Trop J Nat Prod Res, August 2025; 9(8): 4045 - 4055 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Safety Evaluation of Sub-acute and Acute Oral Treatment with Aqueous Extract and Methanol Fraction of *Aloe barbadensis* (*Aloe vera*) Leaves in Wistar Rats

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ARTICLE INFO

Article history:
Received 01 March 2025
Revised 12 July 2025
Accepted 13 July 2025
Published online 01 September 2025

ABSTRACT

Ethnobotanicals continue to gain global relevance due to their therapeutic potential and their role as precursors in pharmaceutical development. *Aloe barbadensis* is one of the most common traditional herbs in Nigeria, particularly for its purported antimalarial properties. The increasing use of traditional remedies in malaria-endemic regions like Nigeria makes it imperative to conduct regular safety assessments of medicinal plants. This study investigates the safety of *A. barbadensis* by assessing its acute and subacute toxicity. The plant was collected from Oke-Aluko farm in Ilorin, Nigeria, and authenticated by a botanical expert. Both aqueous extract and a methanol fraction of *A. barbadensis* leaves were prepared for the study. Wistar rats were administered single oral doses of 2000, 5000, and 6000 mg/kg, followed by a 14-day observation period to assess acute toxicity and potential delayed effects. For the subacute toxicity testing, daily doses of 250, 500, and 750 mg/kg were administered over 28 days. The liver functions (AST, ALT, ALP, GGT), kidney functions (urea, creatinine, sodium, potassium), haematological parameters, and histological analyses of the organs were evaluated. Results showed no significant adverse effects on biochemical or haematological parameters ($p > 0.05$) at all doses in both study phases. However, continuous treatment with the methanol fraction at 750 mg/kg led to histopathological changes in the liver and kidney after 28 days, indicating potential organ toxicity at higher doses. This study advocates for the extract dose regulation and future long-term chronic toxicity studies on the plant.

Keywords: *Aloe barbadensis*, Malaria, Medicinal plants, Nigeria, Toxicity

Introduction

Globally, medicinal plants are recognised as the foundation for pharmaceutical formulations, as medicinal plants are thought to be more culturally acceptable, accessible, affordable, and less toxic with little to no side effects than many synthetic pharmaceutical agents. [1–4]*Aloe barbadensis* is commonly used for the local treatment of fevers in Nigeria.[5,6] In India and Western Uganda, *A. lateritia* and *A. secundiflora* are frequently used to treat malaria and similar symptoms.[7,8] Previous studies have demonstrated the therapeutic potential of *A. barbadensis* to include anticancer activities, digestive and skin protective activity, and antibacterial activities, as well as a possible source of natural herbicides and fungicides.[5,9–11] Anthraquinones, saponins, glycosides, alkaloids, phenolic acids, tannins and flavonoids are common phytochemicals often found in *A. barbadensis* leaves.[12–15] Studies have also indicated local usage for treating fevers and numerous diseases. Previous reports revealed its potential therapeutic actions, which include anti-tumour, anti-inflammatory, anti-parasitic, and immunological regulatory properties.[16–19] The use of murine models for toxicity investigations has been advocated due to their reliability in drawing inferences regarding human biology.[20, 21]

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Citation: Safety Evaluation of Sub-acute and Acute Oral Treatment with Aqueous Extract and Methanol Fraction of *Aloe barbadensis* (*Aloe vera*) Leaves in Wistar Rats. Trop J Nat Prod Res. 2025; 9(8): 4045 – 4055 https://doi.org/10.26538/tjnpr/v9i8.70

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Despite various assertions about the safety of the plant, there have been limited reports on the toxicity of *A. barbadensis.* [18, 22] The reduction in mean corpuscular haemoglobin concentration (MCHC), haematocrit value, and erythrocyte count was previously reported following treatment with a single 500 mg/kg dose of *Aloe barbadensis* gel, while prolonged treatment at 200 mg/kg significantly increased leukocyte and platelet counts. [20,23] Considering the growing local consumption rate of *A. barbadensis* in many communities of Nigeria and several other countries globally, particularly as constituents of cocktails in the local management of malaria and other ailments, it is imperative to ascertain the safety of the plant for human consumption to serve as a guide for the consumers and regulatory authorities in the food and pharmaceutical industries.[3,23] This study investigates the sub-acute and acute oral toxicity of the methanol fraction and aqueous extract of *A. barbadensis* in Wistar rats to ascertain their safety for human consumption.

Materials and Methods

Ethical approval

Ethical approval for this study was sought and obtained from the Research Ethics Committee of the University of Medical Sciences, Ondo State, Nigeria (Protocol Number: NHREC/TR/UNIMED-HREC-Ondo St/22/06/21), with approval granted on the 6th of June, 2022. The care of the rats used in this study was in accordance with institutional guidelines.

Plant Collection and Identification

Fresh leaves of *Aloe barbadensis* were collected in March 2024 from Oke-Aluko Farm, Ilorin, Kwara State, Nigeria, located at coordinates 8.4733°N and 5.6080°E. The plant species was identified and authenticated by Dr. Ojo Samuel of the Department of Plant and Biotechnology, University of Medical Sciences, Ondo, Nigeria. A voucher specimen (UNIMED/PBTH/0067) was deposited in the departmental herbarium for future reference.

Extraction of Plant Materials

The plant leaves were washed with tap water, cleaned with cotton soaked with 70% ethyl alcohol to remove impurities, and thoroughly rinsed with sterile distilled water. They were shade-dried, then oven-dried at 50 °C until adequately dry before being crushed to powder with a lab blender (SKU: CHE03U1H, Shree Bhagwati India). The powdered leaves were weighed, dried, and weighed again until a consistent weight was achieved to ensure dryness. The extract stock was prepared by dissolving 20g of powder in 1000 mL sterile distilled water for forty-eight hours. The contents were filtered using Whatman filter paper no. 1, and the filtrate was evaporated to dryness using a rotary evaporator under reduced pressure at 40 °C. Before analysis, the prepared stocks of aqueous extract and methanol fraction were stored using the method previously adopted by Arsene et al.[24].

Preparation of methanol fraction

The procedure previously documented by Tiwari et al.[25] was modified to fractionate the aqueous crude extract using methanol as the solvent. Briefly, twenty-five grams (25g) of aqueous *A. barbadensis* extract was gently mixed with 500 mL of methanol for 15 minutes, and the filtrate was separated and collected in a 1000 mL beaker as the methanol fraction. The procedure was repeated twice, and the filtrates were pooled together. A rotary evaporator was used to concentrate the pooled filtrate (fraction), which was later dried in an oven at 50°C. Biochemical, histological and haematological toxicity was investigated on the fraction to ascertain the safety for human consumption.

Acute Oral Toxicity Study

The animals, with an average weight of 150 g, were made to acclimatise for two weeks before treatment. The method described by Mohr et al.[26] was adopted to conduct the acute oral toxicity study. Forty (40) sex-matched Wistar rats were grouped into four groups, each group containing ten (10) animals of 5 males and 5 females, in different cages. The animals in the first group were treated with a placebo and used as the control group.

Methanol fraction at a concentration of 2000 mg/kg was administered as a single dose to the rats in the second group using an oral cannula, while the animals in the third and fourth groups were treated with 5000 mg/kg and 6000 mg/kg of the fraction, respectively. Post-treatment observation was conducted on the experimental animals for signs of toxicity and mortality, and LD_{50} was determined. The animals were sacrificed using cervical dislocation after 14 days, following the previous studies.[27–29] Blood specimens were obtained by cardiac puncture, and 2 mL was transferred into plain bottles for biochemical analysis and 2 mL into EDTA anticoagulant bottles for haematological analyses. All samples in the plain bottle were centrifuged at 5000 rpm for 10 minutes, and serum was extracted for liver and kidney function tests. Both organs were excised and fixed in 10% neutral buffered formalin and later processed for histological examination. The same procedure was repeated for aqueous extract with blood samples collected from each animal, transferred into EDTA and plain bottles and processed for haematological and biochemical parameters, while the harvested liver and kidney were processed for histological examination. Briefly, the histopathological investigation was conducted by subjecting both fixed organs to grossing, dehydration, clearing, embedding, sectioning, staining using Hematoxylin and Eosin, mounting and microscopic examination.[30]

Sub-Acute Toxicity Study

Determination of sub-acute oral toxicity in the animal follows the same procedure of animal grouping as explained in the acute oral toxicity study, except that 250, 500 and 750 mg/kg body weight of the fraction were respectively administered to the rats in groups 2, 3, and 4 daily for twenty-eight days. Also, after the experiment, the animals were sacrificed, and blood specimens were collected for haematological and biochemical investigations, while the liver and kidney tissues were harvested and processed for histological examination, as was done in the acute toxicity study. [28, 31, 32]

Statistical analysis

The data obtained were input into the computer and analysed with SPSS software (IBM, Armonk, USA) using the Student T-test and Chi-square test for biochemical and haematological parameters. GraphPad Prism was used for the descriptive statistics of some biochemical parameters. At a 95% confidence level, a probability value less than 0.05 was considered statistically significant.

Results and Discussion

Recent ethnopharmacological and phytotherapeutic reports have indicated a significant increase in public awareness and the therapeutic application of indigenous medicinal plants for the prevention and treatment of various ailments. [4,,33] Frequent consumption of medicinal plants has prompted many researchers to investigate and establish the potential hepatotoxicity and nephrotoxicity of the plants. [29,31,34]. Table 1. depicts the biochemical parameters of the experimental animal following acute exposure to *A. barbadensis* extracts. Creatinine, urea, and electrolyte (Na^+, K^+, Cl^-) levels did not differ significantly at concentrations of 6000 mg/kg, 5000 mg/kg, and 2000 mg/kg ($P > 0.05$), following 14 days of single-dose administration of *A. barbadensis* aqueous extract. Similarly, after treatment with the methanol fraction, urea, creatinine and other electrolyte values were observed to be normal at all doses except potassium levels at 6000 mg/kg ($P < 0.05$) (Table 2). Contrarily, in a study conducted by Abderrahmane et al. [35], significantly elevated urea and creatinine levels after single-dose administration at a concentration of 500 mg/kg were observed. The difference in the findings could probably be attributed to the difference in the parts of the plant used in both studies. While the leaves of the plant were employed in the current study, the gel of *Aloe barbadensis* was used in the previous study. Table 3 depicts no significant variation in all the hepatic parameters evaluated following a single dose of aqueous extract and methanol fraction for 14 days at all concentrations. Administration of *A. barbadensis* product (UP780), leaf juice, and gel in murine models for 14 days has been reported to have an insignificant effect on the levels of urea, aspartate aminotransferase (AST), sodium and alanine aminotransferase (ALT), compared to the control animals without treatment. [36–38]

Consequent upon continuous exposure of the animals to aqueous extract of *A. barbadensis* at concentrations of 250 mg/kg, 500 mg/kg, and 750 mg/kg, our findings revealed no significant difference in urea, creatinine, Na^+ and Cl^- levels ($P > 0.05$). Comparing the K^+ concentrations in the treated animals with the untreated control, the potassium ion values appeared significantly elevated with 750 mg/kg/day treatment (Table 4). The toxicity effect of the methanol fraction of *A. barbadensis* on the mean creatinine and urea values in the rats after 28 days of continuous administration at concentrations of 750 mg/kg, 500 mg/kg, and 250 mg/kg was statistically insignificant compared to the untreated control animals (Table 5). The methanol fraction and aqueous extract of A.*barbadensis* did not significantly alter all the concentrations of all biochemical parameters analysed following 28 days of continuous treatment at dosages of 250 mg/kg, 500 mg/kg, and 750 mg/kg. The findings of the present study contrast those reported by Abderrahmane et al[35], who observed a significant elevation in AST and ALT levels at a dose of 500 mg/kg. This discrepancy may be attributed to the use of *Aloe barbadensis* gel in their study, as opposed to the leaf extract employed in the current investigation. Additionally, variations in animal age, body weight, and experimental conditions may also account for the variation. Haematological indices have been used to define blood-related activities of chemical substances, such as plant extracts. [35,39] Following a single start dose treatment with the methanol fraction of *A. barbadensis* on experimental animals for 14 days, variation in the mean haematological indices in the rats appeared statistically insignificant. No significant difference was observed in total leucocyte and platelet counts, haematocrit, and haemoglobin levels at concentrations of 2000 mg/kg, 5000 mg/kg, and 6000 mg/kg. Furthermore, the haematological indices in the treated animals at the concentrations of 2000 mg/kg, 5000 mg/kg, and 6000 mg/kg did not differ significantly from those of the control groups ($P > 0.05$) (Table 6).

Table 1: Mean values of some renal parameters in Wistar rats after 14 days of single administration with *Aloe barbadensis* aqueous extract

Parameter	Extract (mg/kg)	Test ± SD (mmol/L)	Control ± SD (mmol/L)	T-test	P-value
Creatinine	2000	142.7 ± 08.21	137.42 ± 20.18	0.7761	0.6978
	5000	134.19 ± 22.29	131.68 ± 23.32	0.5642	0.9765
	6000	98.38 ± 11.52	101.95 ± 18.52	1.9460	0.5655
Urea	2000	9.71 ± 4.71	7.67 ± 1.54	1.43655	0.6761
	5000	7.53 ± 1.73	6.32 ± 0.97	0.7862	0.7443
	6000	7.87 ± 1.14	6.79 ± 1.35	0.5876	0.6772
Sodium	2000	137.22 ± 1.15	139.27 ± 0.19	0.8421	0.9552
	5000	137.23 ± 0.19	139.25 ± 0.27	0.8750	1.5710
	6000	137.37 ± 0.53	139.95 ± 0.38	0.9742	0.5112
Potassium	2000	4.18 ± 0.08	4.62 ± 0.07	1.0671	0.90126
	5000	4.27 ± 0.09	4.75 ± 0.05	0.9551	0.7921
	6000	4.82 ± 0.05	4.02± 0.06	7.4233	0.0481
Chloride	2000	95.78 ± 13.13	97.27 ± 0.51	0.7117	0.6897
	5000	98.57 ± 1.19	97.29 ± 0.51	1.0872	0.9891
	6000	98.72 ± 4.07	97.28 ± 0.48	1.2533	0.2577

The acute toxicity effect of *A. barbadensis* aqueous extract on renal parameters. No significant variation in the creatinine levels at concentrations of 2000 mg/kg (T= 0.6978; P> 0.05), 5000 mg/kg (T= 0.9765; P> 0.05), 6000 mg/kg (T= 0.5655; P> 0.05) while the urea values at the administered concentrations of 2000 mg/kg (T= 0.6761; P> 0.05), at 5000 mg/kg (T= 0.7443; P> 0.05), and 6000 mg/kg (T= 0.6772; P> 0.05) was also statistically insignificant compared to the control. Variation in the sodium mean levels is statistically insignificant at all concentrations administered: 2000 mg/kg (T= 0.8421; P> 0.05), 5000 mg/kg (T= 0.8750; P> 0.05), and 6000 mg/kg (T= 0.5112; P> 0.05). The chloride means values depicted no significant variation at concentrations of 2000 mg/kg (T= 0.7117; P> 0.05), 5000 mg/kg (T= 1.0872; P> 0.05) and 6000 mg/kg (T= 1.2533; P>0.05). However, a significant difference was observed in potassium levels at a concentration of 6000 mg/kg/day (T = 7.4233; P < 0.05).

Haematological studies on the effect of *A. barbadensis* methanol fraction after 28 days of continuous administration on the Hb, PCV, WBC, platelet, MCV, MCH, and MCHC values showed no significant variation in the absolute values at concentrations of 750 mg/kg, 500 mg/kg, and 250 mg/kg (P> 0.05) (Table 7). This finding differs from the reports of Nalimu et al.,[40] who documented a reduction in the MCH and MCHC with 400 and 200 mg/kg/bw concentrations of the extract. Our findings also disagree with the report of Akukwu et al. [41,] which documented elevated WBC and platelet levels and a reduction in the PCV and RBC levels. The differences in the mode of administration, doses provided for the experimental animals and the strain of the rats used could probably be responsible for the variations in both studies. Overall, our study demonstrates that consuming *A. barbadensis* extract is reasonably safe as far as haematological parameters are concerned.

Figure 1 also depicts the mean blood Na^+, K^+, and Cl^- concentrations in the rats following 28 days of continuous treatment with the *A. barbadensis* methanol fraction. Electrolyte values showed no significant variation at concentrations of 250 mg/kg and 500 mg/kg of the fractions in treated animals. These findings contrast with those of Nalimu et al.[42], who reported renal toxicity at extract concentrations exceeding 5000 mg/kg. The current findings, however, aligned with the reports of Tong et al.[3] who recorded no fatalities or substance-related toxicity in both acute and subacute toxicity trials, consistent with the absence of mortality in our experimental period. Furthermore, potassium (K^+) level was significantly raised after daily treatment of the rats with 750 mg/kg fraction for 28 days, suggesting that the methanol fraction may not be safe at doses exceeding 500 mg/kg with prolonged use.We reported the histopathological assessment of the liver and kidney in an animal model following acute and subacute treatment with different concentrations of aqueous extract and methanol fraction of *A. barbadensis* to determine their safety for human use. Histological findings in rats treated with the methanol fraction of *A. barbadensis* showed that the liver of the rats of both sexes treated with the methanol fraction of *A. barbadensis* at standard doses of 2000 mg/kg/bw, 5000 mg/kg/bw, and 6000 mg/kg/bw showed normal structures when compared to the untreated control rats, typified by normal histoarchitecture with normal hepatocytes, uninfiltrated and non-congested central vein and sinusoidal space (Figure 2). This present result is consistent with the previous report by Nalimu et al. [40] since acute oral treatment with single dosages of leaf extracts up to 5000 mg/kg induced no significant change in animal behaviour, implying that the fraction is relatively safe. Some other studies reported that aqueous *A. barbadensis* leaf extracts at 600, 400, and 200 mg/kg caused no adverse reactions in the experimental animals. [37,43] Similarly, in agreement with the report of Kwack et al.[44] this study shows that continuous oral treatment of the rats for 28 days with the extract of *A. barbadensis* leaves induced no behavioural alterations or death of the animals throughout the study. Furthermore, our findings agree with the reports of Pranto et al.,[1] who documented the hepatoprotective potential of *A. barbadensis* extracts on liver and renal parameters in carbon tetrachloride (CCL_4) induced liver toxicity.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Mean values of some renal parameters in Wistar rats after 14 days of single administration with the methanol fraction of *A. barbadensis.*

Parameter	Extract (mg/kg)	Test ± SD (mmol/L)	Control ± SD (mmol/L)	T-test	P-value
Creatinine	2000	141.2 ± 22.49	139.25 ± 15.48	0.5672	0.6278
	5000	130.25 ± 15.48	130.25 ± 15.48	0.0000	1.0000
	6000	96.45 ± 9.83	110.25 ± 15.48	2.6067	0.1210
Urea	2000	9.26 ± 2.69	6.72 ± 1.68	1.1326	0.3749
	5000	6.72 ± 1.68	6.72 ± 1.68	0.0000	1.0000
	6000	6.75 ± 1.05	6.72 ± 1.68	0.0250	0.9823
Sodium	2000	135.15 ± 3.18	135.85 ± 0.07	0.3112	0.7851
	5000	135.95 ± 0.13	135.85 ± 0.07	0.2401	1.0000
	6000	135.30 ± 0.71	135.85 ± 0.07	1.0902	0.3895
Potassium	2000	3.95 ± 0.05	3.95 ± 0.05	0.0000	1.0000
	5000	3.95 ± 0.05	3.95 ± 0.05	0.0000	1.0000
	6000	3.35 ± 0.07	3.95 ± 0.05	9.8639	0.0101
Chloride	2000	93.0 ± 11.17	96.65 ± 0.49	0.4617	0.6897
	5000	96.65 ± 0.49	96.65 ± 0.49	0.0000	1.0000
	6000	88.8 ± 7.071	96.65 ± 0.49	1.5665	0.2577

Table 2 shows the mean values of renal parameters in Wistar rats after 14 days of single administration with *A. barbadensis* methanol fraction. Creatinine values were statistically insignificant compared to the control at concentrations of 2000 mg/kg (T= 0.5672; P> 0.05), 5000 mg/kg (T= 0.000; P> 0.05), and 6000 mg/kg (T= 2.6067; P> 0.05), Urea levels show no significant difference in the mean levels at concentrations of 2000mg/kg (T= 1.1326; P> 0.05), 5000 mg/kg (T= 0.000; P> 0.05), and 6000 mg/kg (T= 0.0250; P> 0.05). Sodium mean value was statistically insignificant at concentrations of 2000 mg/kg (T= 0.3112; P> 0.05), at 5000 mg/kg (T= 0.2401; P> 0.05), and at 6000 mg/kg (T= 1.0902; P> 0.05). Also, the chloride mean value shows no significant variation at a concentration of 2000 mg/kg (T= 0.4617; P> 0.05), 5000 mg/kg (T= 0.5410; P> 0.05) and 6000 mg/kg (T= 1.5665; P>0.05). Potassium level was significantly elevated at 6000 mg/kg (T= 9.8639; P< 0.05) while administration at concentrations of 2000 mg/kg (T= 0.1250; P> 0.05), and 5000 mg/kg (T= 0.865; P> 0.05) were statistically insignificant.

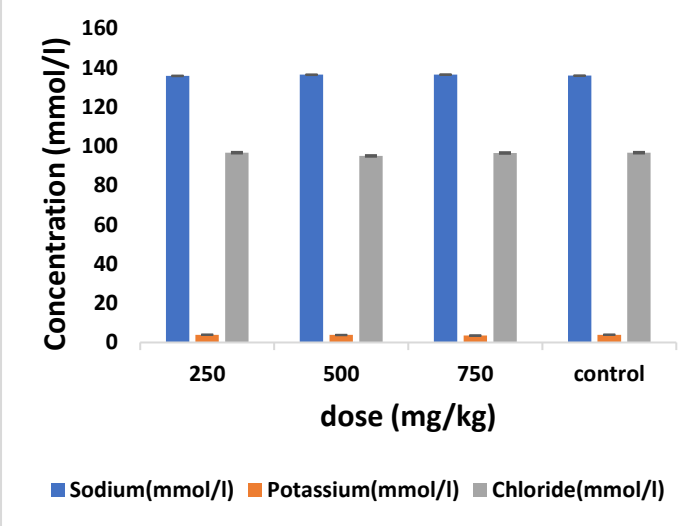

Figure 1: Variation in mean serum Sodium, Potassium and Chloride concentration in Wistar rats after 28 days of continuous administration with *A. barbadensis* methanol fraction. By comparing the mean values of serum Sodium in the test with the mean values in the control without treatment, statistical analysis shows no significant difference at a concentration of 250 mg/kg (T= 0.6389; P> 0.05), at 500 mg/kg (T= 1.0983; P> 0.05), and 750 mg/kg (T= 0.4599; P> 0.05). Also, by comparing the mean serum Potassium with the mean values in the control, statistical analysis shows no significant difference in the mean levels at a concentration of 250 mg/kg (T= 0.7714; P> 0.05), at 500 mg/kg (T= 1.9192; P> 0.05) and at 750 mg/kg (T= 1.5046; P> 0.05). Similarly, the mean serum Chloride when compared to the mean values in the control, shows no significant difference at a concentration of 250 mg/kg (T= 0.3315; P> 0.05), at 500 mg/kg (T= 1.2255; P> 0.05) and at 750mg/kg (T= 0.0584; P>0.05).

Table 3: Hepatic parameters of Wistar rats after 14 days of single administration of aqueous extract and methanol fraction of *A. barbadensis*

Parameter	**Concentrations**				**X^2**
	2000	**5000**	**6000**	**Control**	**P-value**
Aqueous extract					
AST (U/L)	108 ± 1.26	115 ± 1.19	137.24 ± 2.41	103 ± 2.57	
ALT (U/L)	23.51 ± 5.3	26.51 ± 8.32	29.32 ± 1.64	22.27 ± 3.1	
Alb (g/L)	45.26 ±2.11	41.37 ± 1.42	37.71 ±3.06	43.17 ±3.02	0.9301
ALP (U/L)	155.21 ± 14.27	155.4 ± 17.08	149.41 ± 9.18	152.29 ± 2.16	
GGT (U/L)	5.21 ± 0.09	5.90 ± 0.72	3.88 ± 1.24	5.14 ± 0.42	
Methanol fraction					
AST (U/L)	106 ± 1.26	110 ± 4.58	132.5 ± 31.56	103 ± 1.17	
ALT (U/L)	22.72 ± 7.9	24.95 ± 10.69	28.25 ± 4.47	22.23 ± 3.2	
Alb (g/L)	40.21 ±1.01	39.68 ± 1.68	34.65 ±2.29	43.09 ±3.02	0.8672
ALP (U/L)	154.3 ± 33.45	151.1 ± 87.27	147.3 ± 35.79	152.31 ± 2.26	
GGT (U/L)	5.17 ± 0.22	5.89 ± 0.44	3.83 ± 2.58	5.11 ± 0.12	

Table 3. Liver function tests of the Wistar rats, 14 days after a single administration with aqueous extract and the methanol fraction of *A. barbadensis* at concentrations of 2000, 5000, and 6000 mg/kg, compared to untreated control rats. Administration with the *A. barbadensis* aqueous extracts at concentrations of 2000 mg/kg, 5000 mg/kg and 6000 mg/kg showed no significant difference across all the parameters analysed compared to the control (X^2 =1.6723; P>0.05). Treatments with the methanol fraction of *A. barbadensis* after 14 days of a single administration were statistically insignificant on the AST, ALT, ALP, Albumin and GGT levels when compared with the control at concentrations of 2000 mg/kg 5000 mg/kg and 6000 mg/kg (T= 1.5314; P> 0.05).

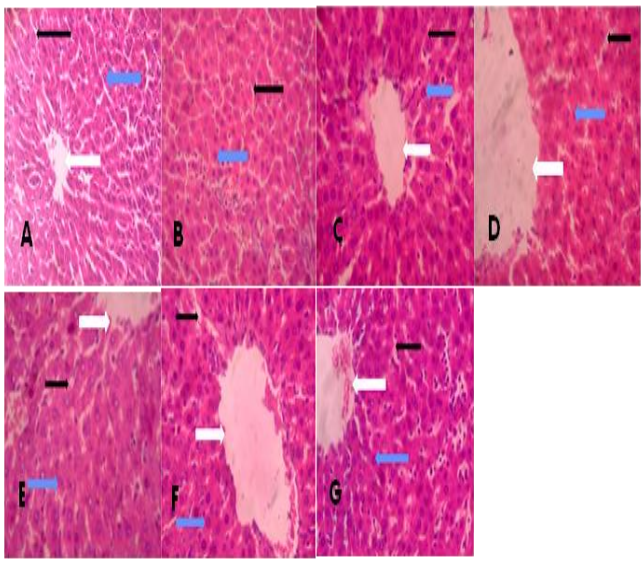

Figure 2: H and E-stained liver section of acutely exposed Wistar rats to the aqueous and methanol fraction of *A. barbadensis* **A.** Control shows normal liver histoarchitecture, the central vein (white arrow) and sinusoids (black arrow) are not congested, while hepatocytes (blue arrow) appear normal. **B.** *A. barbadensis* aqueous extract (2000 mg/kg/bw) in male rats showed normal sinusoids (black arrow) and hepatocytes (blue arrow). **C.** Female rat administered with aqueous extract of *A. barbadensis* fraction (2000 mg/kg) showing the morphology of the hepatocytes (blue arrow), and the sinusoids (black arrow). **D.** Male rat administered with *A. barbadensis* fraction (5000 mg/kg) showing the sinusoids (black arrow), and hepatocytes (blue arrow). **E**. female rat acutely administered with *A. barbadensis* fraction (5000 mg/kg) showing normal sinusoids (black arrow), and hepatocytes (blue arrow). **F.** male rat administered with *A. barbadensis* fraction (6000 mg/kg) showing normal sinusoids (black arrow), hepatocytes (blue arrow). **G.** female rat administered with *A. barbadensis* fraction (6000 mg/kg) showing an un-infiltrated sinusoidal space (black arrow), with the morphology of the hepatocytes appearing normal (blue arrow) (x400).

Table 4: Mean values of some renal parameters in Wistar rats after 28 days of continuous administration with *A. barbadensis* aqueous extract and methanol fraction

Parameter	Extract (mg/kg)	Test ± SD (mmol/L)	Control ± SD (mmol/L)	T-test	P-value
Aqueous extract					
Creatinine	250	141.21± 0.15	137.42 ± 20.18	1.0321	0.7701
	500	131.14± 037	131.68 ± 23.32	0.82761	0.6577
	750	124.26 ± 0.28	101.95 ± 18.52	0.7689	0.7769
Urea	250	9.6 ± 0.13	7.67 ± 1.54	0.8778	0.9532
	500	7.4 ± 0.37	6.32 ± 0.97	1.7710	0.8342
	750	6.5 ± 0.19	6.79 ± 1.35	0.6623	0.6987
Sodium	250	136.17 ± 2.38	135.85 ± 0.07	0.3112	0.7851
	500	135.85 ± 0.07	135.87 ± 0.08	0.0876	1.6577
	750	135.30 ± 0.71	135.85 ± 0.08	1.0902	0.3895
Potassium	250	3.95 ± 0.13	3.97 ± 0.12	0.7021	0.9000
	500	3.95 ± 0.08	3.97 ± 0.14	0.9852	0.8221
	750	3.25 ± 0.10	3.97 ± 0.09	8.8639	0.0241*
Chloride	250	93.0 ± 11.17	96.65 ± 0.49	1.0314	0.5895
	500	96.65 ± 0.493	96.65 ± 0.49	0.4210	0.6233
	750	98.81 ± 7.071	96.65 ± 0.49	1.7565	0.3679
Methanol fraction					
Urea	250	10.3 ± 0.15	137.42 ± 20.18	1.0411	0.6901
	500	12.1 ± 0.08	131.68 ± 23.32	0.9206	0.7537
	750	13.3 ± 0.11	101.95 ± 18.52	0.8186	0.7769
Creatinine	250	105.16 ± 0.21	7.67 ± 1.54	0.8243	0.8512
	500	108.17± 032	6.32 ± 0.97	1.0817	0.7843
	750	113.23 ± 0.13	6.79 ± 1.35	0.8673	0.6937

Table 4 shows the variation in the mean values of urea and creatinine, Na, K^+ and Cl^- across the various treatment groups relative to the control. Treatment with aqueous extract of *A. barbadensis* at concentrations of 250 mg/kg/day, 500 mg/kg/day and 750 mg/kg/day showed no significant variation in the mean urea and creatinine levels (T= 1.8427; P> 0.05) and (T= 0.9732; P> 0.05) respectively. There was no significant variation in the mean level of the sodium at concentrations of 250 mg/kg (T= 0.7851; P> 0.05), at 500 mg/kg (T= 0.08761; P> 0.05), and at 750 mg/kg (T= 1.0902 ; P> 0.05), chloride levels at 250 mg/kg (T= 1.0314; P> 0.05), at 500 mg/kg (T= 0.4210 ; P> 0.05), and 750 mg/kg (T= 1.5665; P> 0.05). However, a significant variation in the mean level of potassium was observed at 750 mg/kg/day concentration of the aqueous extract when compared to the control (T = 8.8639; P < 0.05). Methanol treatment with *A. barbadensis* was also statistically insignificant for the urea (T=1.1786; P> 0.05) and the creatinine levels (T = 0.7214; P> 0.05) at concentrations of 250 mg/kg, 500 mg/kg and 750 mg/kg.

Similarly, no behavioural alterations or death of Wistar rats were recorded by Nalimu et al. [40] following daily administration of 800, 400, and 200 mg/kg for twenty-eight days, which aligns with our findings. Also, the levels of AST and ALB did not increase significantly when the rats were continuously exposed to dosages up to 500 mg/kg. Our findings in this study are at variance with the reports of Ibrahim et al. [22] in which thyroid dysfunction, abdominal discomfort, diarrhoea, hepatocyte inflammation, nausea, and vomiting were associated with *A. barbadensis* extracts. Histological findings in this study showed no lesion in the liver of rats sub-acutely exposed to the aqueous extract and methanol fraction of *A. barbadensis* at varying concentrations of 2000 mg/kg, 5000 mg/kg, and 6000 mg/kg, respectively. At the fifteen days of the single-start dose administration with the methanol fraction of *A. barbadensis*, the kidneys of rats displayed normal histoarchitecture, similar to that of control rats, with normal renal interstitial spaces that were neither congested nor inflamed. There were no visible lesions in the treatment group (Figure 3). The liver of the sub-acutely treated rats with aqueous extract of *A. barbadensis* at 250 mg/kg/bw and 500

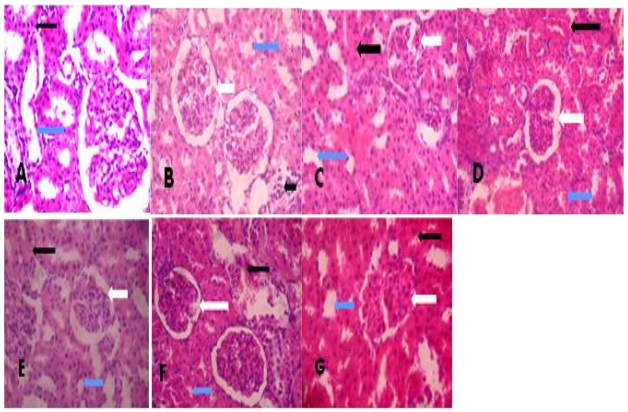

Figure 3: H and E-stained kidney sections of acutely exposed wistar rats to the aqueous extract and methanol fraction of *A.*

barbadensis **A.** control rat showing a normal glomerulus (white arrow) and normal renal tubule (blue arrow), with the interstitial spaces appearing normal and not infiltrated or inflamed (black arrow). **B.** male rat administered with aqueous extract of *A. barbadensis* (2000 mg/kg/bw) showing a normal renal tubule (blue arrow), with non-congested or inflammed renal interstitial spaces (black arrow). **C.** female rat administered with aqueous *A. barbadensis* extract (2000 mg/kg/bw) showing the renal tubule, which appears normal (blue arrow), the renal interstitial space appears normal and not infiltrated (black arrow). **D.** kidney section of a male rat administered with *A. barbadensis* fraction (5000 mg/kg/bw) showing normal renal tubules (blue arrow), the glomerulus (white arrow), renal interstitial space also appears normal (black arrow). **E.** female rat administered with *A. barbadensis* fraction (5000 mg/kg/bw) showing normal renal tubule morphology (blue arrow), glomerulus (white arrow), the renal interstitial space appears normal and not infiltrated (black arrow). **F.** male rat administered with *A. barbadensis* fraction (6000 mg/kg/bw) shows normal renal tubular morphology (blue arrow), the renal interstitial space appears normal and not infiltrated (black arrow). **G.** Female rat administered with *A. barbadensis* fraction (6000 mg/kg/bw) showing normal renal tubule morphology (blue arrow), normal glomerulus (white arrow), the renal interstitial space appears normal and not infiltrated (x400).

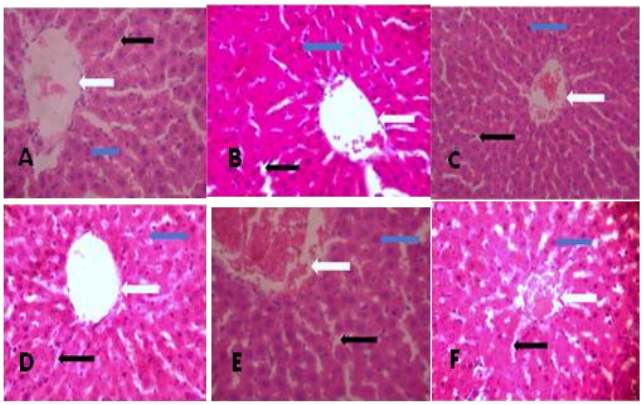

Figure 4. H and E-stained liver section of sub-acute exposed wistar rats administered with aqueous extract and the methanol fraction of *A. barbadensis*. **A.** *A. barbadensis* aqueous administration at 250 mg/kg/bw in male rats showing a normal hepatocyte (blue arrow), non-congested central vein, the sinusoids appear normal and not infiltrated (black arrow). **B**. Female rat administered with aqueous extract *A. barbadensis* (250 mg/kg/bw) showing a normal hepatocyte (blue arrow), the sinusoid appears normal and not infiltrated (black arrow), while the central vein is non-congested (white arrow). **C.** Male rat administered with *A. barbadensis* fraction (500 mg/kg/bw) showing normal hepatocytes (blue arrow) with sinusoids appearing normal and not infiltrated (black arrow), with the central vein appearing slightly congested (white arrow). **D.** The liver section of a female rat subacutely administered with *A. barbadensis* fraction (500 mg/kg/bw) showed normal hepatocytes (blue arrow), the sinusoid, which appears normal and not infiltrated (black arrow), while the central vein is devoid of congestion (white arrow). **E.** male rat subacutely administered with *A. barbadensis* fraction (750 mg/kg/bw) showing the morphology of the hepatocyte, which appears normal (blue arrow), the sinusoid appears normal (black arrow), while the central vein is mildly congested (white arrow). **F.** female rat sub acutely administered with *A. barbadensis* fraction (750 mg/kg/bw) showing the morphology of the hepatocyte, which appeared normal (blue arrow), the sinusoid appeared normal and not infiltrated (black arrow), while the central vein appeared congested (x400).

mg/kg/bw appeared normal with typical characteristics similar to those of control rats, such as normal hepatocytes, no congested or infiltrated central veins, and sinusoids. However, male and female rats treated with aqueous extract of *A. barbadensis* at 750 mg/kg/bw had congested central veins (Figure 4). The kidneys of sub-acutely exposed rats of both sexes treated with aqueous *A. barbadensis* at dosages of 250 mg/kg/bw and 500 mg/kg/bw appeared normal, with normal glomeruli and renal tubules and no congested or infiltrated interstitial space. However, rats administered the aqueous *A. barbadensis* extract at 750 mg/kg/bw had congested interstitium (Figure 5). According to Rajin et al., [45] sub-acute treatment with the methanol fraction of *A. barbadensis* at a concentration of 800 mg/kg/bw is capable of inducing significant toxicity in murine models.

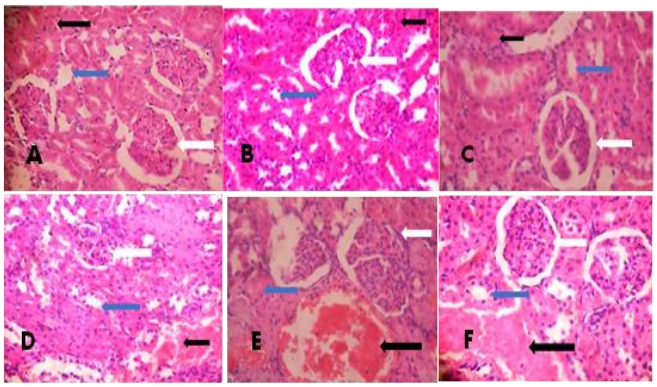

Figure 5: H and E-stained kidney section of sub-acutely exposed wistar rats administered with an aqueous and methanol fraction of *A. barbadensis*. **A**. Extract administered to a male rat at 250 mg/kg/bw showing normal renal tubules (blue arrow), with non-infiltrated or congested interstitial space (black arrow), the glomerulus appears normal (white arrow). **B.** female rat subacutely administered with aqueous *A. barbadensis* extract (250 mg/kg/bw) showing normal renal tubules (blue arrow), the renal interstitial space appears normal without infiltration (black arrow), while the glomerulus appears normal (white arrow). **C.** male rat administered with *A. barbadensis* fraction (500 mg/kg/bw) shows renal tubules with normal morphology (blue arrow), the renal interstitial spaces show mild infiltration (black arrow), while the glomerulus appears normal (white arrow). **D.** female rat administered with *A. barbadensis* fraction (500 mg/kg/bw) showing renal tubules with normal morphology (blue arrow), renal interstitial space is mildly congested (black arrow), while the glomerulus appears normal (white arrow). **E.** the kidney section of a sub-acute male rat administered with *A. barbadensis* fraction (750 mg/kg/bw), showing normal glomeruli (white arrow), the renal interstitial space is moderately congested (black arrow). **F.** The kidney section of a subacute female rat administered with *A. barbadensis* fraction (750 mg/kg/bw) showed normal renal tubules (blue arrow), with mildly congested interstitium (black arrow) (x400).

Post-treatment kidney interstitial inflammation was reported by Nalimu et al.[40] at dosages of 200 and 800 mg/kg of whole leaf extract. Similarly, in a study by Koroye et al.[46] treatment with *A. barbadensis* plus twice daily at volumes of 0.2, 0.4, and 0.8 cm^3 for 14 and 28 days resulted in histological changes in rat kidneys. In this study, continuous treatment of the rats with 750 mg/kg of aqueous and methanol fractions led to interstitial congestion. Table 5 shows the variation in the level of AST, ALT, ALP, GGT and Albumin in Wistar rats after 28 days of continuous administration with *A. barbadensis* aqueous extract and methanol fraction. Statistical analysis depicts no significant difference in the mean levels of AST, ALT, ALP, GGT and Albumin at 250 mg/kg, 500 mg/kg and 750 mg/kg ($P > 0.05$).

Table 6 shows the variation in the level of Mean haematological parameters in Wistar rats after 14 days of single administration with *A. barbadensis* methanol fraction compared to the untreated control group. Toxicity effect of the fraction on WBC and platelet count ($X^2 = 0.7106$; $P < 0.05$); hematocrit and haemoglobin ($X^2 = 0.6917$; $P < 0.05$) at concentrations of 2000 mg/kg, 5000 mg/kg, and 6000 mg/kg single dose depicted no significant difference when compared to the control. When the absolute values (MCH, MCV and MCHC) in the test animals were compared with values in the control, there was no significant difference in the levels of the absolute values of test and control groups after 14 days of single administration at concentrations of 2000 mg/kg, 5000 mg/kg, 6000 mg/kg ($X^2= 0.9774$; $P>0.05$).

Table 5: Variation in hepatic parameters of Wistar rats after 28 days of continuous administration with aqueous extract and methanol fraction of *A. barbadensis*

Parameter	**Concentration**				**X^2**
	250	**500**	**750**	**Control**	**P-value**
Methanol fraction					
AST (U/L)	105 ± 1.22	118 ± 1.09	131.24 ± 2.41	103 ± 2.57	
ALT (U/L)	23.41 ± 5.3	27.31 ± 4.33	28.12 ± 1.02	22.27 ± 3.1	
Alb (g/L)	45.23 ±2.21	41.38 ± 1.20	39.71 ±3.06	43.17 ±3.02	0.9254
ALP (U/L)	155.21 ± 14.27	155.4 ± 17.08	149.41 ± 9.18	152.29 ± 2.16	
GGT (U/L)	5.61 ± 0.09	5.70 ± 0.72	3.98 ± 1.24	5.14 ± 0.42	
Aqueous extract					
AST (U/L)	132.72 ± 9.51	136.3 ± 0.23	137.3 ± 0.23	103 ± 1.17	
ALT (U/L)	139.32 ± 2.16	137.22 ± 0.51	138.4 ± 1.24	22.23 ± 3.2	
Alb (g/L)	56.28 ± 2.42	67 ± 1.98	79.1 ± 2.8	43.09 ±3.02	1.5673
ALP (U/L)	147.42 ± 1.44	148.23 ± 2.26	87.3 ± 3.14	152.31 ± 2.26	
GGT (U/L)	8.65 ± 1.24	7.271 ± 0.84	6.213 ± 0.27	5.11 ± 0.12	

Table 6: Effect of *A. barbadensis* methanol fraction on some haematological parameters after 14 days of single administration

Parameter	**FRACTION CONCENTRATIONS**			**Control**	**X^2-test**	**P-value**
	mg/kg body weight			**(Untreated)**		
	2000	**5000**	**6000**			
HB	12.6±1.41	13.05±0.2	13.9±1.6	12.85±0.07	0.6917	0.2230
PCV	49.5 ±1.2	44.5± 0.3	43.5±3.18	0.435±0.03		
WBC	15.25 ±2.3	13.75±3.1	15.55±1.8	5.8± 0.57		
Platelet	552.5± 27.8	810.5±22.4	939±53.7	424±29.13	0.7106	0.3682
MCV	64.8±4.8	63.9± 2.9	63.5±0.4	60.85±0.25		
MCH	18.35 ±1.05	17.5± 0.25	17.4±0.2	18.2±0.20	0.9774	0.6531
MCHC	301± 79	303±13	304.5±5.5	300.5±4.53		

Table 7: Effect of *A. barbadensis* methanol fraction on some haematological parameters after 28-day continuous administration

Parameter	FRACTION CONCENTRATIONS mg/kg body weight N=5			Control, N=5 (untreated)	X^2-test	P-value
	250	500	750			
HB	14.83± 0.56	15.34± 0.42	14.55± 0.21	12.85±0.07		
PCV	0.4258±0.0071	0.496±0.028	0.46±0.0093	0.435± .0318	1.1875	0.0853
WBC	7.23± 1.56	9.83± 2.54	5.8± 0.57	11.2±0.82		
Platelet	500±216.375	732±255.93	425±229.103	600.5±174	0.9784	0.7661
MCV	59.8±2.7	60.9± 2.8	61.5±1.1	60.85±0.25		
MCH	19.85 ±1.84	17.5± 0.82	18.4±0.29	18.2±0.2	1.5273	0.7590
MCHC	302± 62	303±5.7	301.5±3.6	300.5±4.53		

Table 7 shows the variation in the level of the mean haematological parameters of Wistar rats after 28 days of continuous administration with *A. barbadensis* ethanol fraction. Comparing the values in the experimental animals to the values in the control, there was no significant difference in the levels of the PVC and Haemoglobin ($X^2 = 1.1875$; $P > 0.05$), WBC and Platelets ($X^2 = 0.9784$; $P > 0.05$) and absolute values $X^2 = 1.5273$; $P > 0.05$) at concentrations of 250 mg/kg, 500 mg/kg, 750 mg/kg.

Conclusion

Across the majority of tested concentrations, administration of *Aloe barbadensis* extract and its fraction elicited no mortality or observable signs of behavioural abnormalities in the experimental animals, indicating a favourable preclinical safety profile. By implication, the aqueous extract and methanol fraction of *A. barbadensis* are relatively non-toxic to hepatic and renal parameters when administered as a single start dose for 14 days or a continuous daily dose for 28 days (except potassium). Although the LD_{50} recorded for this extract in this current study was 6000 mg/kg/day. Treatment with a single start dose of 6000 mg/kg and continuous administration with 750 mg/kg daily of the extract and fraction resulted in significant elevation of potassium level, which may cause potassium derangements and lead to cardiovascular disorders. This study further observes that continuous treatment with doses of 750 mg/kg/bw for up to 28 days may be inimical to the anatomical structures of the liver and kidney. Finally, long-term studies to investigate the sub-chronic and chronic toxicity of the plant are suggested, while the safety of the plant in humans through clinical trial studies is advocated.

Conflict of Interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors acknowledge TETFUND/National Research Fund, Nigeria, for funding this research.

References

1. Pranto TI, Ullah MR, Rahman MM, Shohan F, Baroi JA, Bhowmik P, Rupak MA, Chowdhury T, Tashin R. An Assessment of Hepatoprotective Activity of *Aloe barbadensis* on Rat Model with Safety Profile Analysis. Asian J. Res. Rep. Hepatol. 2024;6(1):1-7.
2. Mensah ML, Komlaga G, Forkuo AD, Firempong C, Anning AK, Dickson RA. Toxicity and Safety Implications of Herbal Medicines. Herbal medicine. 2019:63.
3. Tong X, Li M, Li D, Lao C, Chen J, Xu W, Du J, Zhang M, Yang X, Li J. Aloe vera gel extract: Safety evaluation for acute and chronic oral administration in Sprague-Dawley rats and anticancer activity in breast and lung cancer cells. J Ethnopharmacol. 2021; 280:114434.
4. Chaachouay N, Zidane L. Plant-derived natural products: a source for drug discovery and development. Drugs Drug Candidates. 2024;3(1):184-207.
5. Sánchez M, González-Burgos E, Iglesias I, Gómez-Serranillos MP. Pharmacological update properties of aloe vera and its major active constituents. Molecules. 2020;25(6):1–38.
6. Mishra I, Soni H, Soni J, Sing RK. A study on the ethnopharmacological potential of Aloe vera l. Int J Pharmacogn. 2024;11(11):577–83.
7. Kumar S, Yadav M, Yadav A, Rohilla P, Yadav JP. Antiplasmodial potential and quantification of aloin and aloe-emodin in Aloe vera collected from different climatic regions of India. BMC Complement Altern Med. 2017;17:369.
8. Asiimwe, S., Namutebi, A., Borg-Karlson, A. K., Mugisha, M. K., Kakudidi, E. K., Hannington OO. Documentation and consensus of indigenous knowledge on medicinal plants used by the local communities of western Uganda. J Nat Prod Plant Resour. 2014;4(1):34–42.

9. Bendjedid S, Bazine I, Tadjine A, Djelloul R, Boukhari A, Bensouici C. Analysis of Phytochemical Constituents by using LC-MS, Antifungal and Allelopathic Activities of Leaves Extracts of Aloe vera. Jordan J Biol Sci. 2022;15(1):21–28.
10. Adeyemi OF, Okungbowa GE, Ogbeide OU. Aloe vera prevents radiation-induced dermatitis among the black population. Trop J Nat Prod Res. 2018;2(9):433–437.
11. Amro BI, Abu Hajleh MN, Afifi F. Evidence-based potential of some edible, medicinal and aromatic plants as safe cosmetics and cosmeceuticals. Trop J Nat Prod Res. 2021;5(1):16–18.
12. Abeer M. El Sayeda, Shahira M. Ezzata, Moataz M. El Naggarb SSEH. In vivo diabetic wound healing effect and HPLC – DAD – ESI – MS / MS profiling of the methanol extracts of eight Aloe species. Brazillian J Pharmacogn. 2016;26:352–362.
13. Dharajiya D, Pagi N, Jasani H, Patel P. Antimicrobial activity and phytochemical screening of Aloe vera (*Aloe barbadensis* Miller). Int J Curr Microbiol App Sci. 2017; 6(3):2152-2162.
14. Bello R, Muhammad I, Ali H. Quantitative and qualitative phytochemicals and proximate analysis of aloe vera (*Aloe barbadensis* miller). Int J Adv Acad Res. 2020;6(1):95-109.
15. Raad B. Phytochemical screening and biological activities of Aloe vera (L.) Burm. F. Pure Appl Biol. 2021;10(1):360–367.
16. López A, De Tangil MS, Vega-Orellana O, Ramírez AS, Rico M. Phenolic constituents, antioxidant and preliminary antimycoplasmic activities of leaf skin and flowers of Aloe vera (L.) Burm. f.(syn. *A. barbadensis Mill*.) from the Canary Islands (Spain). Molecules. 2013;18(5):4942-4954.
17. Shabnam K, Ram TC, Ashaq M. Aloe Vera : A Systematic Review from the Perspectives of the Food Industries and Medicinal Applications. African J Biol Sci. 2024;6(6):8198–8215.
18. Cordiano R, Caserta S, Minciullo PL, Allegra A, Gangemi S. Anthraquinones and Aloe Vera Extracts as Potential Modulators of Inflammaging Mechanisms: A Translational Approach from Autoimmune to Onco-Hematological Diseases. Molecules. 2025;30(6):1–29.
19. Mensah EO, Adadi P, Asase RV, Kelvin O, Mozhdehi FJ, Amoah I, Agyei D. Aloe vera and its byproducts as sources of valuable bioactive compounds: Extraction, biological activities, and applications in various food industries.PharmaNutrition. 2025;31:100436. Available from: https://doi.org/10.1016/j.phanu.2025.100436
20. OECD 2008. Repeated Dose 28-Day Oral Toxicity Study in Rodents (OECD TG 407). 2008; 477–90.
21. Adebayo G, Ayanda OI, Rottmann M, Ajibaye OS, Oduselu G, Mulindwa J, Ajani OO, Aina O, Mäser P, Adebiyi E. The Importance of Murine Models in Determining In Vivo Pharmacokinetics, Safety, and Efficacy in Antimalarial Drug Discovery. Pharmaceuticals. 2025;18(3):424.
22. Ibrahim AM, Sadah H Al, Ahmad R, Ahmad N, Naqvi AA. Clinical uses and toxicity of aloe vera: An evidence-based comprehensive retrospective review (2007-2017). Pharmacogn J. 2019;11(2):424–428.
23. Guo X, Mei N. Aloe vera: A review of toxicity and adverse clinical effects. J Environ Sci Heal - Part C Environ Carcinog Ecotoxicol Rev. 2016;34(2):77–96. Available from: http://dx.doi.org/10.1080/10590501.2016.1166826
24. Arsene MM, Viktorovna PI, Sergei GV, Hajjar F, Vyacheslavovna YN, Vladimirovna ZA, Aleksandrovna VE, Nikolayevich SA, Sachivkina N. Phytochemical analysis, antibacterial and antibiofilm activities of Aloe vera aqueous extract against selected resistant gram-negative bacteria involved in urinary tract infections. Fermentation. 2022;8(11):626.
25. Tiwari S, Sigdel S, Bhattarai S, Rokaya RK, Pandey J. Phytochemical Screening, Antibacterial - Guided Fractionation, and Thin-Layer Chromatographic Pattern of the Extract Obtained from *Diploknema butyracea*. Pharmacognosy Res. 2021;12(2021):437–443.
26. Mohr BJ, Fakoya FA, Hau J, Souilem O, Anestidou L. The governance of animal care and use for scientific purposes in Africa and the Middle East. ILAR J. 2016;57(3):333–346.
27. Moronkeji A, Akinbo FO. Genotoxic Response and Histological Alterations in Rat Lungs Exposed to Gasoline Generator Exhaust. Sultan Qaboos Univ J Sci [SQUJS]. 2024;29(1):15–27. Available from: https://journals.squ.edu.om/index.php/squjs/article/view/5780
28. Adeniyi TD, Moronkeji, A,Ekundina VO. Histological evaluation of the liver, kidney, and testes of adult male Wistar rats exposed to heavy metals-contaminated waterways. Med Lab J. 2023;17(5):4–8.
29. Abubakar AA, Ismaila NO, Musa MA, Fadairo JK. Combined Extracts of *Bryophyllum pinnatum* and *Aloe barbadensis* Induce Hepato-renal Dysfunctions and Elevated Hematological Indices in Wistar Rats. European J Med Plants. 2014;4(8):990–997.
30. Moronkeji A, Adeniyi T, Olubunmi E, Kayode I, Ajala OJ, Moronkeji AI, Idowu D. Modulatory effects of *Aframomum melegueta* and *Rauvolfia vomitoria* in cadmium-induced renal toxicity in adult rats. J Cell Biotechnol. 2025,11(1):3-13. doi:10.1177/23523689251329719
31. Moronkeji A, Eze IG, Bejide RA, Anwara OA, Igunbor MC. Evaluation of herbal cocktail used in the treatment of malaria on liver tissue of adult Wistar rats. J Med Plants Res. 2018;12(28):508–21.
32. Kinanthi AD, Izza LN, Muhede R, Fitri LE, Handono K, Yudharto HS, Riawan W, Khotimah H. Ethanol Extract from Yellow Pumpkin Modulate Hormonal Levels and Ovarian Follicle Dynamics in Hypoestrogenic Wistar Rats. Trop J Nat Prod Res. 2025;9(3):1001–1010.
33. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Neurol. 2014;4:1–10.
34. Adeniyi TD, Akinpelu M, Akinlami OO, Alaga FO, Adenika MM. Evaluation of the combined extract of *Rauwolfia vomitoria* and *Aframomum melegueta* on the kidney and liver of adult male Wistar rats. Sokoto J Med Lab Sci. 2024;9(2):203–212.
35. Abderrahmane F, Addou S, Labadie I, Kheroua O. Acute toxicity of the aqueous extract of *Aloe barbadensis miller* gel. Bangladesh J Sci Ind Res. 2023;58(1):19–34.
36. Asim Kumar Ghosh, Manasi Banerjee, Tapan Kumar Mandal, Akhilesh Mishra MKB. A Study on Analgesic Efficacy and Adverse Effects of Aloe. Pharmacol 1. 2011;1108:1098 – 1108.
37. Sehgal I, Winters WD, Scott M, David A, Gillis G, Stoufflet T, Nair A, Kousoulas K. Toxicologic assessment of a commercial decolorized whole leaf aloe vera juice, lily of the desert filtered whole leaf juice with aloesorb. J Toxicol. 2013;2013(1):802453.
38. Yimam M, Brownell L, Jia Q. In vivo safety evaluation of UP780, a standardized composition of aloe chromone aloesin formulated with an *Aloe vera* inner leaf fillet. RegulToxicolPharmacol. 2014;69(3):390–7. Available from: http://dx.doi.org/10.1016/j.yrtph.2014.05.001
39. Olaniyan JM, Muhammad HL, Makun HA, Busari MB, Abdullah AS. Acute and sub-acute toxicity studies of aqueous and methanol extracts of *Nelsonia campestris* in rats. J Acute Dis. 2016;5(1):62–70. Available from: http://dx.doi.org/10.1016/j.joad.2015.08.006
40. Nalimu F, Oloro J, Peter EL, Ogwang PE. Acute and sub-acute oral toxicity of aqueous whole leaf and green rind extract of *Aloe vera* in Wistar Rats. BMC Complement Med Ther. 2022;22(1):1-14. Available from: https://doi.org/10.1186/s12906-021-03470-4
41. Darlington Akukwu, Ugochukwu Aguwa, Catherine Akukwu,

Ambrose Agulanna, Obatavwe Ukoba IO. Effects of Aloe vera Gel on the Homeopoietic , Biochemical and Histological Parameters and Bone Marrow of Wistar Rats. South Asian Res J Nat Prod. 2023;6(3):185–95.

42. Nalimu F, Oloro J, Kahwa I, Ogwang PE. Review on the phytochemistry and toxicological profiles of Aloe vera and Aloe ferox. Futur J Pharm Sci. 2021;7:145.
43. Devaraj S, Yimam M, Brownell LA, Jialal I, Singh S, Jia Q. Effects of aloe vera supplementation in subjects with prediabetes/metabolic syndrome. Metab Syndr Relat Disord. 2013;11(1):35–40.
44. Kwack SJ, Do SG, Kim YW, Kim YJ, Gwak HM, Park HJ, Roh T, Shin MK, Lim SK, Kim HS, Lee BM. The no-observed-adverse-effect level (NOAEL) of baby aloe powder (BAP) for nutraceutical application based upon toxicological evaluation. J Toxicol Environ Heal, Part A. 2014;77(22-24):1319-1331.
45. Rajin M, Mawarti H, Asumta MZ. Toxicity of Aloe vera leaf Extract for multidrug-resistant (MDR) of Tuberculosis. J Appl Environ Biol Sci. 2017;7(11):153–156.
46. Koroye OC, Siminialayi IM, Etebu EN. Effects of oral administration of *Aloe vera* plus on the heart and kidney: a subacute toxicity study in rat models. Nig Health J 2010;10(1-2):17-21.