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Effects of Aqueous Wood Extracts of *Azadirachta Indica and Parkia Biglobosa* on the Haematological Profile of *Mus Musculus*

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ABSTRACT

Traditional ethnomedicine often utilizes non-timber plant derivatives like wood ash, yet their long-term toxicological boundaries remain largely uncharacterized. This study evaluated the subchronic (90-day) and chronic (180-day) hematological impacts of aqueous wood-ash extracts from *Azadirachta indica* and *Parkia biglobosa* using a murine model. Male Swiss albino mice (*Mus musculus*) were randomly assigned to seven groups (n = 10/group) and orally administered distilled water (control) and varying concentrations (5, 50, and 100 mg/kg body weight) of *A. indica* and *P. biglobosa* extract daily. Comprehensive hematological profiles were analyzed via retro-orbital phlebotomy at days 90 and 180. Subchronic exposure to both extracts significantly ($P < 0.05$) increased packed cell volume (PCV) and hemoglobin (HGB) at 100 mg/kg, accompanied by localized leukocyte alterations. Particularly, chronic 180-day exposure induced a pronounced, dose-dependent polycythemic effect across all treatment cohorts, marked by marked elevations in red blood cell (RBC) counts, PCV, and HGB, alongside shifted erythrocyte indices (MCV, MCH, MCHC). Conversely, chronic administration of both extracts caused significant, severe thrombocytopenia ($P < 0.05$). Furthermore, *P. biglobosa* induced systemic, persistent leukopenia ($3.40\pm0.06 \times 10^3$ / µL vs. $4.53\pm0.15 \times 10^3$ / µL in controls), whereas high-dose *A. indica* stimulated leukopoiesis. Although both wood-ash extracts possess potent erythropoietic-stimulating activities likely mediated by antioxidant phytocompounds, their chronic administration triggers critical systemic hematotoxicity characterized by thrombocytopenia and line-specific leukocyte suppression. These findings establish strict safety thresholds and caution against unregulated, long-term therapeutic consumption of these ethnomedicinal preparations.

Keywords: *Azadirachta indica*, *Parkia biglobosa*, Wood-ash extract, Hematotoxicity, *Mus musculus*, Safety profile

INTRODUCTION

Plants extract have been used for centuries in the prevention and treatment of diseases especially in developing countries where they serve as an important component of primary health care (Sofowora *et al.,* 2013). The growing interest in herbal medicine has led to increased scientific investigations into safety and therapeutic efficacy of plant derived compounds (Balkrishna *et al.,* 2024) Although many medicinal plants possess pharmacological properties such as antimicrobial, anti- inflammatory, antioxidant and anti-parasitic activities (Nwozo *et al.,* 2023). However, questions remain about their safety and possible toxicological effects especially when consumed over a long period of time or at high doses (Mensah *et al.,* 2019). Therefore, it is important to evaluate their effects on physiological and biochemical parameters in order to ensure their safe use (Grumet *et al.,* 2020).

Azadirachta indica is rich in biologically active compounds such as azadirachtin, nimbin, quercetin and flavonoid which possess antimicrobial, antioxidant,antimalarial and immuno modulatory properties (Devi *et al.,* 2023). Similarly, *Parkia biglobosa* is traditionally utilized in the treatment of hypertension, diarrhoea, infections, inflammation and wounds due to the presence of phenolic compounds, tannins, saponins, alkaloids and flavonoids (Saleh *et al.,* 2021 and Azando, 2016). Despite their extensive traditional use and reported therapeutic benefits, the safety profiles of these plants have not been fully understood (Aliu *et al.,* 2025). Haematological parameters such as red blood cell, white blood cell count, haemoglobin concentration, pack cell volume, platelet count and differential leukocyte count serve as important indicators of the physiological and pathological status of an organism (Banga *et al.,* 2020). Alterations in these parameters may result to anaemia, immune modulation, inflammation, toxicity or bone marrow dysfunction (Sankar *et al.,* 2021).

Mus musculus also referred to the laboratory mouse is extensively used as an experimental model because of its physiological and genetic similarities to humans, making it suitable for toxicological and pharmacological investigations (Perlman, 2016).

Evaluating the haematological effects of aqueous wood extracts of *Azadirachta indica* and *Parkia biglobosa* in *Mus musculus* provides valuable information concerning the safety and potential biological effects of these medicinal plants. Therefore this study seeks to investigate the effects of aqueous wood extracts of *Azadirachta indica* and *Parkia biglobosa* on the haematological profile of *Mus musculus*.

MATERIALS AND METHODS

Collection and Processing of the Woods of *Azadirachta indica* and *Parkia biglobosa* to Ashes

Fresh wood of the stems of *A. indica* (Neem) and *P. biglobosa* (African locust bean) trees were collected from Kakau District, Chikun Local Government Area of Kaduna State, North-Western Nigeria. For proper identification, the stalk of

each plant (carrying leaves and flowers) were collected alongside the stem, and authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria; where voucher specimens *Azadirachta indica* (AI-90051) and *Parkia biglobosa* (PB-324) were deposited.

Preparation of Aqueous Wood-ash Extracts of *Azadirachta indica* and *Parkia biglobosa*

Aqueous wood-ash extracts of *A. indica* and *P. biglobosa* were prepared by percolation method, following standard procedures for hot aqueous extraction of botanicals (Ong *et al.*, 2006). One litre (1 L) of water was added to 5 kg of each wood-ash to increase the moisture content and heated on a hotplate for 2 hrs at 40^0C. The heated wood-ash was cooled to room temperature for 1 hr then transferred into a perforated bowl (underlayed with muslin cloth of 0.025 mm mesh size) and placed on a clean empty container (bowl) for collection of aqueous wood-ash extracts overnight. Aqueous wood-ash extracts were evaporated to dryness with a rotary evaporator (BUCHI Switzerland; Rotavapor R-210) in water bath at 40^0C. The extract residues were placed in a desiccator to remove remnant moisture. Extracts were stored airtight bottles and kept in the laboratory for further analyses and experiments.

Procurement and Acclimatization of *Mus musculus* for Sub-Chronic and Chronic Toxicity Tests

A total of 180 healthy adult *Mus musculus* were purchased from the animal house of Institute for Medical Advance Research and Training (IMRAT), College of Medicine, University of Ibadan, Oyo State for the sub-chronic and chronic toxicity studies. They were housed under standardized (temperature: 28 - 31 ºC, photoperiod: approximately 12 hours natural light per day) animal house conditions and fed with standard mice feed and water *ad libitum*. The animals were randomly selected and kept in their cages to acclimatise for 2 weeks before commencement of each experiment, according to OECD guidelines (2008).

Sub-Chronic Toxicity Test

A 90-day sub-chronic study followed OECD (1998) guidelines with a modification: groups of n = 10 per treatment (rather than n = 5) to increase statistical power given limited prior data. Seventy mice were randomized by weight (≤20% variation within groups) into seven groups (n = 10): control (distilled water); *A. indica* extract at 5, 50, and 100 mg/kg; *P. biglobosa* extract at 5, 50, and 100 mg/kg. Doses were administered orally once daily for 90 days. Animals were observed twice daily during the first week and daily thereafter for clinical signs (general activity, locomotion, feeding/drinking, grooming, posture, fur condition, and respiration, response to handling, tremors, convulsions, and mortality). Individual body weights were recorded prior to dosing and weekly. Surviving animals were weighed immediately before blood collection at termination (OECD, 1998).

Chronic Toxicity Test

A 180-day chronic toxicity study adhered to OECD (1998) guidelines. Seventy mice were randomized by weight (≤20% variation) into seven groups (n = 10): control (distilled water); *A. indica* extract at 5, 50, and 100 mg/kg; *P. biglobosa* extract at 5, 50, and 100 mg/kg. Doses were administered orally once daily for 180 days. Observation schedule, endpoints, and weekly weighing mirrored the sub-chronic protocol. Surviving animals were weighed before blood collection at study termination (OECD, 1998).

Collection of Blood of *Mus musculus*

At the end of the sub – chronic (90-day) and chronic (180-day) exposure periods, blood samples were collected into ethylenediamine tetra-acetic acid (EDTA) bottles by retro-orbital phlebotomy, using capillary tubes from individual mice.

Haematological Analysis

Erthrocytes and leucocyte counts were carried out according to the methods of Blaxhall and Daisley (1973), using the standard haemacytometer. Using the Dacie's dilution fluid (Dacie and Lewis, 1968), the blood sample was introduced into an improved Neubauer counting chamber and the cells were counted under the microscope (x 100). Standard methods (cyanmet-haemoglobin method) described for haemoglobin estimation by Blaxhall and Daisley (1973) were used. For determination of haematocrit percentages (PCV), freshly collected blood samples were drawn into microhaematocrit tubes (75mm long, 1.1-1.2mm internal diameter), which were sealed with plasticine (cristaseal) at one end. The blood in the tube was then centrifuged at 3000 rpm for 5 mins using a Hawskley micro-haematocrit centrifuge. Three readings were taken from each mouse with a micro haematocrit reader. Haematic indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to methods described by Jain (1986).

Statistical Analysis

Data were expressed as mean ± Standard error (SE) of the mean. One way Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) were used for analyses of data. The difference between the groups were considered significant at $p<0.05$. All statistical analyses were conducted with Microsoft Office Excel 2007 and SPSS-16 for Windows.

RESULTS AND DISCUSSION

Haematological Profile of *Mus musculus* after Oral Administration of *Azadirachta indica* and *Parkia biglobosa* Aqueous Wood-ash Extracts for 90 Days

Both extracts induced significant modifications in the hematological parameters compared to the control group (Table 1). Administration of *A. indica* significantly increased PCV, hemoglobin (Hb), MCV, MCH, and MCHC. Notably, while erythrocytic indices improved, RBC counts decreased significantly at the 5 mg/kg dose concentration, and WBC counts were significantly reduced at the highest dose (100 mg/kg).

Similarly, *P. biglobosa* treatment significantly increased Hb, PCV, MCV, and MCH. However, in contrast to *A. indica*, *P. biglobosa* administration resulted in a significant increase in WBC counts. Comparative analysis revealed that *P. biglobosa*-treated groups exhibited lower RBC, PCV, and WBC values than those treated with *A. indica* (Table 1).

Table 1: Changes in Haematological Profile of *Mus musculus* after Oral administration of Varying doses of *Azadirachta indica* Aqueous Wood-ash Extract for 90 Days

Parameters	Experimental Groups (mg/kg bw)			
	0 (DW)	5	50	100
RBC x10^6/µL	8.57±0.24^{ab}	7.85±0.29^{b}	8.89±0.25^{a}	8.95±0.30^{a}
PCV (%)	43.17±1.01^{a}	43.5±1.09^{a}	42.83±1.05^{a}	46.67±1.15^{b}
HGB (g/dL)	14.15±0.24^{a}	14.4±0.37^{a}	14.21±0.33^{a}	15.84±0.52^{b}
Platelets x10^3/µL	952.0±176.10	876.67±65.21	821.0±39.70	855.83±19.92
WBC x 10^3/µL	12.3±0.69^{a}	7.85±0.29^{b}	10.86±0.52^{ab}	11.16±0.82^{ab}
MCV (fl)	50.49±1.27^{a}	55.61±1.47^{b}	48.35±1.89^{a}	52.60±2.76^{ab}
MCH (pg)	16.56±0.42^{ac}	18.42±0.56^{b}	16.04±0.61^{a}	17.88±1.07^{bc}
MCHC (g/dL)	32.81±0.30^{a}	33.10±.14^{a}	33.18±0.09^{a}	33.92±0.44^{b}

Values are expressed as Means ± standard error (n=6). Means in the same row with different superscript letters are significantly ($p<0.05$) different.

DW: Distilled water. WBC: White blood cell. RBC: Red blood cell. PCV: Pack cell volume. HGB: Haemoglobin. MCV: Mean corpuscular volume. MCH: Mean corpuscular haemoglobin. MCHC: Mean corpuscular haemoglobin concentration

Table 2: Changes in Haematological Profile of *Mus musculus* after Oral Administration of Varying doses of *Parkia biglobosa* Aqueous Wood-ash Extract for 90 Days

Parameters	Experimental Groups (mg/kg bw)			
	0 (DW)	5	50	100
RBC x10^6/µL	8.57 ± 0.24	8.53 ± 0.35	8.57 ± 0.50	7.77 ± 0.22
PCV (%)	43.17 ± 1.01^{a}	44.83 ± 1.01^{a}	44.33 ± 1.52^{a}	49.0 ± 0.26^{b}
HGB (g/dL)	14.15 ± 0.24^{a}	15.2 ± 0.19^{b}	15.03 ± 0.59^{b}	16.33 ± 0.13^{c}
Platelets x10^3/µL	952.0 ± 176.10	733.0 ± 25.66	992.5 ± 72.11	867.0 ± 43.05
WBC x 10^3/µL	12.3 ± 0.69^{a}	8.88 ± 0.50^{b}	10.2 ± 1.64^{b}	8.46 ± 0.19^{b}
MCV (fl)	50.49 ± 1.27^{a}	52.80 ± 1.15^{a}	52.45 ± 2.94^{a}	63.38 ± 2.06^{b}
MCH (pg)	16.56 ± 0.42^{a}	17.93 ± 0.51^{a}	17.73 ± 0.88^{a}	21.12 ± 0.73^{b}
MCHC (g/dL)	32.81 ± 0.30^{a}	33.95 ± 0.50^{b}	33.86 ± 0.25^{b}	33.31 ± 0.21^{ab}

Values are expressed as Means ± standard error (n=6). Means in the same row with different superscript letters are significantly ($p<0.05$) different.

DW: Distilled water. WBC: White blood cell. RBC: Red blood cell. PCV: Pack cell volume. HGB: Haemoglobin. MCV: Mean corpuscular volume. MCH: Mean corpuscular haemoglobin. MCHC: Mean corpuscular haemoglobin concentration.

Haematological Profile of *Mus musculus* after Oral Administration of Aqueous Wood-ash Extracts of *Azadirachta indica* and *Parkia biglobosa* for 180 Days

Following 180 days of exposure, both extracts demonstrated a consistent trend in erythrocytic stimulation. Both treatments significantly increased RBC, PCV, Hb, MCV, MCH, and MCHC levels compared to controls (Table 2).

The extracts diverged significantly in their effects on leukocyte and thrombocyte profiles. *A. indica* significantly elevated WBC counts but induced a significant reduction in platelet levels. Conversely, *P. biglobosa* caused significant reductions in both WBC and platelet counts. Inter-extract comparison indicated that *P. biglobosa* yielded higher values for RBC, platelets, and MCHC, whereas *A. indica* resulted in higher levels of PCV, Hb, WBC, and MCH (Table 2).

Table 3: Changes in Haematological Profile of *Mus Musculus* Administered Aqueous Wood-ash Extract of *Azadirachta indica* for 180 Days

Parameters	Doses (mg/kg)			
	Control (0)	5	50	100
RBC x10^6/µL	4.48±0.07^{a}	4.97±0.16^{b}	5.58±0.14^{c}	5.56±0.15^{c}
PCV (%)	21.50±0.43^{a}	32.0±0.81^{b}	34.67±0.42^{c}	34.50±0.67^{c}
HGB (g/dL)	6.90±0.09^{a}	10.58±0.42^{b}	11.50±0.07^{c}	11.70±0.19^{c}
Platelets x10^3/µL	688.33±14.00^{b}	823.33±21.08^{a}	643.33±25..65^{c}	478.3±18.15^{a}
WBC x 10^3/µL	4.48±0.12^{ab}	5.68±0.65^{bc}	3.96±0.22^{a}	6.21±0.92^{c}
MCV (fl)	48.08±1.42^{a}	64.32±0.87^{b}	62.16±1.34^{b}	62.10±0.76^{b}
MCH (pg)	15.43±0.39^{a}	21.27±0.34^{b}	20.65±0.36^{b}	21.07±0.33^{b}
MCHC (g/dL)	32.13±0.47^{a}	33.07±0.34^{ab}	33.19±0.37^{b}	33.94±0.49^{b}

Values are expressed as Means ± standard error (n=6). Means in the same row with different superscript letters are significantly ($P<0.05$) different.

WBC: White blood cell. RBC: Red blood cell. PCV: Pack cell volume. HGB: Haemoglobin. MCV: Mean corpuscular volume. MCH: Mean corpuscular haemoglobin.MCHC: Mean corpuscular haemoglobin concentration.

Table 4: Changes in Haematological Profile of *Mus musculus* Administered Aqueous Wood-ash Extract of *Parkia biglobosa* for 180 Days

Parameters	Doses (mg/kg)			
	Control (0)	5	50	100
RBC x10^6/μL	4.48±0.12^{a}	5.38±0.29^{ab}	5.33±0.05^{b}	5.62±0.52^{b}
PCV (%)	22.00±0.58^{a}	31.00±1.15^{b}	33.67±0.33^{b}	33.00±3.00^{b}
HGB (g/dL)	6.87±0.18^{a}	11.43±1.01^{b}	11.50±0.25^{b}	11.37±0.99^{b}
Platelets x10^3/μL	676.67±20.28^{c}	583.33±37.56^{b}	670.00±36.06^{c}	516.67±21.86^{a}
WBC x 10^3/μL	4.53±0.15^{a}	3.50±0.25^{a}	4.20±0.03^{a}	3.40±0.06^{b}
MCV (fl)	49.27±2.52^{a}	58.15±5.19	63.16±0.19^{c}	58.73±0.09^{c}
MCH (pg)	15.38±0.77^{a}	21.56±2.99^{b}	21.57±0.29^{b}	20.25±0.16^{b}
MCHC (g/dL)	31.21±0.16^{a}	36.74±1.93^{b}	34.15±0.42^{c}	34.48±0.29^{c}

Values are expressed as Means ± standard error (n=10). Means in the same row with different superscript letters are significantly (P<0.05) different.

BW of aqueous wood-ash extract of *P. biglobosa.* WBC: White blood cell count. RBC: Red blood cell count. PCV: Pack cell volume. HGB: Haemoglobin. MCV: Mean corpuscular volume. MCH: Mean corpuscular haemoglobin. MCHC: Mean corpuscular haemoglobin concentration.

Discussion

Haematological indices are reliable indicators of physiological status and are commonly used to monitor erythropoiesis (RBC, Hb, PCV, MCHC, MCH, MCV) and to detect chemical toxicity with translational relevance to humans (Rodrigues & McNeill, 1992; Miyata *et al*., 1994; Ouedraogo *et al*., 2013). In this study, aqueous wood-ash extracts of Azadirachta indica and Parkia biglobosa produced dose-dependent haematological changes after 90-day (sub-chronic) and 180-day (chronic) exposure.

Both extracts increased RBC, PCV and Hb relative to controls, consistent with polycythaemia and stimulated erythropoiesis reported for other plant extracts (Kuppast *et al*., 2009; Okpuzor *et al*., 2009; Mansi & Lahham, 2008; Iranloye, 2002). Antioxidant phytochemicals (e.g., flavonoids, tannins) in the extracts may mediate this effect by protecting erythroid cells or enhancing erythropoietic signalling, as previously suggested (Muriithi *et al*., 2015; Itemobong *et al*., 2010).

WBC responses were mixed. A modest WBC rise at 100 mg/kg (especially for P. biglobosa) suggests an inflammatory or immunostimulatory response, in line with reports that plant phytochemicals (saponins, alkaloids, tannins, phenolics, flavonoids) can act as immunostimulants (Adedapo *et al*., 2007; Dashputre & Naikwade, 2010; Lakshmi *et al*., 2003). Mechanistically, such effects could reflect increased production or sensitivity to colony-stimulating factors and interleukins governing leukopoiesis (Ganong, 2001; Guyton, 2000). Conversely, the observed WBC decreases at low A. indica dose and across P. biglobosa groups after 90 days suggest possible leukocyte suppression or bone marrow suppression at those exposures (Adedapo *et al*., 2004; Osuigwe *et al*., 2007; Jimoh *et al*., 2008; Odesanmi *et al*., 2010). These divergent responses indicate dose- and species-dependent immunomodulation that requires further mechanistic study.

Red cell indices (MCH, MCHC, MCV) were variably affected. Changes in MCH, MCHC and MCV after A. indica dosing indicate altered erythrocyte indices without gross morphological disruption, while in P. biglobosa the 100 mg/kg dose produced increased MCV and decreased MCH, suggesting impaired haemoglobin incorporation into erythrocytes. The concurrent increases in Hb and PCV alongside altered indices raise the possibility of disrupted haemoglobin packaging or changes in erythrocyte size/distribution that could affect oxygen transport (Adebayo *et al*., 2005; Ashafa *et al*., 2009).

CONCLUSION

The findings of this study shows that oral administration of aqueous wood-ash extracts from *Azadirachta indica* and *Parkia biglobosa* in *Mus musculus* reveals a complex, dose-dependent modulation of the mammalian hematopoietic system. Both plant extracts exhibited distinct erythropoietic stimulating properties, characterized by significant elevations in RBC, PCV, and hemoglobin levels. This apparent polycythemic response is likely driven by the antioxidant action of active phytocompounds such as flavonoids and tannins which stabilize erythrocyte membranes and promote positive erythropoiesis. However, the safety profile of these traditional preparations requires careful boundary conditions. While high-dose (100 mg/kg) exposure to *A. indica* appears to safely upregulate immune markers, equivalent chronic doses of *P. biglobosa* induce structural shifts in absolute Wintrobe indices (MCV and MCH), pointing to potential disruption in hemoglobin packaging and reduced peripheral oxygen exchange efficiency. Furthermore, the concentration-dependent suppression of leukocytes at lower subchronic thresholds suggests a latent risk of bone marrow or lymphoid mitigation. Given that altered hematological indices in animal models carry high predictive values for human clinical toxicity, these findings caution against unregulated, long-term therapeutic consumption of wood-ash decoctions. Future molecular studies are required to isolate the distinct phytochemical fractions responsible for this hematological interplay and to map their precise bone marrow signaling pathways.

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