

Median Lethal Dose and Sub-Chronic Toxicity Evaluation of *Adansonia digitata* L. Leaf Methanol Extract on Broiler Chickens

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ABSTRACT

Background and Objective: *Adansonia digitata* L. (Malvaceae) popularly called the baobab tree is widely distributed in hot savannah regions of Sub-Saharan Africa. It is widely used as food and as a medicinal plant in the treatment of many human and poultry diseases. This work was designed to evaluate the safety/toxicity of *A. digitata* leaf methanol extract on broiler chickens. **Materials and Methods:** The chickens were purchased from a commercial hatchery and brooded for three weeks under standard conditions before the commencement of the experiment. The acute and sub-chronic toxicity screening was carried out using the OECD method. A total of fifteen chickens were used for the sub-chronic toxicity evaluation and the chickens were randomly divided into 5 groups of three chickens each. Group 1 served as normal control, while Groups 2 to 5 received 250, 500, 1000 and 1500 mg kg⁻¹ b.wt., of the extract, respectively. **Results:** The results revealed that the LD₅₀ of *A. digitata* leaf methanol extract on broiler chickens is greater than 5000 mg kg⁻¹ b.wt. No mortality or any signs of toxicity was recorded throughout the experiment. The extract significantly reduced the body weight of the chickens, especially in the groups treated with higher doses of the extract, but not significantly in those treated with lower doses. Initially, the average weight of the group that received highest dose of 1,500 mg kg⁻¹ b.wt., was 500 g, after the 4 weeks administration of the extract, the chicken gained weight of an averagely of 2,000 g as against the average weight of the normal control of 3,000 g. The biochemical parameters of the hepatic and renal functions (except for uric acid), as well as hematological indices analyzed in treated groups after the 28 days administration of the extract, did not present significant changes in their levels when compared to the normal control. At higher doses, especially 1,500 mg kg⁻¹ b.wt., of the extract, the level of the uric acid was 8.78±0.32 mg dL⁻¹ compared to 6.01±0.32 mg dL⁻¹ of the control group. **Conclusion:** The *A. digitata* leaf methanol extract may be concluded as practically safe and can be used especially for the treatment of poultry diseases.

KEYWORDS

Adansonia digitata, acute toxicity, LD₅₀, sub-chronic, broiler chickens

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INTRODUCTION

Medicinal plants have long been used for the treatment of certain diseases¹. Plant-derived medicines are used in all civilizations and cultures and, hence, plants have always played a key role in health care systems worldwide. In most developing countries, the indigenous modes of herbal treatment are a part of the culture and the dominant method of healing therapy. These remedies, with a considerable extent of effectiveness, are socially accepted, economically viable and mostly, are the only available source².

In most tropical countries of Africa, the high cost of Western medicine and poor health delivery, as well as the resurgence of phyto-medicine, has necessitated reliance on the use of traditional plant medicine in the treatment of ailment, often without consideration of the toxic effects that these plant products cause to the body³. Many plants have also been reported to be toxic to both humans and animals. It should, therefore, be emphasized that for any traditional use of the medicinal plant, its safety should be ascertained⁴. One of the reasons for the increasing interest in herbal medicines is the belief that because these medicines are natural and have been traditionally used, they are safe and harmless. Nevertheless, their natural origin is not a guarantee of safety, as many reports concerning the risks associated with the use of herbal products have noted⁵.

The purpose of toxicity testing is to provide an adequate database to make decisions concerning the toxicological properties of chemical and commercial products. In some situations, the purpose is to decide whether a material will be safe. Under the conditions of expected use in other situations, the objective is to establish safe limits in the condition of use. This process is called Hazard Evaluation and would contribute to the introduction of new industrial chemicals and household products⁶. *Adansonia digitata* and its related species belong to the family of Malvaceae. The tree is of African origin and known for its medicinal and nutritional value. It has excellent antioxidant and anti-inflammatory properties, various parts of the tree are used to treat different types of ailments⁷. The tree is well known for its medicinal properties as all the parts are screened for bioactive compounds and found to be rich in phytochemicals such as flavonoids and phenols⁸. Medicinally, the leaves of *A. digitata* are used to treat inflammation, diarrhea, dysentery, fever, malaria, fatigue, kidney and bladder diseases⁹ as well as some diseases affecting poultry. Hence, this research work was designed to evaluate the safety/toxicity of *A. digitata* leaf extract on broiler chickens to ascertain the safety of its use in poultry.

MATERIALS AND METHODS

Study area: This research work was conducted between July to December, 2021 in Aliero Town, Nigeria. It was performed in the Biochemistry Research Laboratory, Department of Biochemistry, Faculty of Life Sciences, Kebbi State University of Science and Technology, Aliero, Nigeria.

Experimental animals: Healthy day-old broiler chickens were purchased from a commercial hatchery in Ibadan, Oyo State, Nigeria and brooded under standard conditions for three weeks before the commencement of the study. The chickens were fed with broiler starter feed and water *ad libitum*. Birds were housed in individual cages with proper lighting and heat. The birds were vaccinated against Infectious Bursal Disease (IBD) and Newcastle disease virus with IBD and Lasota, respectively. All experiments were conducted following the principles and guidelines for the care and use of laboratory Animals and approved by the Animals Ethics Committee of Kebbi State University of Science and Technology, Aliero.

Collection and authentication of the plant material: *Adansonia digitata* leaf was collected within Aliero town, Kebbi State, Nigeria. It was authenticated at the herbarium of the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero, Nigeria and a voucher specimen was deposited there.

Preparation of crude *A. digitata* leaf methanol extract: The collected leaf was cleaned with water and air-dried under shade and pulverized using a pestle and mortar. Three kilograms of the powdered leaf were measured and soaked in 7.5 l of 95% methanol. The mixture was then kept at room temperature for 72 hrs and filtered twice, initially with a muslin cloth and later with a Whatman filter paper No. 1. The filtrate was evaporated to dryness using a rotary evaporator.

Toxicity screening of the *A. digitata* leaf methanol extract

Determination of Median Lethal Dose (LD₅₀): Three chickens were used for each dose. A single dose of 1,000 mg kg⁻¹ b.wt., of the extract was initially administered to each chick orally. The treatment followed an overnight fasting period and the body weights of the chickens were determined immediately after the fasting period before administering the extract. The doses were calculated about the body weight. Food was provided to the chickens approximately an hour after treatment. Each chick was observed in detail for mortality and any behavioural changes or sign of toxicity within the first 2, 8, 24 and 48 hrs after the treatment period and then daily for 14 days. When there was no mortality or any sign of toxicity, the dose was increased to 2000 mg kg⁻¹ b.wt. and the same procedure was followed as the previous dose. When a similar result was obtained (no mortality or any signs of toxicity) the dose was increased to 3000 mg kg⁻¹ b.wt. and continued up to 5000 mg kg⁻¹ b.wt.

Sub-chronic toxicity screening: Fifteen chickens were divided into five groups of three chickens each. Daily oral administration of different concentrations of the extract was carried out for 28 days. Weights of the chickens were taken immediately before the commencement of extract administration, then weekly for 4 weeks:

- **Group 1:** Received distilled water orally and served as a normal control for the period of the study
- **Group 2 to 5:** Received graded doses of the extract (250, 500, 1000 and 1500 mg kg⁻¹ b.wt., respectively). The doses were calculated about the body weight

All the groups received the same volume of preparations. The weights of the chickens were taken weekly and detailed observation for the signs of toxicity was done twice daily for 28 days. The chickens fasted overnight on the 28th and the 29th day, thereafter the chickens were anaesthetized with chloroform and sacrificed and then blood samples were collected into EDTA and plain tubes for haematological and biochemical analyses, respectively. The coagulated blood samples for biochemical analyses were centrifuged at 3,000 rpm for 10 min to obtain the sera. The biochemical and haematological analyses were carried out.

Serum biochemical analysis: The following biochemical parameters were analyzed using a commercial kit and the procedures were followed according to the manufacturer's instructions: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total Protein (TP), Albumin (ALB), Total Bilirubin (TB), Direct Bilirubin (DB), Urea, Creatinine, Potassium (K⁺), Sodium (Na⁺), Chloride (Cl⁻) and Uric acid.

Hematological analysis: The following haematological parameters were analyzed using an automatic haematological analyzer (Medonic M32S Cell Counter, India): White Blood Cell (WBC), Red Blood Cell (RBC), Hematocrit Test (HCT), Hemoglobin (HGB), Lymphocytes (LYM), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and platelets.

Data analysis: The data obtained from the study are presented as Mean±SEM and were analyzed using One-way ANOVA with the aid of a Statistical Package (SPSS Version 20).

RESULTS

During the 14 days of observation after oral acute administration of *A. digitata* leaf methanol extract in chickens, there was no mortality or any signs of toxicity recorded even after the administration of the highest dose of 5000 mg kg⁻¹ b.wt., in Table 1. Hence the LD₅₀ of the *A. digitata* leaf methanol extract in chickens is greater than 5000 mg kg⁻¹ b.wt.

Figure 1 shows the weekly progressive weight gain of the broiler chickens for four weeks of administration of the *A. digitata* leaf methanol extract. It was observed that the extract has no significant effect on the weight of the chickens at lower doses of the extract, but reduces the weight at higher doses compared with the normal control. Initially, the average weight of the group that received the highest dose of 1,500 mg kg⁻¹ b.wt., was 500 g, after the four-week administration of the extract, the chicken gained weight of an averagely of 2,000 g as against the average weight of the normal control of 3,000 g.

From Table 2, the levels of each of the hepatic function parameters (except for ALP) from the test groups were not significantly altered statistically ($p > 0.05$) when compared with the normal control group. But, for the ALP, the extract caused a significant increase in its level to 79.12 ± 11.57 U L⁻¹ at the highest dose of 1,500 mg kg⁻¹ b.wt., compared with the normal control level of 33.12 ± 23.80 U L⁻¹.

The levels of all the renal function parameters except for uric acid were not significantly ($p > 0.05$) altered in all extract treatment groups compared with the normal control group in Table 3. The uric acid level

Table 1: LD₅₀ of *A. digitata* leaf methanol extract in broiler chickens

Dose of extract (mg kg ⁻¹ b.wt.)	Number of chickens used	Number of death	Number of Survival
1000	3	0	3
2000	3	0	3
3000	3	0	3
4000	3	0	3
5000	3	0	3

LD₅₀ of the extract was determined to be >5000 mg kg⁻¹ b.wt.

Table 2: Effect of *A. digitata* leaf methanol extract on the broiler chickens hepatic function parameters after 28 days of administration

Parameters	Control	250 mg kg ⁻¹ b.wt.	500 mg kg ⁻¹ b.wt.	1000 mg kg ⁻¹ b.wt.	1500 mg kg ⁻¹ b.wt.
AST (U L ⁻¹)	195.10 ± 2.01 ^a	192.84 ± 5.12 ^a	174.68 ± 6.67 ^a	170.49 ± 2.86 ^a	191.61 ± 1.25 ^a
ALT (U L ⁻¹)	12.91 ± 5.29 ^{ab}	13.23 ± 5.61 ^{ab}	10.03 ± 1.37 ^a	10.95 ± 0.68 ^a	11.99 ± 2.86 ^{ab}
ALP (U L ⁻¹)	33.12 ± 23.80 ^a	55.20 ± 13.61 ^{ab}	38.80 ± 11.53 ^a	59.80 ± 0.74 ^{ab}	79.12 ± 11.57 ^b
TP (g L ⁻¹)	4.56 ± 0.54 ^{ab}	3.40 ± 0.39 ^a	3.31 ± 0.17 ^a	3.31 ± 0.70 ^a	3.94 ± 0.38 ^a
ALB (g L ⁻¹)	1.83 ± 0.09 ^b	1.94 ± 0.10 ^b	1.77 ± 0.14 ^b	1.39 ± 0.12 ^a	1.59 ± 0.06 ^{ab}
TB (mg dL ⁻¹)	1.47 ± 0.14 ^{ab}	1.29 ± 0.17 ^a	1.09 ± 0.31 ^a	1.64 ± 0.25 ^{ab}	1.00 ± 0.10 ^a
DB (mg dL ⁻¹)	2.67 ± 1.41 ^b	0.42 ± 0.21 ^a	0.50 ± 0.15 ^a	1.03 ± 0.03 ^{ab}	0.87 ± 0.0 ^{ab}

Values are presented as mean ± SEM (n = 3), value having similar superscript along the rows are not significantly different at ($p > 0.05$) analysed using One-Way ANOVA, followed by Duncan Multiple Comparison Test with SPSS Version 20.0, AST: Aspartate amino transferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, ALB: Albumin, TP: Total protein, TB: Total bilirubin and DB: Direct bilirubin

Table 3: Effect of *A. digitata* leaf methanol extract on the broiler chickens renal function parameters after 28 days of administration

Parameters	Control	250 mg kg ⁻¹ b.wt.	500 mg kg ⁻¹ b.wt.	1000 mg kg ⁻¹ b.wt.	1500 mg kg ⁻¹ b.wt.
Creatinine (mg dL ⁻¹)	9.85 ± 0.33 ^{ab}	10.51 ± 2.07 ^{ab}	9.54 ± 1.23 ^a	10.68 ± 0.21 ^{ab}	8.63 ± 0.54 ^a
Urea (mmol L ⁻¹)	25.53 ± 1.76 ^{ab}	28.89 ± 2.60 ^{ab}	32.05 ± 2.75 ^b	19.24 ± 2.95 ^a	21.94 ± 3.04 ^a
Uric acid (mg dL ⁻¹)	6.01 ± 0.32 ^{ab}	4.75 ± 0.10 ^a	6.34 ± 0.68 ^b	7.06 ± 0.38 ^b	8.78 ± 0.32 ^c
K ⁺ (mmol L ⁻¹)	12.50 ± 1.44 ^a	12.50 ± 1.44 ^a	15.00 ± 2.89 ^{ab}	15.00 ± 2.87 ^{ab}	15.00 ± 2.89 ^{ab}
Na ⁺ (mmol L ⁻¹)	100.00 ± 2.89 ^a	100.00 ± 5.77 ^a	145.00 ± 2.87 ^{ab}	156.67 ± 4.41 ^{ab}	118.33 ± 6.01 ^a
Cl ⁻ (mmol L ⁻¹)	88.73 ± 10.25 ^{ab}	65.08 ± 5.92 ^a	65.42 ± 29.92 ^a	71.00 ± 10.25 ^a	94.67 ± 5.92 ^{ab}

Values are presented as mean ± SEM (n = 3), values having similar superscript along the rows are not significantly different at ($p > 0.05$) using One-Way ANOVA, followed by Duncan Multiple Comparison Test with SPSS Version 20.0, Potassium (K⁺), Sodium (Na⁺) and Chloride (Cl⁻)

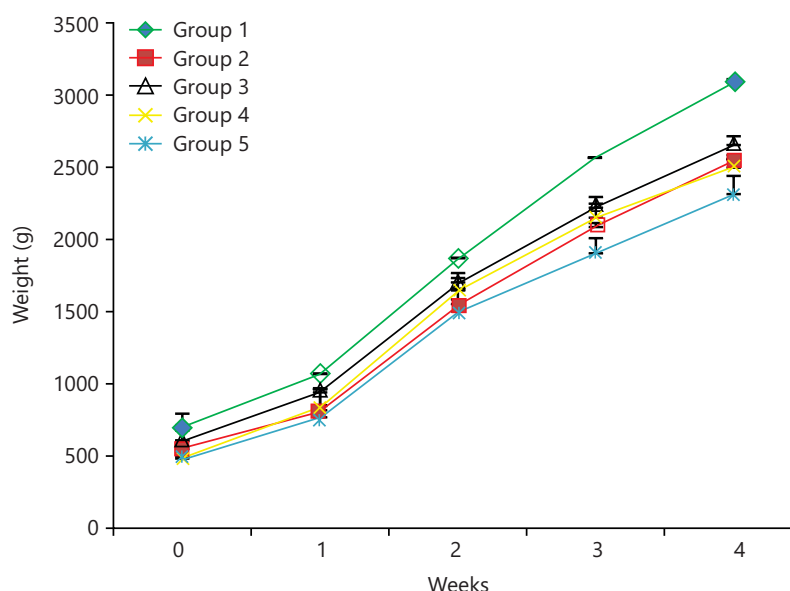


Fig. 1: Weight of chickens administered with *A. digitata* leaf methanol extract for four weeks

Group 1: Normal control, Group 2: 250 mg kg⁻¹ b.wt., Group 3: 500 mg kg⁻¹ b.wt., Group 4: 1000 mg kg⁻¹ b.wt. and Group 5: 1500 mg kg⁻¹ b.wt.

Table 4: Effect of *A. digitata* leaf methanol extract on haematological parameters in broiler chickens for 28 days administration

Parameters	Control	250 mg kg ⁻¹ b.wt.	500 mg kg ⁻¹ b.wt.	1000 mg kg ⁻¹ b.wt.	1500 mg kg ⁻¹ b.wt.
WBC ($\times 10^9$ L ⁻¹)	35.97 \pm 1.25 ^b	27.38 \pm 4.19 ^a	32.57 \pm 0.29 ^{ab}	30.79 \pm 1.15 ^{ab}	31.77 \pm 0.99 ^{ab}
LYM (%)	88.93 \pm 1.70 ^a	91.67 \pm 0.99 ^a	91.07 \pm 0.84 ^a	92.90 \pm 0.49 ^{ab}	90.40 \pm 1.01 ^a
RBC (%)	2.78 \pm 0.06 ^a	2.58 \pm 0.11 ^a	2.52 \pm 0.10 ^a	2.62 \pm 0.05 ^a	2.55 \pm 0.07 ^a
HGB (g dL ⁻¹)	16.73 \pm 0.38 ^{ab}	15.37 \pm 0.49 ^a	15.27 \pm 0.43 ^a	15.43 \pm 0.13 ^a	15.10 \pm 0.44 ^a
HCT (%)	39.20 \pm 1.55 ^{ab}	34.30 \pm 1.23 ^a	34.53 \pm 0.68 ^a	34.60 \pm 0.59 ^a	33.00 \pm 0.84 ^a
MCV (%)	134.97 \pm 2.53 ^{ab}	133.10 \pm 1.08 ^a	137.43 \pm 3.18 ^{ab}	131.83 \pm 0.64 ^a	129.40 \pm 1.58 ^a
MCH (%)	60.17 \pm 1.01 ^a	59.63 \pm 0.82 ^a	60.73 \pm 0.40 ^a	58.87 \pm 0.73 ^a	59.17 \pm 0.69 ^a
MCHC (%)	42.67 \pm 0.84 ^a	44.80 \pm 0.31 ^{ab}	44.67 \pm 0.55 ^{ab}	44.67 \pm 0.42 ^{ab}	45.70 \pm 0.20 ^{ab}
PLT (%)	7.33 \pm 0.88 ^a	10.33 \pm 1.86 ^{ab}	7.00 \pm 1.53 ^a	6.00 \pm 1.15 ^a	11.00 \pm 1.53 ^{ab}

Values are presented as mean \pm SEM (n = 3), values having similar superscript across the rows are not significantly different at (p<0.05) using One-Way ANOVA, followed by Duncan Multiple Comparison Test with SPSS Version 20.0, RBC: Red blood count, MCV: Mean Cell volume, LYM: Lymphocytes, HGB: Hemoglobin, MCH: Mean corpuscular Hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelets, WBC: White blood count and HCT: Hematocrit

(significant, p<0.05) increased in the groups treated with higher doses of the extract (500 to 1500 mg kg⁻¹ b.wt.) compared to the normal control group. At 1,500 mg kg⁻¹ b.wt., of the extract, the level of the uric acid was 8.78 \pm 0.32 mg dL⁻¹ compared to 6.01 \pm 0.32 mg dL⁻¹ of the control group.

From Table 4, the levels of each of the haematological parameters from the test groups were not significantly altered (statistically, p>0.05) when compared with the normal control group.

DISCUSSION

The findings revealed that *Adansonia digitata* leaf methanol extract is safe based on the toxicity study due to the higher LD₅₀ of >5000 mg kg⁻¹ b.wt., observed in this research. There were no signs of toxicity or mortality observed after the 28 days sub-chronic toxicity study. Shehu *et al.*⁹ reported that no mortality was recorded in petroleum ether, ethanolic and aqueous extracts of stem bark of *A. digitata* using albino Wister rats, in any of the experimental groups for 24 hrs and up to 2 weeks after oral administration of 5000 mg kg⁻¹ of each of the extracts. Another finding was reported by Muhammad *et al.*¹⁰, who reported that the LD₅₀ of *A. digitata* fruit pulp extract was greater than 5000 mg kg⁻¹ b.wt., in albino rats. The safety of *A. digitata* leaf extract at 5000 mg kg⁻¹ b.wt., was also confirmed by the findings of Eghoi and Paul¹¹,

who observed mortality only after 46 hrs of administration in mice that received 6000 mg kg⁻¹ b.wt., of the plant leaves extract. The nontoxic effect of *A. digitata* explains why most of its parts, seeds, fruit pulps, stem-bark and leaves are consumed by many communities⁸. As a standard, any substance that is not toxic at less than or equal to 5000 mg kg⁻¹ body weight is considered relatively safe¹².

From the sub-chronic toxicity study, the liver enzymes (AST, ALT and ALP) which are the liver biomarkers were analyzed and the results revealed that there was no significant difference in all the experimental groups compared to the normal control group ($p < 0.05$). AST and ALT are found primarily in the liver. When the liver is injured or inflamed after exposure to various forms of toxic substances, the level of ALT and AST in the blood is usually increased. The serum activities of these enzymes are directly related to the extent of the hepatic tissue damage¹³. In hepatotoxicity, the serum aminotransferases such as Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) indicate the concentration of hepatic intracellular enzymes that have leaked into the circulation. These are the markers for hepatocellular injury¹⁴. This research revealed that the administration of *A. digitata* leaf extract did not elevate the activities of these enzymes and therefore the extract is not hepatotoxic. For Total Protein (TP) and Albumin (ALB), there was no significant alteration in their levels compared to the normal control group. The results also revealed that there was no significant alteration in the levels of Direct Bilirubin (DB) and Total Bilirubin (TB) across all the treated groups compared to the control group. These findings also confirmed the non-hepatotoxic effect of the leaf extract on the broiler chickens.

The results on the renal function parameters (except for uric acid) showed that there was no significant difference in the serum levels of Creatinine, Urea, K⁺, Na⁺ and Cl⁻ in all the treated groups compared to the normal control group. Creatinine is an important marker for testing renal function, it is a breakdown product of creatinine phosphate in muscle. When elevated it may indicate renal dysfunction. The increased creatinine and urea concentrations in the blood serum are often associated with the impairment of the kidneys due to the failure of glomeruli to reabsorb and prevent the leakage of proteins and urea into the blood. Increased creatinine level in the blood is a sign of abnormal kidney function due to decreased excretion of creatinine in urine¹⁵. In cases of acute or chronic renal toxicity, these two parameters (urea and creatinine) are usually markedly increased to four or five times higher than the normal values in control animals⁵. Hence, their levels in this research confirm the nontoxic effect of the extract on the renal tissue of the chickens. At a doses of 1,500 mg kg⁻¹ b.wt., of the extract, the level of uric acid was 8.78±0.32 mg dL⁻¹ compared to 6.01±0.32 mg dL⁻¹ of the control group. This implies that, high doses of the extract cause increase in the level of serum uric acid which might be a result of the increase in protein/amino acids metabolism due to the high protein content of the *A. digitata* leaf¹⁶.

As observed in this research, the extract significantly reduced the body weight of the chickens at higher doses. At the initial stage, the average weight of the group that received the highest dose of 1,500 mg kg⁻¹ b.wt., was 500 g, but, after the four-week administration of the extract, the chicken in that group gained a weight of an averagely of 2,000 g which is lower than the average weight of the normal control group of 3,000 g. This implies that breaking down of the protein content of the extract was more than the utilization of the protein content of the extract¹⁶ by the chickens as evidently observed through the increase in the level of serum uric acid (as the end-product of protein metabolism in chickens) and decreased in the bodyweight of the chickens as a result of reduced protein intake leading to less muscle mass buildup¹⁷.

The results for the haematological indices showed that *A. digitata* leaf methanol extract did not cause any significant alteration ($p > 0.05$) in the levels of the indices among all the treated groups when compared to the normal control. HGB, RBC and HCT are associated with the total population of red blood cells while MCV, MCH and MCHC relate to individual red blood cells⁴. These findings also confirmed the non-hemato-toxic effect of the leaf extract on the broiler chickens.

CONCLUSION

Conclusively, *Adansonia digitata* leaf methanol extract practically safe and can be used especially for the treatment of poultry diseases such as coccidiosis.

SIGNIFICANCE STATEMENT

The *Adansonia digitata* leaf is well known to be used in the treatment of some diseases, especially in poultry farming, such as coccidiosis. To the knowledge of the authors, no research on the toxicity evaluation of the leaf of this plant was conducted on broiler chickens. Therefore, this research has ascertained the safety of *A. digitata* leaf extract, hence, can be used in the treatment of some diseases like coccidiosis in poultry farming.

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