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EFFECTS OF RAW AND BOILED LEAVES OF ADANSONIA DIGITATA ON ALUMINIUM CHLORIDE-INDUCED ANAEMIC RATS

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ABSTRACT

Adansonia digitata leaf owns a vast collection of therapeutic and nutritional uses. In this study, the effect of feed fortified with raw and boiled leaves of *A. digitata* on aluminum chloride-induced anaemia in rats was determined. A total of twenty-one rats were assigned into seven groups (n=3), anaemia was induced in groups 2 to 6 with aluminum chloride. Groups 4, 5, 6 and 7 were fed with rat chow fortified with 20% and 40% of raw and boiled *A. digitata* leaves while groups (1-3) were tagged normal, negative and standard. Reduction in feed intake and body weight of rats in the untreated group as compared to the normal control was observed. However, the group treated with boiled leaves demonstrated increased body weight (133.44 ± 0.68 g) as compared to the normal group (127.99 ± 1.63 g). Anaemia induction brought about a significant decrease ($p < 0.05$) in RBC, MCV, WBC, neutrophils and PCV in rats when compared with the normal rats. Although, treatment with 40% fortified boiled leaves of *A. digitata* reversed these altered parameters. This study indicates that *A. digitata* leaves possess anti-anaemic activities and it can be implored further for the management of anaemia.

Keywords*:* White blood cell, Packed-cell volume, Anti-anaemic, Red blood cells.

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INTRODUCTION

Anaemia is a state in which a human's haemoglobin (Hb) concentration/red blood cell (RBC) count descends below normal and inadequate to meet their biological requirements [1]. About 1/3 of the globe's population are suffering from anaemia [2] with high percentage in women of reproductive age and preschool children [3]. The higher morbidity and mortality in children and women has been linked to this disease [4, 5], likewise, poor birth condition [6, 7], lower work output in grown person [8], reduced cognitive and behavioral development in children [4, 5] have been reported to be symptoms of anaemia. About 400 different types of anaemia have been reported and each of them are uncommon which is characterized by low red blood cell counts [9].

In 2019, 58.6 million of people suffered from disability worldwide as a result of anaemia with highest burden in Western Africa, South Asia and Central Africa [9]. The three top contributing factors worldwide are iron insufficiency (Dietary), deficiency in vitamin A, and beta-thalassemia trait [10].

Aluminum in various forms (chloride, nitrate, phosphate) has no known biological function in its ionic state, but its accumulation in the system causes kidney problem [11], liver damage [12], anaemia and bone problem [13]. Component of industrial waste and products like cement producing factories, cookware, food additives, cosmetics and toothpaste, pharmaceutical products such as antacids, buffered aspirin, vaccines and injectable allergens have been reported to be the sources of Aluminium chloride. Al has also been exhibited a direct effect on haematopoiesis [14], high erythropoiesis impairment couple with iron (Fe) deficiency anaemia. [15, 16] and disrupting Fe homeostasis. [17].

Most chelating agents such as Dimercaptosuccinic acid (chemet), British anti-Lewiste (dimercaprol), Disodium EDTA used to address anaemia due to metal-induced toxicity showed many disadvantages; which include prooxidant effect, toxic effect, high cost and poor effectiveness. Consequently therefore, necessitating the search for alternatives from natural sources.

Natural products particularly those of plant origin e.g., *Moringa oleifera* [33], and *Mucuna pruriens* [29] have successfully healed a number of ailments, including anaemia. Plants have always provided food and medicine to humans [18]. Secondary metabolites are produced by plants in response to infectious pathogens and environmental conditions which are believed to be responsible for the medicinal properties plant elicit. Processing of the leaves of plants is a common method of preparing and preserving them for human consumption. It is critical in improving the shelf life of leaves while also making them fit for ingestion. Different processing methods either maintain the bioactive compounds or reduce the anti-nutrients inherent in the plants. Air drying is the process of removing moisture from the leaves of plants thereby preventing spoilage and inhibiting the growth of microorganisms. It has been shown to be a good method for retention of bioactive contents and it is done at room temperature [19].

Adansonia digitata (known as African baobab in English, "bokki" in Fulani and "kuka" in Hausa) is amongst the numerous plants believed to have blood boosting effect. *A. digitata* is a deciduous, vast, big tree that may grow up to 25 m tall and can survive for hundreds of years. The African baobab (*A. digitata* L.) is a versatile tree species, native to the dry savannah zone of the sub-Saharan Africa, where it plays an imperative role in the sustenance of food security and daily income of rural communities [20], and is intimately linked with cultural uniqueness and local credence [21]. The African baobab is said to have originated in West Africa, from which it spread rapidly throughout

Africa's savannahs and Madagascar [22]. The parts commonly utilized for enhancing food and nutrition security are the fruit pulp, leaves, and seeds. Other parts of the baobab (bark, flowers, and root) have been proven to be helpful and of medicinal value [23, 24, 25]. Baobab pulp is claimed to be used superficially with buttermilk to cure looseness of the bowels and dysentery in India, while the young leaves are mashed and used to heal aching swellings [26]. The leaves and fruit pulp are used as an antipyretic as well as an immune tonic [27]. The leaves are also used in the northern part of Nigeria as soup for many dishes.

The aim of this study is to investigate the effect of raw and boiled leaves of *A. digitata* on aluminum chloride-induced anaemia in albino rats. The precise objectives of this study are to determine; the variations in food consumption, bodyweight and haematological parameters of - anaemic rats induced with Aluminium chloride.

MATERIAL AND METHODS

Materials

Plant Sample Collection and Identification

The leaves of *A. digitata* were collected in Pyawu village, Minna, Niger State, Nigeria. The leaves were verified by Dr. Daudu Yusuf of Plant Biology Department, Federal University of Technology Minna, Nigeria.

Experimental Animals

Albino rats between 155 to 175 g bodyweight were gotten from the Department of Biochemistry, Federal University of Technology, Minna. They were housed in plastic cages with unlimited access to commercial pelletized rats' food and allowed to acclimatize for a period of 14 days before the commencement of the experiment.

METHODS

Sample Preparation and Leaf Processing Methods

Preparation of Plant Material

The leaves of the plant were removed from the stalk, rinse with distil water and divided into two parts before subjected both to air dried and boiled; then air-drying. Air-dried, *A. digitata* leaves were dried at ambient temperature (27 ^{o}C) for 7 days, then pulverized with an electric blender and stored in an opaque bottle at room temperature [28]. The second part of the leaves were boiled for 10 minutes at 100°C, drain and dried for 7 days ambient temperature (27 °C). The dried boiled leaves were further pulverized as former before being stored in an opaque bottle then kept in the laboratory shelf at temperature before uses.

Experimental Design

Three Wistar rats per group of seven amount to total of twenty-one (21) rats were used for the experiment. Anaemia was induced in rats with $AlCl_3$ as described by Naman *et al*. [29]. Oral daily administration of $AlCl_2$ 00 mg/kg body weight was given to each group of rats for 14 days with the exception of the normal group. The treatment groups were further fed with rat pellets fortified with (20 and 40) % raw and boiled *A. digitata* leaves while standard group was fed with rat pellet fortified with ferrous sulphate (200 mg of per 100g of the feed). The experiment lasted for 14 days. The groups were as follows:

Group1: Normal (rat chow + water)
Group2: Negative (200 mg/kg bodyweight $AlCl_3$ only)
Group 3: Standard ($AlCl_3$ + $FeSO_4$
Group 4: - $AlCl_3$+ 20% Raw *A. digitata* fortified-feed)
Group 5: - $AlCl_3$+ 20% Boiled *A. digitata* fortified-feed)
Group 6:- $AlCl_3$+ 40% Raw *A. digitata* fortified-feed)
Group 7: - $AlCl_3$+ 40% Boiled *A. digitata* fortified-feed).

Blood Sample Collection

On the 15th day, the experimental animals were sacrificed under mild anaesthesia

(diethyl ether). Blood samples were collected and subsequently deposited in heparinized vials. The haematological parameters were analyzed as reported [29].

Data analysis

The results were presented as means ± standard error mean (SEM). A significant difference was established at $p < 0.05$ using one-way analysis of variance (ANOVA), and the level of significance was tested using Duncan's Multiple Range Test.

RESULTS

Effect of Raw and Boiled Leaves of *A. digitata* on the Feed intake of Aluminium Chloride-induced Anaemic rats

Table 1 represents the effect of raw and boiled *A. digitata* leaves on the feed intake of aluminium chloride-induced anaemic rats. As the experiment proceeded, the meal consumption of the negative group dropped when compared to all other groups. The group fed with 40% boiled *A. digitata* fortified-feed demonstrated a substantial rise in feed consumption within day 0 to day 14 compared to the other treatment groups.

Effect of Raw and Boiled Leaves of *A. digitata* on the Body weight of Aluminium Chloride-induced Anaemic Rats

The result of the effect of raw and boiled leaves of *A. digitata* on the body weights of aluminum chloride-induced anaemic rats is shown in Table 2. The result showed that there was a steady decline in the body weight of rats in the negative control group from beginning of the experiment to the end on a daily basis. The group fed with 40% of raw *A. digitata* leaf-fortified feed showed a more significant increase in body weight as compared to negative group.

3.3

Effect of Raw and Boiled Leaves of *A. digitata* on the Haematological Parameters of Aluminum Chloride-induced Anaemic Rats

The effects of raw and boiled *A. digitata* leaves on haematological parameters in aluminum chloride-induced anaemic rats are presented in Table 3. The result revealed that haematological parameters of the group fed with the raw and boiled leaves of *A. digitata* improved significantly. The haemoglobin concentration (HB), packed cell volume (PCV) and neutrophils were observed to be highest in the group fed with 40% bodyweight of the raw and boiled leave.

DISCUSSION

The effect of *A. digitata* on the overall feed intake in the treatment groups when compared to the negative (toxicant) groups showed an increase in the feed intake with the boiled leaves showing a more significant increase. This may perhaps be because the boiled leaves of *A. digitata* did not have negative effects on the appetite of the animals rather it had the ability to stimulate hunger and thus improving their appetite.

A significant decrease in the weight for the rats in the negative group, (anaemic but not treated) might be as a result of the reduction in food intake. The groups fed with the feed fortified with processed leaves of *A. digitata* at 20 and 40 % which showed an increase in their average weight correlates with the increase in the feed intake. This is in concurrence with the study performed by Ebaid *et al*. [30] which indicates that oral administration of *A. digitata* leaf extract improved the bodyweight in diabetic rats signifying an enhancement in their urge for food intake.

Neutrophil(N), Hemoglobin (HB), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), Packed Cell Volume (PCV), Red Blood Cells (RBC), Mean Corpuscular Hemoglobin Concentration (MCHC), and Total white blood cells (TWBC) are all markers in judging haematological deficiencies in living organisms. In this present study, it was noted that administration of aluminum chloride

caused a significant reduction in the concentrations of HB, PCV, RBC, MCHC, MCH, MCV, and neutrophils in the anaemic rats when compared to the groups that were administered processed leaves. This possibly will be that aluminum chloride produces peroxidative variations in erythrocyte membrane leading to haemolysis. For this reason, the decreased RBCs count in the animal models having anaemia due to intoxication with aluminum chloride might be due to the consequences of both the haemolytic action of aluminum chloride as well as erythrocyte survival time shortening as observed [31].

Administration of air-dried and boiled processed leaves of *A. digitata* significantly ($p < 0.05$) reversed the aluminum chloride-induced anaemic distress in the rats by increasing the haematological parameters in a dose dependent manner. *A. digitata* might be capable of aiding the biosynthesis of erythrocytes and haemoglobin via stimulation of hormone erythropoietin; responsible for erythrocyte synthesis in the bone marrow. One more reason which was supported by Kerharo and Adam [32] is that *A. digitata* has high content of iron; amineral element required for the production of haemoglobin and RBCs. Specifically, significant increase was observed in the levels of HB, PCV, MCV, MCHC, TWBC, RBC and neutrophils. This is comparable to the result obtained previously [33], after ethanol extract of *Moringa oleifera* leaves were administered to aluminum-induced anaemic rats.

The increase in RBC and haemoglobin by the processed leaves indicates the amelioratory effect of the leaves. It has been reported that rats with anaemia resulting from aluminum chloride toxicity often have decreased reticulocytes counts, MCVand MCHC [32]. The pulp, leaves, and seed of *A. digitata* have been shown to contain vitamins and Fe in substantial amount [34] and this might be accountable for the increase in the levels of RBC and HB. Although both processing methods (air-drying and boiling) improved the RBC, PCV and HB, the boiled leaves seem better at the highest fortification of 40% used in this study and this could be ascribed to the fact that boiling might have improved the nutritional composition of the leaves especially the mineral content through its extraction by heat, thus improving PCV, Hb and RBC as observed.

White blood cells count elevation could be consequential in stimulating the immune system of animals and may be due to a combination of non-specific immune and protective response to metal stress [35]. The stabilization or increase in WBC count suggests that the leaves exhibited the potential of treating inflammation caused by aluminum chloride-induced anaemia.

Taking the above into account, the current study has shown that aluminum chloride-induced anaemia can be reversed by supplementation with *A. digitata* leaves.

CONCLUSION

According to the findings, raw and boiled leaves of *A. digitata* showed a significant amelioratory impact on $AlCl_3$-induced variations in haematological parameters, signifying that these raw and boiled leaves could be used in the management of anaemia.

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Table 1: Effect of Raw and Boiled Leaves of *A. digitata* on the Feed Intake of Aluminum Chloride-induced Anaemic rats

Time (days)	Grouping						
	Normal	Negative (Anaemic)	Standard	ADA 20%	ADA 40%	ADB 20%	ADB 40%
1	54.92±2.12^{c}	40.32±1.08^{b}	45.84±1.60^{b}	28.83±0.98^{a}	35.97±1.21^{a}	30.64±1.04^{a}	29.99±0.90^{a}
2	60.55±2.56^{cd}	44.76±1.34^{b}	48.93±1.67^{b}	26.94±0.87^{a}	30.34±1.02^{a}	32.87±1.22^{a}	30.54±1.00^{a}
3	65.45±2.66^{d}	49.56±1.56^{c}	47.56±1.64^{b}	32.95±1.07^{a}	45.12±1.42^{c}	38.56±1.08^{b}	43.76±1.42^{bc}
4	60.22±2.56^{cd}	50.23±1.98^{c}	45.95±1.08^{b}	30.51±1.03^{a}	48.22±1.57^{c}	35.87±1.03^{a}	46.90±1.53^{c}
5	63.98±2.65^{cd}	38.54±0.98^{b}	42.19±1.02^{b}	35.48±1.22^{a}	43.12±1.43^{bc}	43.87±1.44^{b}	42.67±1.38^{b}
6	61.64±2.48^{c}	34.66±0.92^{b}	47.64±1.34^{c}	39.22±1.28^{b}	40.65±1.31^{b}	40.21±1.32^{b}	48.09±1.58^{c}
7	61.64±2.48^{c}	34.66±0.22^{b}	47.64±1.34^{c}	39.22±1.28^{b}	40.65±1.32^{b}	40.21±1.32^{b}	48.09±1.76^{c}
8	63.53±2.65^{c}	32.77±0.89^{b}	45.75±1.26^{b}	40.39±1.32^{b}	41.92±1.34^{b}	42.08±1.33^{b}	48.41±1.78^{c}
9	62.66±2.16^{c}	30.98±0.94^{b}	47.64±1.34^{c}	41.37±1.41^{b}	42.9±1.42^{b}	43.06±1.45^{b}	49.39±1.80^{c}
10	60.77±2.08^{c}	29.09±0.86^{a}	46.52±1.28^{c}	42.35±1.43^{b}	43.66±1.45^{b}	44.04±1.56^{bc}	50.50±2.00^{c}
11	61.78±2.22^{cd}	27.50±0.84^{a}	44.63±1.18^{b}	43.44±1.44^{b}	44.64±1.54^{bc}	45.02±1.62^{c}	51.48±2.11^{c}
12	63.70±2.63^{c}	25.610±0.78^{a}	46.73±1.32^{c}	44.34±1.46^{bc}	45.62±1.50^{c}	46.00±1.67^{c}	52.46±2.34^{c}
13	61.81±2.22^{c}	24.42±0.74^{a}	44.84±1.11^{b}	45.32±1.49^{c}	46.60±1.52^{c}	47.20±1.68^{c}	53.32±2.43^{cd}
14	63.80±2.87^{cd}	22.53±0.81^{a}	46.81±1.21^{c}	46.30±1.51^{c}	47.58±1.58^{c}	48.18±1.72^{c}	54.30±2.50^{d}

Values are presented in Mean ± Standard Error Mean. Values with different superscripts across the rows are significantly different at $p<0.05$.

Key:

ADA 20% = 20% Raw *A. digitata* fortified-feed ADA 40% = 40% Raw *A. digitata* fortified-feed

ADB 20% = 20% Boiled *A. digitata* fortified-feed ADB 40% = 40% Boiled *A. digitata* fortified-feed

Table 2: Effects of Raw and boiled leaves of *A. digitata* on the body weights of aluminum chloride-induced anaemic rats

Grouping							
Time (days)	**Normal**	**Negative (Anaemic)**	**Standard**	**ADA 20%**	**ADA 40%**	**ADB 20%**	**ADB 40%**
0	118.47± 0.99^{a}	123.58±2.27^{c}	121.28±3.62^{a}	122.57±1.73^{b}	123.82±2.064^{b}	122.26± 3.04^{c}	121.39± 2.51^{b}
3	122.10±1.46^{a}	115.14±2.15^{b}	118.09±4.52^{a}	115.62± 1.65^{a}	119.50±1.09^{a}	116.57±3.63^{a}	116.57±2.08^{a}
6	125.91± 1.51^{a}	111.03±3.70^{b}	120.83±4.17^{a}	118.16±1.59^{ab}	123.24±0.92^{b}	117.61±3.57^{a}	117.61±3.57^{a}
9	130.35±1.58^{b}	107.04±3.42^{b}	123.88±4.39^{b}	121.03±1.95^{ab}	126.23±0.88^{b}	119.12±3.73^{b}	119.12±3.73^{a}
12	134.08±0.99^{c}	102.13±3.76^{a}	126.81±5.01^{b}	123.23±1.95^{b}	128.90±0.78^{b}	120.26±3.53^{b}	120.26±3.53^{b}
15	127.99±1.63^{b}	96.44±3.17^{a}	129.92±3.83^{c}	125.32±1.31^{c}	133.44±0.68^{c}	124.46±3.53^{c}	130.34±4.47^{c}

Values are presented in Mean± Standard Error Mean. Values with different superscripts across the rows are significant different at $p<0.05$.

Key:

ADA 20% = 20% Raw *A. digitata* fortified-feed
ADA 40% = 40% Raw *A. digitata* fortified-feed
ADB 20% = 20% Boiled *A. digitata* fortified-feed
ADB 40% = 40% Boiled *A. digitata* fortified-feed

Table 3: Effect of Raw and Boiled Leaves of *A. digitata* on the Haematological Parameters of Aluminum Chloride-induced Anaemic rats

Haematological Parameters	Normal	Negative (Anaemic)	Standard	ADB 20%	ADB 40%	ADA 20%	ADA 40%
Hb (g/dl)	13.72±1.02^{c}	6.13±0.19^{a}	13.26±0.47^{c}	9.17±0.63^{a}	11.36±0.47^{b}	11.13±0.56^{b}	12.15±0.25^{b}
PCV (%)	42.50±2.50^{c}	35.50±1.50^{b}	39.50± 0.50^{c}	34.00± 1.00^{a}	40.50±0.50^{c}	37.00± 1.00^{b}	41.00± 1.00^{c}
MCV(g/dl)	43.50±1.50^{c}	37.50±1.50^{b}	44.50± 1.50^{c}	40.00±2.00^{b}	41.50±1.50^{b}	41.00± 1.00^{b}	39.50± 0.50^{b}
MCH10^{12}/L	17.00±1.00^{d}	13.00±1.00^{b}	17.00±1.00^{d}	11.00± 2.00^{a}	14.50±1.50^{c}	13.00± 100^{b}	17.50± 1.50^{d}
MCHC10^{6}/L	46.00±2.00^{d}	35.50±1.50^{a}	41.00±1.00^{c}	35.50± 1.50^{a}	38.00±1.00^{b}	39.00± 1.00^{b}	36.50± 2.50^{b}
RBC10^{12}/L	7.00±0.20^{b}	4.15±0.65^{a}	8.05±0.25^{c}	5.85± 0.45^{a}	6.35±0.45^{b}	5.95± 0.75^{a}	7.20± 0.30^{b}
PLC10^{6}/L	155.00±1.00^{d}	131.00±3.00^{b}	135.00±3.00^{c}	129.00± 3.00^{b}	139.50±2.50^{c}	126.50±2.50^{b}	120.00± 3.00^{a}
TWBC10^{12}/L	6.05±0.35^{b}	6.30± 0.40^{b}	6.55±0.35^{b}	7.25± 0.35^{c}	5.95±0.55^{a}	8.35±0.55^{d}	5.35± 0.55^{a}
N%	36.50±2.50^{b}	33.00±0.55^{a}	35.50±1.50^{b}	33.50± 1.50^{a}	41.00±2.00^{c}	37.50± 1.50^{b}	44.00± 2.00^{d}
E, B%	33.00±3.00^{b}	30.00±1.00^{c}	32.00±2.00^{b}	30.50± 2.50^{a}	26.50± 1.50^{a}	34.50± 1.00^{a}	32.50± 2.50^{b}

Values are presented in Mean ± Standard Error Mean. Values with different superscripts across the rows are significant different at $p < 0.05$

Key:

ADA 20% = 20% Raw *A. digitata* fortified-feed
ADA 40% = 40% Raw *A. digitata* fortified-feed
ADB 20% = 20% Boiled *A. digitata* fortified-feed
ADB 40% = 40% Boiled *A. digitata* fortified-feed