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Effects of *Anacardium occidentale* leaves on haematological and serum biochemical parameters in experimentally induced Haemorrhagic anaemia of Wistar rats

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Abstract

In this study the haematological benefits of crude ethanol extracts of leaves of *Anacardium occidentale* were evaluated on experimentally induced hemorrhagic anaemia in Wistar rats. Twenty-five (25) adult female rats were randomly shared into five groups of five rats each. Group A: normal saline at 2 ml/kg (normal control), Group B: untreated anemic rats (negative control), Group C: anaemic rats, 2 ml/kg of Ranferon®, Group D: anaemic rats, 1000 mg/kg plant extracts and Group E: anaemic rats, combination of 1000 mg/kg plant extracts and 2 ml/kg of Ranferon® orally for 14 days. Preliminary phytochemical screening revealed Phenols (5.33%), while flavonoids, cardiac glycosides, steroids, saponins, alkaloids, tannins and terpenoids were present. Haematological and serum biochemical analysis by day 3 and 14 showed significant increases ($p < 0.05$) in packed cell volume ($37.20 \pm 0.7\%$), haemoglobin concentration ($13.04 \pm 0.5\text{g/dl}$), and red blood cell counts ($6.50 \pm 0.1 \times 10^{12}/\text{L}$) Group C, Group D and Group E when compared to the control group while the white blood cell counts ($5.36 \pm 0.2 \times 10^9/\text{L}$) and neutrophils ($1.75 \pm 0.1 \times 10^9/\text{L}$), decreased significantly ($p < 0.05$) across all groups when compared to the normal control. The treatments showed no adverse effects on the liver and kidneys.

Keywords: Haemorrhagic Anaemia; *Anacardium occidentale*; Haematology; Serum Biochemistry

1. Introduction

Anaemia is a condition characterized by a decrease in red blood cells or hemoglobin, leading to inadequate oxygen delivery to tissues [1]. In animals, anaemia may produce clinical signs such as shock if a third of the blood volume is rapidly lost and even death if not replaced. Rapid blood loss could result in signs like increased heart rates, pale gums and low blood pressure. Animals are jaundiced if red blood cells are being destroyed. In chronic cases, the signs develop slower and may include: pale gums, lethargy and loss of appetite [2].

Anaemia is a public health concern affecting millions of people worldwide, particularly in developing countries. The World Health Organization [3] projected that almost two billion people or 25% of the world's population is anemic, with roughly half of them having iron deficiency anaemia [4]. The incidence of anaemia is higher in the third world countries than in developed countries due to the presence of many aggravating factors such as poor nutrition, high prevalence of blood parasites for example, plasmodium, trypanosomes and helminthes infestation ([3, 5].

In animals, causes of anaemia include: parasitic diseases, non-infectious diseases, bacterial and viral causes, which correlated with season, age, breed and sex of dogs [6].

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Anaemia may occur in the following forms: Haemolytic anaemia (red blood cell destruction), aplastic anaemia (bone marrow failure) and haemorrhagic anaemia (blood loss anaemia) [7]. Haemorrhagic anaemia is a severe and potentially life-threatening condition characterized by fever, bleeding, and shock [3]. Haemorrhage can be caused by trauma, parasites and medications such as NSAIDs that can increase bleeding risks, especially in the gastrointestinal tracts [7]. Haemorrhagic anaemia may present in the following forms: acute (2-3 days), characterized by consistent decrease in PCV, inconsistent decrease in platelets and plasma protein and lack of regenerative response, subchronic (3-10 days), characterized by regenerative response, inconsistent increase and decrease of platelets and plasma protein. In chronic (10 days) haemorrhagic anaemia, there may be regenerative, non-regenerative or iron deficient anaemia [8].

Anaemia treatment depends on the underlying cause [9]. Addressing underlying conditions like infections and chronic diseases is also crucial. Furthermore, public health initiatives like education and awareness campaigns can help reduce the prevalence of anaemia [10]. Strategies for management of anaemia may include dietary changes and nutritional supplementation programs such as iron, folic acid, and vitamin B12 [9, 11]. Medications such as Epoetin alfa and blood transfusions may also be necessary [9, 10]. In severe cases, hospitalization may be required to manage symptoms and prevent complications [12]. More recent treatments may include gene therapy for sickle cell anaemia and beta-thalassaemias and iron chelators [9].

Traditional medicine has long utilized plant-based remedies to treat anaemia, with *Anacardium occidentale* being one such plant [13].



Figure 1 Picture of *Anacardium occidentale*

Anacardium occidentale, also known as cashew belongs to the family Anacardiaceae, characterized by tropical and subtropical trees and shrubs. There are more than sixty genera and four hundred species related to the genus of *Anacardium* [14]. The common names of *Anacardium occidentale* include: Cashew in English, “Kashu” in Igbo, “Yazawa” in Hausa, “Kaju” in Yoruba, “Shase” in Tiv, “Angala” in Agatu, “Ikachwu” in Igede [15].

The plant is native to West Africa and has been traditionally used in folk medicine to treat various ailments, including anaemia [16]. The plant's stem bark extract has been shown to increase packed cell volume and reduce inflammation in mice [17]. The plant contains various bioactive compounds, such as flavonoids, phenolic acids, and terpenoids, which have been shown to have antioxidant, anti-inflammatory, haematopoietic, antidiabetic and antihypertensive properties, [18, 19, 20].

Anacardium occidentale as a medicinal plant has been studied but there is insufficient information whether the leaves possess haematinic effects that can ameliorate the effects of haemorrhagic anaemia of any cause in the body system. The effect of the leaves of this plant on haemorrhagic anaemia needs to be assessed in other to see if this plant can be used as an alternative in ameliorating the effects of haemorrhaging of red blood cells in certain disease conditions that result in haemorrhagic anaemia. This would offer a cheaper and more readily available alternative treatment in third world countries such as Nigeria. The study was conducted to evaluate the hematological benefits of crude ethanol extracts of leaves of *Anacardium occidentale* in experimentally induced haemorrhagic anaemia in Wistar rats.

2. Materials and methods

2.1. Plant Collection and Preparation

Anacardium occidentale leaves and stalks were collected in the month of June 2024 from Behind the Veterinary Teaching Hospital, North Bank, Makurdi, Nigeria. The plant was authenticated in the Herbarium of the Department of Forestry of Joseph Sarwuan Tarka University, Makurdi, where a voucher number UAM/FH/0563 was assigned to the plant and stored. The leaves were separated from their stalk, air-dried at room temperature for two weeks, then pulverized to powder form after drying using a mortar and pestle.

2.2. Preparation of crude ethanol extract of the *Anacardium occidentale*

The leaf of *Anacardium occidentale* was extracted by cold maceration method [21]. The powder materials were weighed and then mixed with ethanol in a ratio of 1:5 w/v in an Erlenmeyer flask for 24 h. The mixture was filtered and the filtrate concentrated in vacuo at 50 °C in a rotatory evaporator coupled to a thermocirculator. The resultant extract was weighed and reconstituted with distilled water to obtain a 1% stock solution.

2.3. Preliminary Phytochemical Screening

2.3.1. Qualitative Phytochemical Screening of Crude Ethanol Extract of *Anacardium occidentale*

Standard protocols [22, 23] were used in detecting the presence of different phytochemical constituents in the crude ethanol extract.

2.4. Quantitative Phytochemical Screening of Crude Ethanol Extract of *Anogeissus leiocarpus*

The percentage yield of alkaloids, flavonoids, saponins, tannins and phenols in the crude extract were determined using standard procedures as described by [24, 25, 26, 27] respectively.

2.5. Animals used

Twenty-five (25) adult female rats between 100-120g were obtained from the Veterinary Teaching Annex, North Bank, Makurdi. They were grouped into five groups (5) each group containing five (5) rats. Each group were housed in cages and kept in the Veterinary Teaching Hospital Annex, Joseph Sarwuan Tarka University, Makurdi. They were fed with chicken grower's mash and given water *ad libitum*. The rats were acclimatized to the environment for 3 weeks before commencement of the experiments

2.6. Induction of Anaemia

Haemorrhagic anaemia was induced in the rats by withdrawing 2 mls of blood from the tail vein of the rats twice a week for 2 weeks, and packed cell volume (PCV) was tested to confirm the presence of anaemia [28].

2.6.1. Experimental Design Animal Groups Mode of Administration

- The rats were weighed and assigned randomly into 5 groups of n=5 as follows:
- Group A: Normal saline at 2 ml/kg orally and served as normal control.
- Group B: Anaemic rats were left untreated and served as negative control

- Group C: Anaemic rats were treated with 2 ml/kg of Ranferon® orally for 14days
- Group D: Anaemic rats were treated with 1000 mg/kg plant extracts was administered orally for 14 days
- Group E: Anaemic rats were treated with a combination of 1000 mg/kg plant extracts and with 2 ml/kg of Ranferon® orally for 14days

The crude ethanol extracts was reconstituted by dissolving 1 g of extract in 2.5 ml distilled water to obtain a concentration of 400 mg/ml. All administrations were given at 9:00 am daily for fourteen days after confirmation of anaemia.

2.7. Sample Collection

At day 3 and 14, about 1 ml of blood sample was collected via the canthus with the aid of capillary tube into EDTA tube for haematological analysis and 2 ml of blood was discharged into a sterile anticoagulant free bottle for serum extraction for serum biochemical analysis.

2.8. Hematological and Serum Biochemical Studies:

Haematocrit values, erythrocyte, total and differential leucocyte counts were determined using the methods described by Weiss and Wardrop [29].

Determination of serum biochemical changes Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), proteins and creatinine levels were determined using the methods described by Davidson *et al.* [30]

3. Results and Discussion

3.1. Results for preliminary phytochemical screening of leaf of *Anacardium occidentale*

The results of the preliminary phytochemical screening on the powdered leaf of *Anacardium occidentale* (Table 1), revealed the presence of phytochemicals. Phenols were observed to be the highest phytochemical components in the leaf of the plant (5.33%), followed by flavonoids (4.56%), and cardiac glycosides (4.39 %). Steroids were reported to be 2.78 % while saponins were (2.31 %). Alkaloids (1.9 %), tannins (1.5 %) and terpenoids (0.59 %) were also detected in the leaf extracts of *Anacardium occidentale*.

Table 1 Preliminary phytochemical screening of ethanol leaf extract of *Anacardium occidentale*

Phytochemical components	Quantitative	Qualitative%
Flavonoids	+++	4.36
Tannins	+	1.50
Alkaloids	+	1.90
Saponins	+	2.31
Cardiac glycosides	++	4.39
Terpenoids	+	0.59
Phenolics	+++	5.33
Steroids	+	2.78

Key: + = present

3.2. Effect of administration of ethanol leaf extract of *Anacardium occidentale* on haematological parameters 3 days post induction of haemorrhagic anaemia

The effect of administration of ethanol leaf extract of *Anacardium occidentale* on haematological parameters 3 days post induction of haemorrhagic anaemia are shown in Table 2. The results showed a significant decrease in the packed cell volume (PCV), haemoglobin concentration (Hb), and red blood cell counts (RBC) in the untreated anaemic rats (Group B) when compared to normal control (Group A) and all the other treated groups. However, there was a significant increase ($p < 0.05$) in Ranferon® treated group (Group C), extract treated group (Group D) and combination of Ranferon®

and extract treated group (Group E) when compared to the control group after 3 days of treatment. There was a significant increase ($p < 0.05$) in mean cell volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) in group B while no significant differences were observed with group A, C, D and E. The white blood cell counts (WBC) and neutrophils, decreased with significant ($p < 0.05$) difference, across all groups when compared to the normal control. Lymphocytes also decreased with significant ($p < 0.05$) difference, across all groups except in group D where there was no significant difference ($p > 0.05$) when compared to the normal control.

Table 2 Effect of administration of ethanol leaf extract of *Anacardium occidentale* on haematological parameters 3 days post induction of haemorrhagic anaemia

Parameters/Units	A Normal control	B Negative control	C 2ml/kg ranferon	D 1000mg/kg extract	E 1000mg/kg Extract + 2ml/kg Ranferon
PCV (%)	31.25 ± 0.7	20.60 ± 0.7	31.60 ± 0.7	31.80 ± 0.7	37.20 ± 0.7 ^a
Hb(g/dL)	10.40 ± 0.5	8.20 ± 0.5	10.5 ± 0.5	10.60 ± 0.5	13.04 ± 0.5 ^a
RBC($\times 10^{12}$ /L)	5.20 ± 0.1	3.10 ± 0.1	5.30 ± 0.1	5.40 ± 0.1	6.50 ± 0.1
MCV (fL)	60.00 ± 1.0	66.45 ± 0.4	59.00 ± 1.0	58.00 ± 1.0	60.00 ± 1.0
MCH(Pg)	19.00 ± 0.45	21.58 ± 0.5	19 ± 0.5	19.00 ± 0.5	20.00 ± 0.5
MCHC(g/l)	33.30 ± 0.4	39.81 ± 0.4 ^a	33.3 ± 0.4	33.30 ± 0.4	33.20 ± 0.4
WBC($\times 10^9$ /L)	6.55 ± 0.2	4.60 ± 0.2	5.3 ± 0.2	6.06 ± 0.2	5.36 ± 0.2
Neutrophils($\times 10^9$ /L)	2.44 ± 0.1	1.32 ± 0.1	1.60 ± 0.1	1.89 ± 0.1	1.75 ± 0.1
Lymphocytes($\times 10^9$ /L)	3.19 ± 0.1	3.18 ± 0.1	3.59 ± 0.1	4.01 ± 0.1	3.43 ± 0.1
Monocytes($\times 10^9$ /L)	0 ± 0.05	0.02 ± 0.01	0.02 ± 0.05	0.01 ± 0.05	0.01 ± 0.05
Eosinophils($\times 10^9$ /L)	0.06 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.15 ± 0.01	0.12 ± 0.01

KEY: PCV: Packed cell volume, Hb: Haemoglobin, RBC: Red blood cell, MCV: Mean Cell Volume MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration WBC: White Blood Cell a: mean values are significantly different when compared to control $P < 0.05$

3.3. Effect of administration of ethanol leaf extract of *Anacardium occidentale* on haematological parameters 14 days post induction of haemorrhagic anaemia

The effect of administration of ethanol leaf extract of *Anacardium occidentale* on haematological parameters 14 days post induction of haemorrhagic anaemia are shown in table 3. The rats in anaemic untreated group had all died by day 14 of the experiment. The PCV and Hb in groups C and E was significantly increased ($p < 0.05$) when compared to the normal control group, while there was no significant difference ($p > 0.05$) in PCV between group D and normal control. Haemoglobin decreased significantly ($p > 0.05$) in group D when compared to normal control. There was no significant difference in red cell counts between all the treatment groups when compared to the normal control. The MCV in extract treated group (D) indicated a significant increase ($p > 0.05$) when compared to the normal group. MCH across treated group showed no significant difference ($p > 0.05$) when compared to the normal control. The MCHC also significantly decreased in group C and E when compared to the normal control. White blood cells (WBC) significantly decreased ($p < 0.05$), in group D and E when compared with normal control while there was no significant difference between group D when compared with the normal control. Neutrophils increased with significant difference ($p < 0.05$) across treated groups when compared with the normal control. Lymphocytes showed no significant difference ($p > 0.05$) across treated groups when compared with the normal control. Monocyte in group C and D increased with significant difference ($p < 0.05$) but Monocyte in group E showed no significant difference ($p > 0.05$) when compared with the normal control. Eosinophil in group C showed no significant difference ($p > 0.05$), Eosinophil group D and E increased with significant difference ($p < 0.05$) when compared to the normal control. Basophil showed no significant difference across treated groups when compared to the normal control.

Table 3 Effect of administration of ethanol leaf extract of *Anacardium occidentale* on haematological parameters 14 days post induction of haemorrhagic anaemia

Parameters	A Normal control	B Negative control	C 2ml/kg ranferon	D 1000mg/kg extract	E 1000mg/kg Extract + 2ml/kg Ranferon
PCV (%)	38.25 ± 0.5	-	39.2 ± 0.5	38.80 ± 0.5	41.80 ± 0.5 ^a
Hb(g/dL)	12.73 ± 0.55	-	13.04 ± 0.55	11.56 ± 0.55	13.9 ± 0.55 ^a
RBC($\times 10^{12}$ /L)	6.33 ± 0.25	-	6.5 ± 0.25	5.76 ± 0.25	6.92 ± 0.25
MCV (fL)	60.42 ± 0.20	-	60.30 ± 0.20	67.36 ± 0.20	60.40 ± 0.20
MCH(Pg)	20.11 ± 0.20	-	20.06 ± 0.20	20.07 ± 0.20	20.09 ± 0.20
MCHC(g/l)	33.28 ± 0.75	-	33.27 ± 0.75	29.79 ± 0.75	31.10 ± 0.75
WBC($\times 10^9$ /L)	7.00 ± 0.10	-	6.74 ± 0.1	7.00 ± 0.1	6.8 ± 0.1
Neutrophils($\times 10^9$ /L)	1.96 ± 0.05	-	2.27 ± 0.05	2.39 ± 0.05	2.16 ± 0.05
Lymphocytes($\times 10^9$ /L)	4.89 ± 0.08	-	4.33 ± 0.08	4.71 ± 0.08	4.44 ± 0.08
Monocytes($\times 10^9$ /L)	0 ± 0.01	-	0.01 ± 0.01	0.02 ± 0.01	0 ± 0.01
Eosinophils($\times 10^9$ /L)	0.15 ± 0.01	-	0.11 ± 0.01	0.21 ± 0.01	0.20 ± 0.01

KEY: Values are \pm SEM of 4 replicates determinations. a: mean values are significantly different when compared to control P<0.05

3.4. Effect of Administration of Ethanol Leaf Extract of *Anacardium occidentale* on serum biochemical Parameters

After 14 days of treatments, there was a significant decrease ($p < 0.05$) in ALT across all the treatment groups when compared to the normal control. There was also a significant decrease ($p < 0.05$) in AST in group C and D when compared to the normal control group but in group E there was a significant increase in AST. Total protein and albumin increased with significant difference ($P < 0.05$) across treated groups when compared with normal control. Creatinine levels in group C increased with significant difference ($p < 0.05$), while creatinine in group D showed no significant difference ($p > 0.05$) and Creatinine in group E decreased with significant difference ($p < 0.05$) when compared to the normal control.

Table 4 Effect of administration of ethanol leaf extract of *Anacardium occidentale* n serum biochemistry 14 days' post induction of haemorrhagic anaemia

Parameters	A Normal control	B Negative control	C 2ml/kg Ranferon®	D 1000mg/kg extract	E 1000mg/kg Extract + 2ml/kg Ranferon®
ALT(μ /L)	9.60 ± 0.3	-	7.20 ± 0.3	7.60 ± 0.3	8.00 ± 0.3
AST(μ /L)	17.80 ± 0.3	-	13.40 ± 0.3	16.20 ± 0.3	19.50 ± 0.3
Total Protein(g/L)	51.60 ± 0.3	-	67.00 ± 0.3	72.80 ± 0.3	67.50 ± 0.3
Albumin(g/L)	30.20 ± 0.3	-	40.20 ± 0.3	41.00 ± 0.3	46.00 ± 0.3
Creatinine(mg/dL)	1.568 ± 0.3	-	2.25 ± 0.3	1.53 ± 0.3	1.35 ± 0.3
Urea (mg/dl)	0.50 ± 0.1	-	1.00 ± 0.3	1.20 ± 0.6	0.70 ± 0.2

KEY: ALT = Alanine amino transferase, AST = Aspartate amino transferase

4. Discussion

Preliminary phytochemical screening of the crude ethanol extracts of leaves of *Anacardium occidentale* showed that it had high amounts of Phenols, flavonoids and cardiac glycosides. Phenolic compounds gained massive attention due to their functional and therapeutic potential showing antioxidant, anti-cancerous and radio-protective activities [31, 32, and 33]. Flavonoids on the other hand, have been shown to possess antioxidant properties, reduce the prevalence of cardiovascular diseases and protect red blood cell haemolysis through antioxidant effects and their impacts on iron

metabolism [34, 35]. Flavonoids compete with haemoglobin for oxygen, thereby resulting in hypoxia which in turn stimulates the release of Erythropoietin [36]. They have also been shown to improve the levels of red blood cells, white blood cells, hemoglobin, and hematocrit [37, 38]. Cardiac glycosides may increase haemoglobin concentration and improve red blood cell characteristics [39]. These bioactive constituents maybe responsible for the observed haematinic effect of the plant. Thus, the increase in the haematological indices observed following treatment with *Anacardium occidentale* leaf extract might be associated with the phytochemicals present in the leaves of this plant.

In the current study, it was observed that by the third day of administration of the crude ethanol leaf extract of *Anacardium occidentale*, the haemogram showed an improvement in the group treated with the leaf extract when compared to the control group. By the 14th day of administration, all the rats in the untreated groups had died so comparisons were made between the normal control group and the treatment groups. The haematinic effect of *Anacardium occidentale* and the standard drug, in this study, were comparable while the combination of showed greatly improved haematinic effect. So many medicinal plants have been reported to possess anti-anaemic properties [39]. Some of these plants include: *Phyllanthus emblica*, *Moringa oleifera*, *Beta vulgaris*, *Amaranthus* species and *Cajanus cajan* [40]. Others that have been studied include: *Jatropha tanjorensis*, *Telfairia occidentalis*, *Carica papaya* and *Aloe vera* [41]. Most of these plants have been shown to either have phyto-nutrient rich components which are essential for the formation of red blood cells and or possess phytochemicals which serve are antioxidants that protect the red cells from reactive oxygen species [40, 41]

In the current study, Serum enzymes in treatment groups showed no significant increase when compared to the control group as well as comparison between treated groups these. Donsogo *et al.*, [42] and Kailani,[43] reported that some of the phytochemical components of plants had hepato-protective abilities and also protected the kidneys through their antioxidant activities. Such phytochemicals have also been reported in this plant. A significant increase in total proteins was observed the treated groups. These could be attributed to the increased protein synthesis as the body is trying to increase augment the protein lost to frank blood loss.

5. Conclusion

It is thus concluded that *Anacardium occidentale* has an efficacious stimulation of erythropoiesis hence the haematinic effect of the plant observed in experimentally induced hemorrhagic anaemia in Wistar rats. The study shows that the extract produced similar haematological responses to treatment of anaemia with the standard drug. However, the plant extract in combination with a standard anti-anaemic drug showed a higher haematinic response in anaemic rats. The plant also showed no adverse effect on the liver, and kidneys and so it can be considered safe and a cheaper more available option in cases of haemorrhagic anaemia.

Anacardium occidentale is recommended as a good candidate for further research on specific anaemias and specific hepato-protective and kidney health potentials. It is of utmost importance that the efficacy of *Anacardium occidentale* is further analyzed for the treatment of anaemia by standardizing and evaluating the use of its active plant-derived compounds.

Compliance with ethical standards

All procedures carried out on animals in this study were approved and performed in accordance with the ethical standards of the Ethical committee of the Joseph Sarwuan Tarka University Makurdi.

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Disclosure of conflict of interest

The authors declare no conflict of interests

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