Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Biology and Health

Phytochemical and Anti-Anemic Properties of the Aqueous Extract of *Pseudarthria hookeri* (Fabaceae) Leaves

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DOI: 10.36347/sajb.2023.v11i01.001 | **Received:** 18.11.2022 | **Accepted:** 24.12.2022 | **Published:** 04.01.2023

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Abstract **Original Research Article**

In order to contribute to the valorization of plants used in African traditional medicine for the improvement of the health of the populations, we undertook this study to evaluate the therapeutic effectiveness of an aqueous extract of leaves of *Pseudarthria hookeri* (Fabaceae), a plant reputed to be anti-anemic. Qualitative phytochemical tests performed with the aqueous extract of *Pseudarthria hookeri* (Fabaceae) leaves revealed the presence of sterols, polyterpenes, polyphenols, catechic tannins, flavonoids, alkaloids, quinones and saponosides. The acute toxicity study of the aqueous extract of *Pseudarthria hookeri* leaves in female mice gave an estimate, according to the OECD-423 guidelines, of the 50% lethal dose (LD50). The results show that the administration of aqueous extract of *Pseudarthria hookeri* leaves at a dose of 2000 mg/kg bw and then at a dose of 5000 mg/kg bw did not induce any mortality in these animals during 14 days of observation; this allows us to conclude that aqueous extract of *Pseudarthria hookeri* leaves is not toxic by the oral route. Anemia was induced by administration of phenylhydrazine hydrochloride (4 mg/kg bw) for 6 days. Oral administration of aqueous extract of *Pseudarthria hookeri* leaves at doses of 50, 200, 1000 and 2000 mg/kg bw and the reference treatment (Ranferia hookeri) for 6 days did not induce anaemia. and the reference treatment (Ranferon®) resulted in normalization of red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular haemoglobin volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular haemoglobin level (MCHL) in anaemic rats compared to the white control rats. In conclusion, this study showed that *Pseudarthria hookeri* leaves have similar properties to Ranferon® in rats, which justifies the traditional use of this plant in the treatment of anaemia.

Keywords: Anemia, haematological parameters, phenylhydrazine, *Pseudarthria hookeri*.

INTRODUCTION

Anaemia is a global public health problem affecting both developing and developed countries, with major consequences for human health and social and economic development. It occurs at all stages of the life cycle, but is most common in pregnant women and young children. Around 35% of the world's population suffers from anaemia [1].

The World Health Organization (WHO) defines anaemia as a decrease in haemoglobin (Hb) concentration below 13 g/dL in men or 12 g/dL in women [2].

Multiple triggers for anaemia can occur in isolation but more frequently co-occur. These include genetic factors such as haemoglobinopathies, infectious factors such as malaria, intestinal helminthiasis, chronic infections and nutritional factors [3].

Nutritional factors in anaemia include iron deficiency as well as deficiencies of other vitamins and minerals such as folic acid, vitamins A and B12, and copper [4].

Treatment varies according to the type of anaemia. Treatment may include oral iron, vitamin B12 or B9, immunosuppressive or corticosteroid therapy, erythropoietin injections, blood transfusion, or even bone marrow transplantation [5].

Taking into account the high cost of modern medicines in pharmacies, the WHO encourages the search for alternatives.

The therapeutic use of plants (herbal medicine) is currently experiencing a resurgence of interest among the population. About 80% of the world's population uses herbal medicine [6].

In emerging countries, between 70 and 95% of the population rely mainly on medicinal plants for their primary care [7], due to poverty, lack of access to modern medicine and also because plants are effective.

Ethnopharmacological information has shown that the use of various medicinal plants for the treatment of anaemia is common [8].

The leaves of *Pseudarthria hookeri* are used in West and East Africa for the treatment of cough and diarrhoea [9] while the roots are used in Central and East Africa for the treatment of malaria and tuberculosis [10]. Previous biological studies on plants of the genus Pseudarthria have found interesting activities such as oestrogenic, antidiabetic, cytotoxic, antifungal and antibacterial activity [11], as well as blood disorders [12].

MATERIALS AND METHODS

Plant Material

The plant material consisted of leaves of *Pseudarthria hookeri* (Fabaceae) collected and dried in the town of Korhogo in northern Côte d'Ivoire. This plant was identified at the Centre National de Floristique (CNF) of the Université Felix Houphouët Boigny by the herbarium UCJ 010930 of this centre.

Animal Material

It consists of rats of the species *Rattus norvegicus* (Muridae), of Wistar strain and mice of the species Mus musculus (Muridae), of Swiss strain. The rats weighed between 150 and 200 g, while the mice weighed between 20 and 26 g. These animals were reared in the vivarium of the Ecole Normale Supérieure d'Abidjan, where the photoperiod is 12/24 hours with a relative humidity of 70% and the average temperature is 28°± 3°C. These animals have free access to water and food.

Pharmacological Substances

The chemical substances used in this work are phenylhydrazine hydrochloride 99% used for induction of anaemia; Ranferon® (Sun Pharmaceutical Ind. Ltd., India), which is the reference drug used to treat anaemia.

Methods

Preparation of Aqueous Extract of *Pseudarthria Hookeri* Leaves

For the preparation of aqueous extract of *Pseudarthria hookeri* leaves, 100 g of crushed dry leaves were boiled for 15 minutes in 2 L of distilled water. The resulting decoctate was double filtered on cotton wool, followed by double filtration on whatman paper. The filtrate obtained was dried for 72 h, at 50°C in an oven. The aqueous extract of *Pseudarthria hookeri* leaves is presented as a powder.

Phytochemical Screening

Phytochemical screening was carried out in order to identify chemical constituents of pharmacological interest such as sterols and polytherpenes, polyphenols, flavonoids, tannins, quinone compounds, saponosides, alkaloids via qualitative analysis techniques described in the literature [13].

Method for Studying Acute Oral Toxicity

The acute oral toxicity study was conducted on female rats using the Organisation for Economic Co-operation and Development protocol 423 [14].

Method of Study for Anaemia

Induction of Anaemia

Anemia was induced by phenylhydrazine hydrochloride [15; 16].

Phenylhydrazine, previously dissolved in distilled water [2] was administered to rats by gavage (per os) at a dose of 4 mg / kg body weight / day for 6 days.

Treatment

Rats weighing between 150-200 g, without any pre-treatment were divided into seven (7) batches of 4 rats per batch. The aqueous extract of *Pseudarthria hookeri* was dissolved in distilled water to obtain the required concentrations administered by gavage (per os).

- **Batch 1 (White Control):** Normal animals given distilled water only.
- **Batch 2 (Negative Control):** Anaemic animals given distilled water only.
- **Batch 3 (Positive Control):** Anaemic animals treated with 5 mg/kg bw of the reference treatment (Ranferon®) for 14 days.
- **Batch 4 (D1):** Anaemic animals treated with Dose 1 (50 mg/kg bw) of the aqueous extract of *Pseudarthria hookeri* leaves for 14 days.
- **Batch (D2):** Anaemic animals treated with Dose 2 (200 mg/kg bw) of the aqueous extract of *Pseudarthria hookeri* leaves for 14 days.
- **Batch (D3):** Anaemic animals treated with Dose 3 (1000 mg/kg bw) of the aqueous extract of *Pseudarthria hookeri* leaves for 14 days.
- **Batch 7 (D4):** Anaemic animals treated with Dose 4 (2000 mg/kg bw) of the aqueous extract of *Pseudarthria hookeri* leaves for 14 days.

Measurement of Haematological Parameters

Approximately 2 mL of blood was collected in tubes containing the anticoagulant ethyldiaminetetraacetic acid (EDTA) by puncturing the retro-orbital sinus with a sterile Pasteur pipette after the rats were anaesthetised with ether, prior to

phenylhydrazine administration, and then on days D0 (after PHZ), D7, and D14.

Haematological parameters were measured in each batch of rats using an automatic blood cell counter (URIT 3000 PLUS).

Processing the Results

The statistical analysis of the values and the graphical representation of the data were carried out using Graph Pad Prism 8 software (San Diego, California, USA). The statistical difference between the results was carried out using the analysis of variances (ANOVA), followed by the Tukey-Kramer multiple comparison test, with a significance level of $p < 0.05$; with $p > 0.05$: not significant; $p < 0.05$: (*) slightly significant; $p < 0.001$: (**) significant; $p < 0.001$: (***) highly significant; $p < 0.0001$: (****) highly significant. All values are presented as mean ± SEM (Standard Error on the Mean).

RESULTS

Phytochemical Study of the Aqueous Extract of Dry Leaves of *Pseudarthria Hookeri*

Table I shows the result of the phytochemical screening of the aqueous extract of dry leaves of *Pseudarthria hookeri*. This test reveals the presence of sterols, polyterpenes, polyphenols, catechic tannins, flavonoids, alkaloids, quinones and saponosides, but gall tannins are absent in the aqueous extract of *Pseudarthria hookeri*.

Table I: Phytochemical screening of the aqueous extract of *Pseudarthria hookeri* leaves

Chemical compounds sought	Reactions / Reagents	Results
Polyphenols	Reaction with ferric chloride	+
Sterols and polyterpenes	Liebermann-Bouchard reaction	+
Flavonoids	Reaction with cyanidine	+
Saponosides	Vigorous agitation	+
Quinonic compounds	Bouchardât reagent	+
Alkaloids	Dragendorff's reagent	+
	Bouchardât's reagent	+
Catechic tannins	Stiasny's reagent	+
Gallic tannins	Reaction with ferric chloride	-

+ : positive tests; - : negative tests

Acute Toxicity Study of Aqueous Extract of *Pseudarthria Hookeri* Leaves by Gavage

Table II shows the number of mice and the percentage mortality as a function of the dose of aqueous extract of *Pseudarthria hookeri* leaves administered by gavage.

Administration of the aqueous leaf extract of *Pseudarthria hookeri* to mice by gavage at a dose of 2000 mg/kg bw did not result in any deaths of animals or changes in their behaviour. However, the maximum dose of 5000 mg/kg bw administered to the mice caused a decrease in motor skills and clustering of the animals in a corner of the cage. After a period of about 30 minutes, their behaviour normalised. During the 14 days of observation, no deaths of mice were recorded for either the 2000mg/kg bw dose or the 5000mg/kg bw dose.

Table II: Number of mice and percentage mortality as a function of the dose of aqueous extract of *Pseudarthria hookeri* (AEPh) leaves administered by gavage.

Batches	AEPh doses (mg/kg bw)	Number of mice tested	Number of dead mice	Percentage dead
1	Distilled water	3	0	0
2	2000	3	0	0
3	5000	3	0	0

Effect of Phenylhydrazine Hydrochloride on Haematological Parameters

The mean values of the haematological parameters, i.e. red blood cells, haemoglobin concentration, haematocrit level, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin level (MCHL), before administration of phenylhydrazine hydrochloride (PHZ) are 7.23 ± 0.31 x109/mm^3, respectively; 12.8 ± 0.82 g/l; 38.9 ± 1.53%; 53.8 ± 1.96 fl; 17.4 ± 1.15 pg; 34.2 ± 1.31 g/dl respectively.

After administration of phenylhydrazine at a dose of 4 mg / kg / day bw, there was a highly significant ($P < 0.0001$) decrease in red blood cells (3.62 ± 0.63 x109/mm^3 ; corresponding to a decrease of 49.93%), haemoglobin (9.3 ± 1.04 g/dl ; corresponding to a decrease of 27.34%), haematocrit (30.5 ± 1.28% ; corresponding to a decrease of 21.59%) as well as a very significant decrease ($P < 0.001$) of the mean corpuscular haemoglobin concentration (30.5 ± 1.57 g/dl; corresponding to a decrease of 10.82%) and, on the other hand, a highly significant increase ($P < 0.0001$) of the mean corpuscular volume (73.1 ± 2.14 fl; corresponding to an increase of 35.87%) and mean corpuscular

haemoglobin level (23.3 ± 1.26 pg corresponding to an increase of 33.9 %) (Table III).

Table III: Effect of phenylhydrazine on haematological parameters

Before treatment with PHZ			Afer treatment with PHZ		EP	
	NR	**TR**	**NR**	**TR**	**NR**	**TR**
RBC (10^9/mm^3)	6.97±0.35	7.23±0.11	7.4±0.12	3.62±0.63	+1.64%	-49.93%
Hg (g/dl)	13.4±0.2	12.3±0.4	13.7±0.66	9.3±1.04	+3.78%	-27.34%
Hmt (%)	39.6±0.4	38.5±0.8	40.6±0.80	30.5±1.28	+2.52%	-21.59%
MCHL (pg)	17.9±1.1	17.0±0.3	18.8±0.21	23.3±1.26	+5.02%	+33.90%
MCHC (g/dl)	33.4±0.3	35.0±0.7	34.0±0.10	30.5±1.57	-0.14%	-10.82%
MCV (fl)	55.3±0.90	51.8±0.4	55.1±0.7	73.1±2.14	-0.36%	+35.87%

RBC: red blood cells; Hg: haemoglobin; **Hmt**: haematocrit; **MCHL**: mean corpuscular haemoglobin level; **MCHC**: mean corpuscular haemoglobin concentration; **MCV**: mean corpuscular volume; **PHZ**: phenylhydrazine; **EP**: Evolution's Percentage; **NR**: normal rats; **TR**: Test rats

Effects of Aqueous Extract of *Pseudarthria Hookeri* Leaves (AEPh) on Red Blood Cell Count

Figure 1 shows the effect of aqueous extract of *Pseudarthria hookeri* leaves on red blood cell counts.

At day 0, the number of red blood cells for all phenylhydrazine-treated batches (Batch 2, Batch 3, Batch 4, Batch 5, Batch 6 and Batch 7) decreased significantly ($p < 0.0001$) compared to Batch 1 (white control batch). The red blood cell count for these treated batches was approximately 3.62 ± 0.63 x10^9/mm^3 while that of the blank control batch was 7.4 ± 0.18 x10^9/mm^3.

Administration of the aqueous extract of *Pseudarthria hookeri* leaves by gavage resulted in an increase in red blood cell count at day 7. For the batches treated with 1000 and 2000 mg/kg bw of the aqueous extract of *Pseudarthria hookeri* leaves, the number of red blood cells was 6.04 ± 0.21 x10^9/mm^3 ; 6.57 ± 0.64 x10^9/mm^3 respectively. These values are not significant ($p > 0.05$) compared to the white control group (7.38 ± 0.45 x10^9/mm^3). On the other hand, for the lower doses (50 and 200 mg/kg bw) of the aqueous extract of *Pseudarthria hookeri* leaves as well as for the rats treated with Ranferon®, we obtain respectively 5.5 ± 0.21 x10^9/mm^3 ; 5.55 ± 0.36 x10^9/mm^3 ; 5.81 ± 0.38 x10^9/mm^3, which show a non-significant difference ($p < 0.05$) compared to the white control group.

On day 14, the red blood cell count was 6.5 ± 0.15 x10^9/mm^3 for the Ranferon® treated batch and 6.24 ± 0.33 x10^9/mm^3; 6.48 ± 0.29 x10^9/mm^3; 6.56 ± 0.19 x10^9/mm^3; 7.18 ± 0.38 x10^9/mm^3 for the 50, 200, 1000, 2000 mg/kg bw of the aqueous extract of *Pseudarthria hookeri* leaves respectively.

These values are insignificant compared to the white control.

The red blood cell count in the negative control (untreated anaemic) rats on day 7 and 14 was 4.91 ± 0.22 x10^9/mm^3 and 5.95 ± 0.17 x10^9/mm^3 respectively. These values are significant ($p < 0.001$) compared to those of the blank control at these same dates.

The results obtained suggest a dose-dependent effect of the aqueous extract of *Pseudarthria hookeri* leaves.

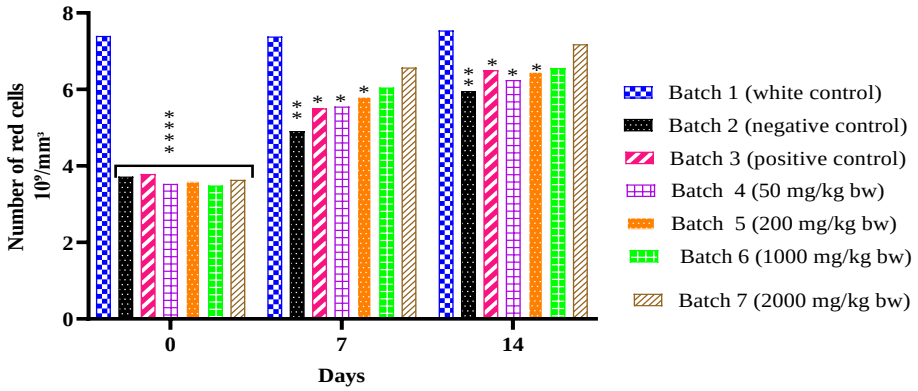

Figure 1: Effect of aqueous extract of *Pseudarthria hookeri* leaves on red blood cell count

Values expressed represent the mean; with n=4 in each group. *P<0.05; **P<0.01; ***P<0.001; **** P < 0.0001; compared to white control group (Batch 1).

Effects of Aqueous Extract of *Pseudarthria Hookeri* Leaves (AEPh) On Haemoglobin Levels

Figure 2 shows the effects of aqueous extract of *Pseudarthria hookeri* leaves on haemoglobin levels.

At day 0, the haemoglobin concentration of all batches (batch 2, batch 3, batch 4, batch 5, batch 6 and batch 7) treated with phenylhydrazine is about 9.3 ± 1.04 g/dl and that of the blank control batch is (13.7 ± 0.8 g/dl). These values are significant ($p < 0.0001$) compared to the blank control.

Administration of aqueous extract of *Pseudarthria hookeri* leaves at doses of 50, 200, 1000 and 2000 mg/kg bw and Ranferon® by gavage resulted in an increase in haemoglobin levels on day 7.

The values obtained on day 7 of treatment were 11.63 ± 0.9 g/dl in the Ranferon® -treated rats and 13.0 ± 1.3 g/dl; 13.1 ± 1.3 g/dl; 13.1 ± 1.7 g/dl; 13.2 ± 1.2 g/dl with the 50, 200, 1000, 2000 mg/kg bw AEPh doses respectively.

The haemoglobin levels obtained on day 14 in the Ranferon® -treated rats were 13.8±0.9 g/dl ($p < 0.05$) and 13 ± 0.5 g/dl ($p > 0.05$); 12.55 ± 0.5 g/dl ($p > 0.05$) ; 13.25±0.3 g/dl ($p > 0.05$); 13.8 ± 0.4 g/dl ($p < 0.05$); for 50, 200, 1000, 2000 mg/kg bw of the aqueous extract of *Pseudarthria hookeri* leaves, respectively.

In rats from the negative control lots, the haemoglobin level on day 7 was 10.4 ± 0.5 g/dl and 11.6 ± 0.7 g/dl on day 14. The averages obtained for this batch show a non-significant difference ($p < 0.05$) at day 7, and a non-significant difference ($p > 0.05$) at day 14 compared to the white control batch.

In view of these results, the aqueous extract of *Pseudarthria hookeri* leaves leads to a rapid increase in haemoglobin levels. Ranferon® and the dose of 2000 mg/kg bw induced a stronger increase in haemoglobin concentration.

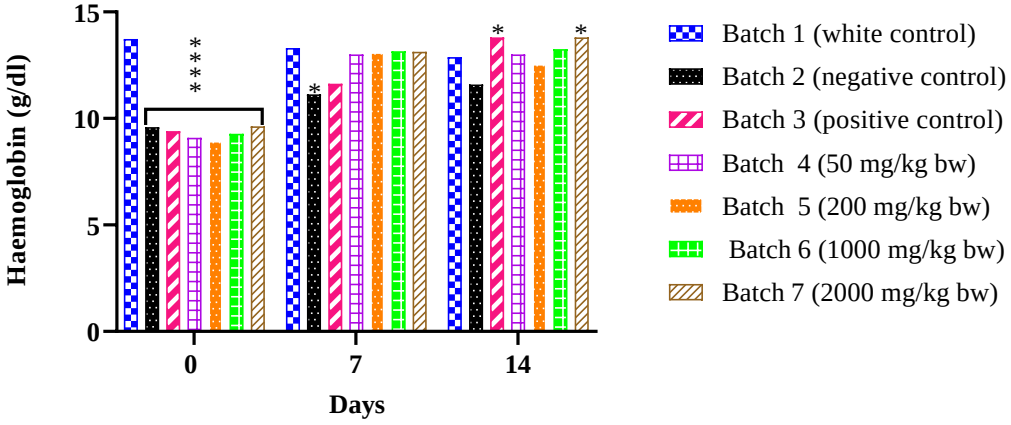

Figure 2: Effect of aqueous extract of *Pseudarthria hookeri* leaves on haemoglobin concentration

Values expressed represent the mean; with n=4 in each group. *P<0.05; **P<0.01; ***P<0.001; **** P < 0.0001; compared to the blank control group (Batch 1).

Effects of Aqueous Extract of *Pseudarthria Hookeri* Leaves on Haematocrit Levels

Figure 3 shows the effects of aqueous extract of *Pseudarthria hookeri* leaves on haematocrit levels.

On day 0, the haematocrit level of batches 2, 3, 4, 5, 6 and 7 was approximately 30.5 ± 1.28%. This is a highly significant difference ($p < 0.0001$) compared to the blank control (40.6 ± 0.4 %).

The administration of the aqueous extract of *Pseudarthria hookeri* leaves by gavage resulted in an increase in the haematocrit level on day 7. Thus, in the treated groups, the haematocrit level was 37.9 ± 0. 7 % ($p > 0.05$); 34.12 ± 0.36 % ($p < 0.05$); 34.33 ± 1.31 % ($p < 0.05$); 34.75 ± 1.4 % ($p < 0.05$); 38.11 ± 1.2 % ($p > 0.05$) respectively with Ranferon® and the doses of 50, 200, 1000, 2000 mg/kg bw of aqueous leaf extract of *Pseudarthria hookeri.*

On day 14, the haematocrit level was 45.2 ± 0.7% ($p < 0.05$) for the Ranferon® -treated batch and 38.9 ± 2.3% ($p > 0.05$); 37.36 ± 2.11% ($p < 0.05$); 39.83 ± 0.6% ($p > 0.05$); 39.6 ± 1.1% ($p > 0.05$); for the respective doses of 50, 200, 1000, 2000 mg/kg bw of the aqueous extract of *Pseudarthria hookeri* leaves.

The haematocrit levels in the negative control and white control rats on day 7 were 35.33 ± 0.2% ($p > 0.05$) and 39.5 ± 0.45% ($p > 0.05$), respectively; and 36.21 ± 0.8% ($p > 0.05$); 40.25 ± 0.12% ($p > 0.05$) on day 14.

Based on these results, the 1000 and 2000 mg/kg bw doses of the aqueous extract of *Pseudarthria*

hookeri leaves resulted in an increase in haematocrit levels close to that of the blank control lot after 14 days. Ranferon® resulted in a more rapid increase after 14 days.

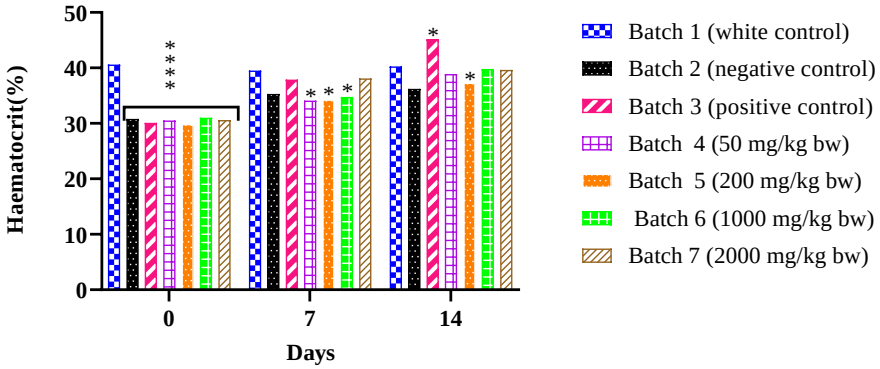

Figure 3: Effect of aqueous extract of *Pseudarthria hookeri* leaves on haematocrit level
Values expressed represent the mean; with n=4 in each group. *P<0.05; **P<0.01; ***P<0.001; **** P < 0.0001; compared to the blank control group (Batch 1).

Effects of Aqueous Extract of *Pseudarthria Hookeri* Leaves on Mean Corpuscular Haemoglobin Level

Figure 4 shows the effects of aqueous extract of *Pseudarthria hookeri* leaves on the mean corpuscular haemoglobin level.

On day 0, the mean corpuscular haemoglobin level of batches 2, 3, 4, 5, 6 and 7 is approximately 23.3 ± 1.26 pg, ($p < 0.001$) compared to the white control batch (18.8 ± 0.1 pg).

Administration of aqueous extract of *Pseudarthria hookeri* leaves by gavage resulted in a small decrease in the mean corpuscular haemoglobin content on day 7 of treatment. The values obtained for the doses of 50, 200, 1000, 2000 mg/kg bw were significant ($p < 0.01$) compared to the blank control. They are respectively 23.8 ± 1.9 pg; 23.1 ± 1.8 pg 22.4 ± 0.9 pg; 22.3 ± 1.6 pg. On the same day, the mean corpuscular haemoglobin level of the negative control batch was 22.6 ± 0.2 pg. This value is significant ($p < 0.001$) compared to the white control. In contrast, the Ranferon® -treated rats had a mean corpuscular haemoglobin level of 20.2 ± 0.4 pg. This level was non-significant ($p > 0.05$) compared to the white control (19.6 ± 1.1 pg).

On day 14, in the groups treated with Ranferon® and the different doses of the aqueous extract of *Pseudarthria hookeri* leaves, there was a significant decrease in the mean corpuscular haemoglobin level, with a non-significant difference ($p < 0.05$) compared to the white control group, whose mean corpuscular haemoglobin level was 19.5 ± 0.1 pg. The means obtained for Ranferon® and the doses of 50, 200, 1000, 2000 mg/kg bw are 20.2 ± 0.5 pg; 20.7 ± 0.2 pg; 19.9 ± 1.1 pg; 20.8 ± 0.1 pg; 20.7 ± 0.7 pg respectively.

The mean corpuscular haemoglobin level of the negative control group was 21.3 ± 0.2 pg. This content is highly significant ($p < 0.001$) compared to that of the white control group.

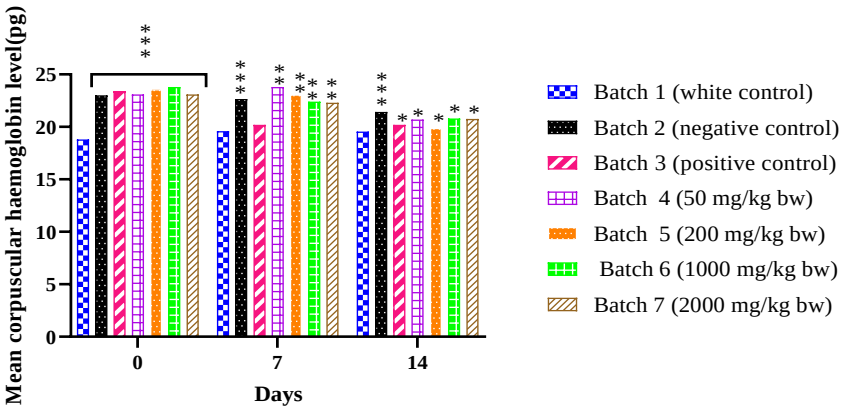

Figure 4: Effect of aqueous extract of *Pseudarthria hookeri leaves* on the mean corpuscular haemoglobin level
Values expressed represent the mean; with n=4 in each group. *P<0.05; **P<0.01; ***P<0.001; **** P < 0.0001; compared to the white control group (Batch 1).

Effects of Aqueous Extract of *Pseudarthria Hookeri* Leaves on the Mean Corpuscular Haemoglobin Concentration (MCHC)

Figure 5 shows the effects of aqueous extract of *Pseudarthria hookeri* leaves on the mean corpuscular haemoglobin concentration.

The mean corpuscular haemoglobin concentration of batches 2, 3, 4, 5, 6 and 7 of 30.5 ± 1.57 g/dl was significantly different ($p < 0.001$) from the white control batch with a mean corpuscular haemoglobin concentration of 34.0 ± 0.2 g/dl.

In the groups treated with the aqueous extract of *Pseudarthria hookeri* leaves and Ranferon®, an increase in the mean corpuscular haemoglobin concentration was observed on day 7, with a statistically non-significant difference ($p > 0.05$) compared to the white control group.

The values obtained for the white control, the negative control, Ranferon® and for the doses of 50, 200, 1000, 2000 mg/kg bw of the aqueous extract of *Pseudarthria hookeri* leaves are respectively 38.3 ± 0.9 g/dl, 34.7 ± 0.2 g/dl, 31.8 ± 0.6 g/dl; 33.7 ± 2.1 g/dl; 34.8 ± 2.5 g/dl; 36.9 ± 0.4 g/dl; 35.4 ± 1.8 g/dl.

On day 14, a slight decrease in the mean corpuscular haemoglobin concentration was observed in batches 2, 3, 4, 5, 6 and 7 with a statistically insignificant difference ($p > 0.05$) compared to the white control group whose mean corpuscular haemoglobin concentration was 32.0 ± 0.1 g/dl. The means obtained for the negative control group, the Ranferon® treated group and the groups treated with 50, 200, 1000, 2000 mg/kg bw of the aqueous extract of *Pseudarthria hookeri* leaves were 33.0 ± 0.1 g/dl; 31.2 ± 0.2 g/dl; 30.9 ± 0.7 g/dl; 32.1 ± 0.3 g/dl; 32.0 ± 0.4 g/dl and 32.9 ± 1.5 g/dl, respectively.

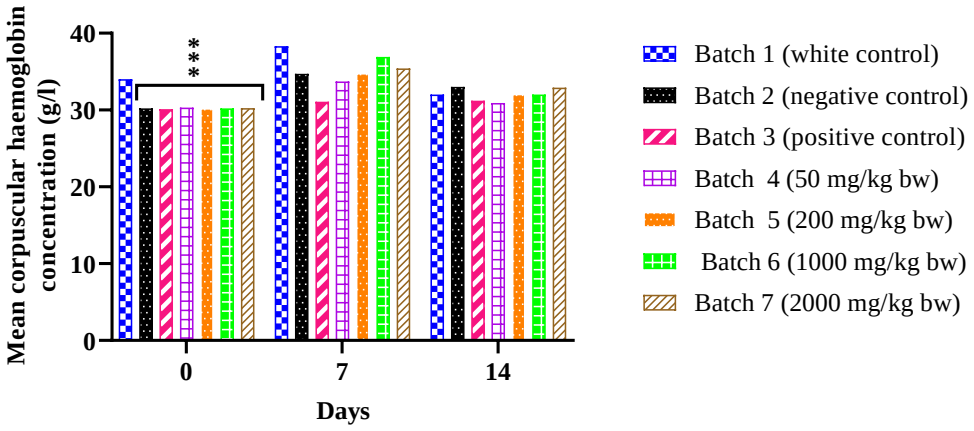

Figure 5: Effect of aqueous extract of *Pseudarthria hookeri* leaves on the mean corpuscular haemoglobin concentration
Values expressed represent the mean; with n=4 in each group. *P<0.05; **P<0.01; ***P<0.001; **** P < 0.0001; compared to the blank control group (Batch 1).

Effects of Aqueous Extract of *Pseudarthria Hookeri* Leaves on Mean Corpuscular Volume

Figure 6 shows the effects of aqueous extract of *Pseudarthria hookeri* leaves on mean corpuscular volume.

On day 0, the mean blood volume of batches 2, 3, 4, 5, 6 and 7 is approximately 73.1 ± 2.14 fl, with a highly significant difference ($p < 0.0001$) compared to the white control batch whose mean corpuscular volume value is 55.1 ± 0.41 fl.

The administration by gavage of the aqueous extract of *Pseudarthria hookeri* leaves leads to a decrease in the mean corpuscular volume in treated rats. On day 7 of treatment, we obtained mean corpuscular volume values of 72.0 ± 0.3 fl; 70.5 ± 1.7 fl; 62.0 ± 2.1 fl; 70.8 ± 1.8 fl; 63.3 ± 2.1 fl for Ranferon® and doses of 50, 200, 1000, 2000 mg/kg bw of the aqueous extract of *Pseudarthria hookeri* leaves, respectively.

For these different doses, we obtained a significant difference ($p < 0.01$) compared to the white control batch.

On day 14 of the treatment the mean corpuscular volume of batches 2, 3, 4, 5, 6 and 7 continued to decrease with a significant difference ($p < 0.01$) in the groups treated with aqueous extract of *Pseudarthria hookeri* leaves and a highly significant difference ($p < 0.001$) for the group treated with Ranferon® compared to the white control group. The values obtained for Ranferon® as well as for the doses of 50, 200, 1000, 2000 mg/kg bw of the aqueous extract of *Pseudarthria hookeri* leaves are 69.4 ± 0.29 fl; 64.6 ± 1.9 fl; 61.4 ± 2.2 fl; 64.4 ± 0.5 fl; 61.2 ± 1.6 fl respectively.

The mean corpuscular volume of the negative control batch on day 7 and day 14 was significantly different ($p < 0.0001$) compared to the white control lot.

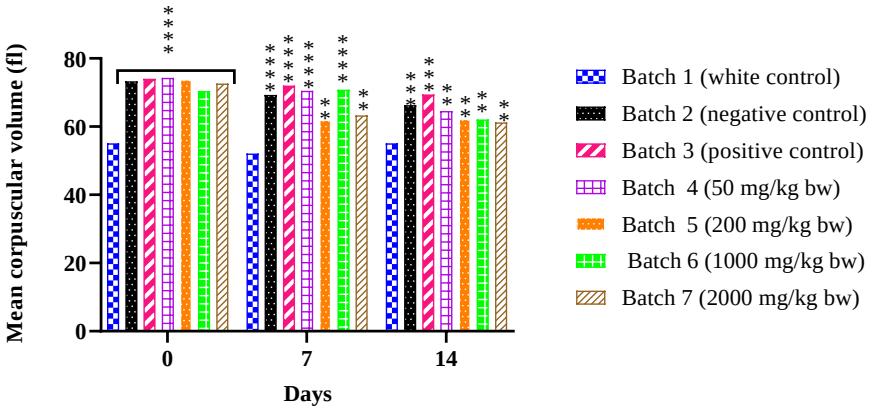

Figure 6: Effect of aqueous extract of *Pseudarthria hookeri* leaves mean corpuscular volume
Values expressed represent the mean; with n=4 in each group. *P<0.05; **P<0.01; ***P<0.001; **** P < 0.0001; compared to the blank control group (Batch 1).

DISCUSSION

The qualitative phytochemical tests carried out with the aqueous extract of *Pseudarthria hookeri* (Fabaceae) leaves revealed the presence of sterols, polyterpenes, polyphenols, catechic tannins, flavonoids, alkaloids, quinones and saponosides. However, gall tannins are absent in this extract. A study carried out on the aqueous extract of *Justicia carnea* leaves, used for the treatment of anaemia, revealed the same compounds observed in the aqueous extract of *Pseudarthria hookeri* leaves, with the exception of quinone compounds [17]. These phytochemical constituents are thought to be the source of the potential pharmacological effects of the aqueous extract of *Pseudarthria hookeri* leaves.

The acute oral toxicity study of the aqueous extract of *Pseudarthria hookeri* leaves, conducted according to OECD guideline 423 [14], shows that this extract administered by gavage does not cause any deaths in mice at doses of 2000 and 5000 mg/kg bw for 14 days of observation. The fact that the aqueous extract of *Pseudarthria hookeri* leaves is not lethal at the maximum dose of 5000 mg/kg bw indicates that its LD50 is well above this value. All substances with an LD50 between 50 and 500 mg/kg bw are toxic and those with an LD50 above 5000 mg/kg bw are non-toxic [18]. Thus, the aqueous extract of *Pseudarthria hookeri* leaves with an LD50 unquestionably above 5000 mg/kg bw is non-toxic when administered orally. This absence of toxicity by gavage observed with the aqueous extract of *Pseudarthria hookeri* leaves is also observed with other plants of the African traditional pharmacopoeia such as *Calotropis procera* (Apocynaceae) by [19], *Pterocarpus erinaceus* (Fabaceae) by [20] and *Mitragyna inennis* (rubiaceae) by [21].

The study of the pharmacological effects of aqueous extract of *Pseudarthria hookeri* leaves on haematological parameters shows that, before administration of phenylhydrazine, the values of haematological parameters in the Wistar rats used ranged from 6.97 ± 0, 35 x10^9/mm^3 to 7.23 ± 0.11 x10^9/mm^3 for the number of red blood cells, from 12.3 ± 0.4 g/dl to 13.4 ± 0.2 g/dl for the haemoglobin concentration, from 38.5 ± 0.8% to 39.6 ± 0.4% for the haematocrit level, from 51.8 ± 0.4 fl to 55.3 ± 0.9 fl for the mean corpuscular volume; from 17.0 ± 0.3 pg to 17.9 ± 1.1 pg for the mean corpuscular haemoglobin level and from 33.4 ± 0.3 g/dl to 35.0 ± 0.7 g/dl for the mean corpuscular haemoglobin concentration. These values are consistent with those of normal rats. These results demonstrate that blood cell counts can vary within the same population, or from one environment to another [22].

Administration of phenylhydrazine resulted in significant mean decreases in haemoglobin, red blood cell count, haematocrit and mean corpuscular haemoglobin concentration. However, there were significant increases in the mean corpuscular haemoglobin level and mean corpuscular volume compared to control rats. These results would confirm the induction of haemolytic anaemia in rats. Our results are similar to those of [15, 23, 24], who observed a decrease in red blood cell count, haemoglobin and haematocrit and an increase in the mean corpuscular haemoglobin level and mean corpuscular volume with phenylhydrazine administration at 40 mg/kg/d for 4 days and at 10 mg/kg/d for 8 days in rats.

Oral treatment of anaemic rats with aqueous extract of *Pseudarthria hookeri* leaves at doses of 50, 200, 1000 and 2000 mg/kg bw resulted in a highly significant increase in red blood cell level, haemoglobin concentration and haematocrit level. In addition, the effect of the aqueous extract of *Pseudarthria hookeri* leaves is substantially similar to that of the standard anti-anemic treatment (Ranferon®) used in this study during the treatment period. Both the aqueous extract of *Pseudarthria hookeri* leaves and Ranferon® ameliorate phenylhydrazine -induced disturbances in rats. These results would indicate that aqueous extract of *Pseudarthria hookeri* leaves stimulated haematopoiesis

via erythropoietin. Erythropoietin is a hormone that regulates the production of red blood cells. It increases the number of sensitive erythroblasts in the bone marrow that are converted into reticulocytes and later into mature erythrocytes [25]. Similar results were obtained after treatment of anaemic rats with an aqueous extract of *Solanum torvum* fruit at 250 mg/kg bw [26], *Sorghum bicolor* seed at 200 and 300 mg/kg bw [27] and *Trema guineensis* leaves at 200 mg/kg bw [28]. In addition, aqueous extract of *Pseudarthria hookeri* leaves caused fluctuations in mean corpuscular volume, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin level values deteriorated by phenylhydrazine. However, the values obtained in this study during treatment are within the reference values described by [29].

Aqueous extract of *Pseudarthria hookeri* leaves induced almost normalization of mean corpuscular volume, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin level. This means that aqueous extract of *Pseudarthria hookeri* leaves normalizes red blood cell size, charge and haemoglobin content. Our results are different from those of [30]. In their work, they revealed a significant decrease in the mean corpuscular haemoglobin concentration and mean corpuscular volume during different periods in anaemic rats treated with 300 mg/kg bw of an aqueous extract of the calyxes of *Hibiscus sabdarifa* during 15 days.

In this study, the aqueous extract of *Pseudarthria hookeri* leaves was effective against phenylhydrazine-induced anaemia. This result tells us that the bioactive molecules contained in the aqueous extract of *Pseudarthria hookeri* leaves would be able to repair the damage caused by phenylhydrazine.

Tannins, saponosides, flavonoids and alkaloids have anti-anemic properties. They promote tissue regeneration, reduce the permeability of blood capillaries and increase their resistance to haemolysis [31]. The effect of aqueous extract of *Pseudarthria hookeri* leaves on phenylhydrazine-induced haemolytic anaemia in rats could be partly attributed to its phytochemical constituents.

CONCLUSION

Aqueous extract of *Pseudarthria hookeri* leaves has an anti-anemic effect in rats made anemic by phenylhydrazine. The effects of aqueous extract of *Pseudarthria hookeri* leaves on haematological parameters are similar to those of Ranferon®, a reference substance used for the treatment of anaemia.

The effects of aqueous extract of *Pseudarthria hookeri* leaves on induced anaemia can be attributed to the chemical compounds it contains, including sterols, polyterpenes, polyphenols, catechic tannins, flavonoids, alkaloids, quinones and saponosides, which are believed to possess anti-anaemic properties.

The results provide a scientific basis for the traditional use of *Pseudarthria hookeri* (Fabaceae) leaves in the treatment of anaemia.

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