Absent

Table 2: Proximate and mineral composition of *Securidaca longepedunculata* rootbark

Nutrients	Quantity
Crude protein (%)	5.64±0.05
Fat (%)	1.05±0.02
Ash (%)	2.30±0.02
Fiber (%)	34.29±0.01
Moisture (%)	11.24±0.01
Sodium (%)	0.013±0.002
Potassium (%)	0.067±0.001
Calcium (%)	0.018±0.001
Phosphorus (%)	0.018±0.001
Magnesium (%)	0.163±0.001
Zinc (mg/kg)	17.65±0.150
Copper (mg/kg)	3.3±0.150
Iron (mg/kg)	21.4±0.150

Values are mean±SE, n=2. SE: Standard error

The mineral composition also showed that sodium (Na), potassium (K), calcium (Ca), phosphorous (p), magnesium (Mg), zinc (Zn), copper (Cu), and iron (Fe) are present in varying percentages with 17.65% the highest value found in zinc while 0.013% the least value was for sodium.

Paw edema of Treated and Untreated Formalin-induced Inflammation in Rats

The mean paw edema of rats before induction ranged between 2.37 mm (Group 7) and 2.76 mm (Group 1) [Table 3]. There was no significant difference ($P < 0.05$) in paw size prior induction. Injection of 0.1 ml of 2% formalin caused marked increase in paw size 1 h after induction, with paw size ranging from 7.95 mm (Group 2) to 7.45 mm (Group 3). A slight decrease was observed in the paw size after 3 h of induction with Group 2 reduced to 7.47 mm and Group 3 to 6.67 mm, whereas the paw size of the control remained unchanged (2.37 mm). The treatment of animals with various doses of *S. longepedunculata* extract resulted in a reverse-dose-dependent decrease in paw edema after the 6 days of treatment. Rats treated with 100 mg/kg *S. longepedunculata* had the highest percentage inhibition (93.01%), while rats treated with 300 mg/kg had the least percentage inhibition (77.25%). Rats treated with the standard

Table 3: Effect of oral administration of *Securidaca longepedunculata* extract on formalin-induced edema in rats

Induction of paw edema (mm)				Treatment of paw edema (mm)					
Animal groups (n=5)	Paw diameter before induction	Paw diameter 1 h after induction	Paw diameter 3 h after induction	Day 1	Day 2 (%)	Day 3 (%)	Day 4 (%)	Day 5 (%)	Day 6 (%)
Group 1 100 mg/kg (extract)	2.76±0.16	7.71±0.56	7.60±0.42	8.33±0.54	6.55±0.24 (35.82)	4.91±0.31 (58.18)	4.22±0.35 (70.94)	3.25±0.21 (89.59)	2.95±0.23 (93.01)
Group 2 200 mg/kg (extract)	2.58±0.06	7.95±0.36	7.47±0.47	8.93±0.28	6.26±0.03 (37.52)	5.43±0.37 (42.20)	4.77±0.44 (56.11)	3.55±0.66 (78.13)	2.91±0.09 (91.21)
Group 3 300 mg/kg (extract)	2.48±0.16	7.45±0.15	6.67±0.24	8.61±0.31	6.33±0.31 (34.46)	5.25±0.24 (45.17)	3.88±0.43 (71.94)	3.75±0.02* (75.15)	3.61±0.10* (77.25)
Group 4 10 mg/kg (indomethacin)	2.74±0.16	7.60±0.39	7.26±0.35	7.87±0.34	6.32±0.19 (39.21)	5.00±0.45 (55.42)	3.88±0.11 (77.15)	3.11±0.26* (85.77)	2.56±0.20* (95.49)
Group 5 (untreated)	2.37±0.05	7.69±0.29	7.18±0.36	8.63±0.82	8.26±0.41*	7.44±0.12*	7.36±0.17*	7.07±0.46*	6.81±0.31*
Group 6 (extract only)	NI	NI	NI	NI	NI	NI	NI	NI	NI
Group 7 (control)	2.37±0.04	2.37±0.04*	2.37±0.04*	2.37±0.04*	2.37±0.04*	2.37±0.04*	2.37±0.04*	2.37±0.04*	2.37±0.04*

Mean±SE, n≤5. Values within a column having (*) are significantly different LSD fishers at P≤0.05. Values in bracket () with percentage indicate rate of inhibition. NI: Not induced, SE: Standard error, LSD: Least significant difference

drug; indomethacin had a higher percentage inhibition (95.49%) compared to rats treated with the extract. Edema was observed in the paws of the positive control (Group 5) rats throughout the experiment. Overall, the percentage inhibition of edema in the treated rats was in the order: 95.49% (10 mg/kg of indomethacin) >93.01% (100 mg/kg of extract) >91.21% (200 mg/kg of extract) >77.25% (300 mg/kg of extract). The least concentration of the extract (100 mg/kg) gave the highest anti-inflammatory activity in rats.

Effect of Oral Administration of *S. longependunculata* Extract and Indomethacin on the Hematological Parameter of Rats

Treatment with methanol extract of *S. longependunculata* induced increased hematopoiesis in a reverse-dose-dependent order compared with the untreated and control groups [Table 4]. Rats treated with the extract alone had the highest values (46.00, 14.25, and 7.87) whereas rats treated with indomethacin had the least values (29.00, 9.50, and 4.35) for PCV, Hb, and RBC, respectively. WBC counts were higher in rats treated with an extract of *S. longependunculata* than the untreated and control rats. WBC count was reduced with increasing dose among rats treated with *S. longependunculata*. Rats treated with indomethacin had the highest lymphocyte count. Counts of lymphocytes were, however, higher in most treated rats compared to the untreated. Neutrophils and monocytes counts were higher in untreated rats than in most of the treated rats. Treatment with indomethacin induced dose-dependent increase in neutrophil counts. There was however no significant difference ($P < 0.05$) in neutrophil counts in the treated and untreated rats. The highest eosinophils level (3.00%) was recorded for Group 6 (200 mg/kg of extract only).

Effect of Oral Administration of *S. longependunculata* on Liver Enzymes

The results of liver function test presented in Table 5 show that rats treated with various doses of the extract had lower AST, ALT, and GGT levels compared with the control. There was however, a dose-dependent increase in AST, ALT, and GGT levels in rats treated with the extract. Treatment with the extract however induced higher ALP and total bilirubin levels compared with the control group. The GGT value was highest in Group 5 (2.07 IU/L) and least in Group 6 (0.55 IU/L).

Histological Studies

The result of the histopathological studies of the section of the liver for treated, untreated, and control rats are presented in Plate 1a-f. Liver of the control group [Plate 1a] showed moderate hepatocellular vacuolar change with no visible lesion. Moderate vacuolar change of the periportal hepatocytes was observed in the liver of the induced but untreated rats [Plate 1e]. Liver of rats treated with the extract [Plates 1b-d] show hepatocellular vacuolar change; Plate 1b shows uniformly-sized cytoplasmic vacuoles in the cytoplasm of the hepatocytes. Plate 1c shows moderate congestion of blood vessels as well as lymphocytic perivascular cuffs. Moreover, Plate 1d and e shows a severe widespread vacuolar change of the hepatocytes. The indomethacin [Plate 1f] treated rats show multiple foci of coagulation necrosis of the hepatocytes as well as widespread hepatocellular vacuolar change.

DISCUSSION

The methanol extract of *S. longepedunculata* showed anti-inflammatory activity in the second phase of the formalin-induced paw edema in rats in this study. The magnitude of activity obtained at the three dose levels of extracts used indicated high anti-inflammatory effect compared to indomethacin after 6 days of treatment. On the final day of the experiment, the percentage inhibition recorded for rats treated with the various doses of the extract compared favorably with indomethacin. Earlier studies by previous authors [27,28] on the leaf, stem bark and root bark of *S. longepedunculata* reported that various doses of the methanol extract exhibited anti-inflammatory activity >70% in carrageenan-induced paw

Table 4: Effect of oral administration of *Securidaca longependunculata* extract and indomethacin on the hematological parameter of rats

Animal groups (n=5)	PCV %	Hb g/dl	RBC ($\times10^6/\mu l$)	Platelet ($\times10^3/\mu l$)	WBC ($\times10^3$/ul)	Lymph (%)	Neutrphil (%)	Monocyte (%)	Eosinophil (%)
Group 1 100 mg/kg (extract)	44.20±1.65*	14.50±0.53	7.34±0.24*	111.00±7.72	70.80±5.17*	73.20±4.16	23.80±4.03	1.80±0.37	1.20±0.49
Group 2 200 mg/kg (extract)	43.50±3.50	14.00±1.50	7.07±0.48	112.00±9.00	67.25±2.75	73.50±0.50	24.00±1.00	1.00±0.00	1.50±0.50
Group 3 300 mg/kg (extract)	41.00±1.73	13.50±0.58	6.83±0.28	123.00±4.72	56.83±9.33	53.33±9.52	44.33±9.20	1.33±0.33	1.00±0.57
Group 4 indomethacin (10 mg/kg)	29.00±6.00*	9.50±2.10*	4.35±0.97*	102.00±8.00	48.00±9.00*	76.00±4.00	20.00±2.00	3.00±1.00*	1.00±1.00
Group 5 (untreated)	39.75±4.11	13.07±1.52	6.48±0.71	99.75±9.04	64.00±3.93	55.75±8.94	40.00±8.15	2.00±0.48	1.00±0.40
Group 6 (extract only)	46.00±1.00*	14.25±0.35	7.87±0.38*	105.00±4.00	58.00±1.00	67.50±2.50	33.00±2.00	1.50±0.50	3.00±0.02*
Group 7 (control)	34.25±4.71	11.22±1.67	5.58±0.82	99.00±10.66	59.25±3.90	63.75±9.95	33.75±9.03	1.25±0.47	1.25±0.47

Values within a column having (*) are significantly different at $P\leq0.05$. PCV: Packed cell volume, Hb: Hemoglobin, RBC: Red blood cell, WBC: White blood cell, Lymph: Lymphocytes

Table 5: Effect of oral administration of *Securidaca longependunculata* and indomethacin on liver enzymes of experimental rats

Animal groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	Total bilirubin (μmol/L)
Group 1 100 mg/kg	40.80±1.93	29.60±1.72	110.60±6.25	0.90±0.62	0.94±0.23
Group 2 200 mg/kg	41.00±6.00	30.50±2.50	106.50±12.50	1.70±1.50	1.95±1.65
Group 3 300 mg/kg	43.00±3.60	30.67±1.76	113.00±5.29	2.83±0.85	0.36±0.03
Group 4 indomethacin	41.00±2.00	29.50±2.50	111.50±15.50	1.60±0.00	0.25±0.05
Group 5 untreated	44.75±1.31	32.00±1.22	115.50±3.59	2.07±0.81	1.10±0.80
Group 6 extract only	42.50±0.50	30.50±0.50	120.50±1.50	0.55±0.25*	0.70±0.50
Group 7 control	43.25±2.65	31.75±1.31	105.00±9.57	1.52±0.11	0.27±0.04

Values within a column having (*) are significantly different at $P\leq0.05$. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphate, GGT: Gamma glutamate peptidase

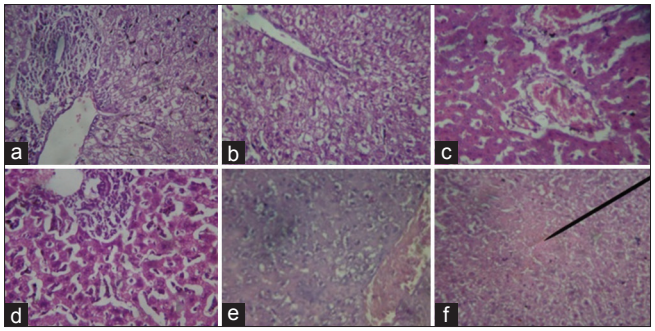

Plate 1: Effects of extract and drug on the liver of treated and untreated rats. (a) Control; (b) 100 mg/kg of extract; (c) 200 mg/kg of extract; (d) 300 mg/kg of extract; (e) induced but untreated rats; (f) 10 mg/kg of indomethacin

edema. These findings support the traditional use of this plant as an anti-inflammatory agent.

Our pharmacological screening of root bark of *S. longepedunculata* revealed that the root bark contains phytochemical components (alkaloids, saponins, tannins, phlobatannins, phenols, glycosides, and flavonoids) which agree with the findings of previous authors [29,30]. The bioactivity of a plant species is based on its bioactive components and could vary due to the types of solvents used for extraction and methods of extraction. The family *Polygalaceae* to which *S. longepedunculata* belongs is known traditionally to be involved in the management of diseases such as epilepsy, headache, inflammation, infertility problems, constipation, and toothache [19,31].

The phytochemical components could have accounted for potent anti-inflammatory activity in the topical and systemic models of acute inflammation [32,33]. The constituents in the extract may have inhibited the release of pro-inflammatory mediators of acute inflammation such as histamine and prostaglandin [34]. Acute inflammation induced by formaldehyde results from cell damage, which provokes the production of endogenous mediators, such as, histamine, serotonin, prostaglandins, and bradykinin [35]. Inhibition of formalin-induced edema in rats is therefore one of the most suitable test procedures to screen anti-inflammatory agents as it closely resembles human arthritis. Furthermore, earlier reports by Yang *et al.* [36] confirmed the presence of anti-inflammatory constituents such as salicylic and benzoic acids in *Securidaca inappendiculata* (Hassk.) a species related to *S. longepedunculata*. The possible presence of these constituents in *S. longepedunculata* might have contributed to the anti-inflammatory activity. Centrally acting analgesics are

also known to inhibit the two phases, in contrast to NSAIDs like indomethacin that only inhibit the late phase [37]. In this study, the methanol extract of the plant though not administered in the first phase, showed better activity at the later phase of the formalin-induced inflammation. Therefore, isolation of the active constituent in *S. longepedunculata* is likely to reveal the active or potent anti-inflammatory agent.

The consumption of drugs (either synthetic or phytomedicine) may bring about significant changes in the structure, function, and metabolic transformation of all classes of biomolecules, enzymes, and metabolic pathways. These alterations that could be rapid or slow may lead to different biochemical mechanisms producing similar pathological, clinical and laboratory findings. In the present study, the oral administration of the extract and indomethacin on rats for 6 days produced some contrasting results. The various dosage of methanol extract of *S. longepedunculata* significantly increased the PCV, Hb, RBC, WBC, and platelets values with a decrease in the group treated with indomethacin when compared with the control which is in accordance with the report of Owoyele *et al*. [38]. The increase in RBC count is beneficial to the animal since this will allow for efficient transfer of oxygen from the lungs into the tissues [39]. The greatly elevated levels of platelets and lymphocytes may indicate the presence of factors that stimulate the production of these cells. The elevated level of platelets confirmed the use of the plant as hemostatic agent [40] because Group 6 which was administered with extract only showed an increase in these parameters. An evident decrease of these hematological parameters in Group 4 (10 mg/kg indomethacin) was in agreement with the reports of previous authors, that NSAIDs are potential hemolytic agents which when taken in excess or in large doses could lead to gastric intolerance, bone marrow depression, dizziness, headache, and stomach cramps [41].

WBCs are generally important in the development and propagation of inflammation. Neutrophils in particular play a crucial role manifestation of inflammation and cytokines produced by neutrophils are the major source of free radicals at the site of inflammation [42]. The reduced levels of neutrophils in most of the treated rats suggest their inflammatory activities. Eosinophils are granules containing leukocytes that synthesize and release lipid-derived mediators which stimulate inflammatory responses in tissues. They also produce cytokines such as interleukins (IL-3, IL-5) and granulocyte macrophage stimulating factor that contribute to inflammatory responses [42]. Lymphocytes are the predominant cell in chronic inflammation. It can cause permanent distortion of the tissue, interfering with its function. Elevated level of eosinophils and lymphocytes in the treated groups is a confirmation of their roles in combating inflammation.

Increase in plasma levels of liver enzymes is an indication of hepatic impairment. It is diagnostic of hepatocellular damage, as seen in disorders that might lead to hepatic necrosis such as acute hepatitis and prolonged collapse of the circulatory system (shock) when the liver is deprived of fresh blood bringing oxygen and nutrients [43]. ALP, an ectoenzyme of the hepatocyte plasma membrane, is a "marker" enzyme for the assessment of the integrity of the hepatocyte's plasma membrane [44,45]. Any alteration in the serum level of the enzyme would be indicative of likely damage to the external boundary of cells (plasma membrane). The increase in serum enzymes levels in all rats injected with formalin suggests that the integrity of the hepatic plasma membrane was compromised and also indicated acute liver damage. These observations are further supported by the histopathological examination of the liver of these rats which revealed moderate hepatic vacuolations. Treatment with our extract, however, ameliorated some of these pathologies as observed by a marked reduction of liver enzymes particularly AST, ALT, and GGT in the treated rats.

CONCLUSION

The root bark extract of *S. longepedunculata* showed anti-inflammatory activity in rats. *S. longepedunculata* could be a good alternative NSAIDs in the management of inflammatory diseases. The proximate and nutritional components of the plant might have complemented its phytochemical constituents in the anti-inflammatory activity. Furthermore, the plant displayed hematopoietic activity by increasing the level of blood indices in rats treated with *S. longepedunculata* root extract.

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