Original Research Article

Anti-anemic activity of aqueous root bark extract of *Morinda lucida* Benth against phenylhydrazine-induced hemolytic anemia in Wistar rats

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ABSTRACT

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| **Aims:** evaluate the anti-anemic potential of aqueous root bark extract of *Morinda lucida* against induced hemolytic anemia in Wistar rats in addition to its total phenol content and its acute toxicity.**Study design:** Experimental Design.**Place and Duration of Study:** Laboratory of Biology and Health, Training and Research Unit Biosciences, Félix Houphouët-Boigny University of Abidjan, June 2024 to September 2024.**Methodology:** Total phenol content of aqueous root bark extract of *Morinda lucida* was determined using Folin-Ciocalteu reagent. The acute toxicity test was performed according to OECD guideline no. 425. Anemia was induced in Wistar rats by intraperitoneal administration of phenylhydrazine at 40 mg/Kg bw for two days. The rats were orally treated during 21 days with 100 and 200 mg/kg bw of *Morinda lucida* extract and Ranferon® (anti-anemic drug) at 50 mg/kg bw. Blood was collected from all rats before and after induction of anemia, and then weekly during the treatment period to monitor changes in hematological parameters such as hemoglobin level, red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration.**Results:** The results show that the aqueous root bark extract of *Morinda lucida* contains phenolic compounds, estimated at 0.169 ± 0.015 mg gallic acid equivalent/g extract, and has low toxicity. In anti-anemic test, phenylhydrazine caused a significant reduction in hemoglobin level in rats, from 13.33 to 8.33 g/dL, with a disturbance in the other hematological parameters. After 15 days of treatment, hemoglobin levels significantly increased in rats treated with *M. lucida* extract at 100 mg/kg (13.27±0.26 g/dL) and Ranferon® (12.93±0.145 g/dL), comparable to normal controls (13.17±0.03 g/dL).There were also improvements in the other hematological parameters after two to three weeks treatment.**Conclusion:** These results show that *Morinda lucida* is effective in repairing phenylhydrazine-induced erythrocyte damage and highlight its anti-anemic potential. |

*Keywords: anemia, phenylhydrazine, Morinda lucida, anti-anemic activity.*

1. INTRODUCTION

Anemia is a condition characterized by an abnormal drop in the number of red blood cells or the hemoglobin concentration within them. It is a serious global public health problem that particularly affects young children, menstruating adolescent girls and women, and pregnant and postpartum women (WHO, 2023). According to WHO (2023), 40% of children 6-59 months of age, 37% of pregnant women, and 30% of women 15-49 years of age worldwide are anemic. Several factors contribute to the development of anemia: nutrient deficiencies through inadequate diets or inadequate absorption of nutrients, infections (e.g. malaria, parasitic infections, tuberculosis, HIV), inflammation, chronic diseases, gynecological and obstetric conditions, and inherited red blood cell disorders. The most common nutritional cause of anemia is iron deficiency, although deficiencies in folate, vitamin B12 and vitamin A are also important causes (WHO, 2023).

There are many types of anemia such as iron-deficiency anemia, pernicious anemia, aplastic anemia, sickle cell anemia and hemolytic anemia due to malaria infection. Among these, iron-deficiency anemia and hemolytic anemia are the most common. Treatment varies depending on the type of anemia. It may include a supply of iron, vitamin B12 or vitamin B9 orally, treatment with immunosuppressors or corticosteroids, erythropoietin injections, blood transfusion or even bone marrow transplantation (Movaffaghi et al., 2006). Dietary changes, iron supplementation and blood transfusions are commonly used to treat anemia. Iron supplementation has many disadvantages, as the body has few mechanisms for eliminating this trace element, so it accumulates easily and can lead to serious health complications, such as certain neurogenic disorders or cancer (Saha et al., 2018). Meanwhile, blood transfusions often present risks of infection and incompatibility (Pelletier, 2018; Ainley and Hewitt, 2018). Given the undesirable side-effects of available therapies and especially their high cost, attention should be focused on the use of medicinal plants for the treatment of anemia.

*Morinda lucida* Benth (Rubiaceae) is a medicinal plant whose different parts are used in traditional medicine to treat various diseases in Africa. For example, preparations based on the leaves of this plant were used against fever, to treat malaria and jaundice, as well as to treat sickle cell disease. The stem barks are used to make bitters, treat malaria and jaundice, and treat hypertension. In addition, the roots are used to treat itching and ringworm and as an antihypertensive (Adewole et al., 2021).

In experimental studies, phenylhydrazine (PHZ) has been used to induce hemolytic anemia in animal models. Its auto-oxidation produces reactive oxygen species and PHZ-derived radicals, which can lead to a number of harmful cellular reactions, including hemolytic anemia (Sung et al., 2013). On the other hand, phenolic compounds can prevent and repair free radical/reactive oxygen species-induced cellular oxidative damage (Shen et al., 2022). They show considerable ability to combat and protect against the effects of oxidative stress. Phenolic compounds have been shown to act as active antioxidants even at low concentrations, although most evidence regarding antioxidant capacity comes from *in vitro* studies (Kruk et al., 2022). Toxicity studies on medicinal plants, even if they are effective against pathologies, are also essential for the assessment of their potential adverse effects and to ensure their safe use. Thus, this study was conducted to investigate the effect of aqueous root bark extract of *Morinda lucida* against phenylhydrazine-induced anemia in Wistar rats in addition to its total phenol content and its acute toxicity.

2. material and methods

**2.1 Plant material**

Root barks of *Morinda lucida* Benth (Rubiaceae) constituted the plant material. Roots of this plant were collected in the region of N’douci, Southern Côte d’Ivoire. Samples were sent to the National Floristic Center, Félix Houphouët-Boigny University of Abidjan, and were authenticated by comparison with specimens registered under number UCJ019084.

**2.2 Animal material**

White *Mus musculus* mice, aged 8 weeks and weighing between 15 and 22 g, were used for the acute toxicity study. Wistar albino rats of the species *Rattus norvegicus*, aged 3 to 4 months and weighing between 150 and 200 g, were used to assess anemic activity. These animals came from the animal house of the Normal High School of Abidjan where they were bred. They were kept at room temperature with 12 h of light during the day and 12 h of darkness in the night, fed pellets and had free access to water. All experimental procedures have been examined and approved by the Ethical Committee of Health Sciences, Félix Houphouët-Boigny University of Abidjan.

**2.3 Extract preparation**

Root barks of *Morinda lucida* previously harvested were shade at room temperature for 3 weeks and dried samples were later grounded to a powder using a grinder. One hundred (100) grams of plant powder were shaken in 1 L of distilled water and the mixture was homogenized for 5 min using an electronic mixer. The homogenate was then successively filtered twice on cotton and once on Whatman filter paper (3 mm) (Zirihi et al., 2003). This operation (homogenization and filtration) was repeated 3 times and the filtrates obtained were concentrated to dryness under reduced pressure at 30°C using a rotary evaporator (BÜCHI). The resulting extract constituted the aqueous root bark extract of *Morinda lucida*, which was stored at 4° C for subsequent analysis.

**2.4 Determination of total phenol content**

The Folin-Ciocalteu reagent was used to determine the total phenol content of the aqueous root bark extract of *Morinda lucida*. For this purpose, 5 mL of Folin-Ciocalteu reagent (1:10 with distilled water) and 4 mL of sodium carbonate (1 M) were added to 0.5 mL of plant extract (0.1 g/mL). The reaction mixture was shaken, incubated at room temperature for 15 min and the absorbance measured at 765 nm. As a standard, gallic acid was used at concentrations ranging from 0 to 250 mg/L in methanol/water (50:50, v/v). The total phenol content was expressed as mg of gallic acid equivalents (GAE) per gram of extract using gallic acid standard curve. All measurements were repeated three times (Mc Donald et al., 2001).

**2.5 Acute oral toxicity**

The acute oral toxicity study of the aqueous root bark extract of *Morinda lucida* was performed according to the Organization for Economic Co-operation and Development (OECD) guideline no. 425 for the testing of chemicals (OECD, 2001). Two groups of 3 mice were formed. The first group (control group) received distilled water, while the second group was treated with a single dose of 2000 mg/kg b.w. of aqueous root bark extract of *M. lucida*. The animals were fasted overnight before administration of the extract. After treatment, the animals were observed regularly for 24 h, with particular attention paid to the first 4 h, and then daily for 14 days. Observations focused on the following symptoms: agitation, convulsion, torsion, diarrhea and mobility.

**2.6 Anti-anemic activity**

The anti-anemic activity of aqueous root bark extract of *Morinda lucida* was evaluated in rats according to the method of Ryu and Yook (2001) with few modifications. The study involved determining the hematological parameters of rats before and after induction of anemia.

**2.6.1 Induction of anemia**

Anemia was induced in rats by intraperitoneal (ip) injection of phenylhydrazine at a dose of 40 mg/kg bw per day (Naughton et al., 1989) for two consecutive days (D0 and D1). The phenylhydrazine was first dissolved in dimethyl sulfoxide (DMSO) diluted 1:10 in distilled water. On day 3 (D2), rats with a hemoglobin concentration of less than 9 g/dL were considered anemic and selected for further study.

**2.6.2 Treatment and monitoring of animals**

Twenty-five (25) rats were divided into 5 groups of 5 rats each. Group 1, which had previously received 1:10 diluted DMSO intraperitoneally, consisted of non-anemic rats, whereas groups 2, 3, 4 and 5 consisted of anemic rats. These different groups of animals were treated daily for 21 days (D2 to D22) as follows:

- Groups 1 (normal control) and 2 (anemic control) received orally distilled water daily.

- Group 3 (reference control) was treated orally with the standard anti-anemic product (Ranferon®) at 50 mg/kg bw.

- Groups 4 and 5 were treated orally with the aqueous root bark extract of *Morinda lucida* at doses of 100 and 200 mg/kg bw respectively.

During the experiment, blood samples were collected by eye puncture after the rats were anaesthetized. Blood was taken before induction of anemia (D0) and then at D2, D8, D15 and D22 and collected separately in EDTA tubes for determination of hematological parameters.

**2.6.3 Determination of hematological parameters**

Hematological parameters were determined using an automated hematological analyzer (URIT-3000 Plus, China) according to the manufacturer's instructions. The parameters determined were hemoglobin level, red blood cell (RBC) count, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

**2.7 Statistical analysis**

Statistical analysis was performed using Graph Pad Prism 9.0 (Microsoft USA) software. Data were subjected to one-way analysis of variance (ANOVA) followed by Dunett's multiple comparison test (post-test). The difference between means was considered significant at a *P*-value of less than 5% (*P* < .05).

3. results

**3.1 Total phenol content**

The total phenol content of aqueous root bark extract of *Morinda lucida* was expressed as mg gallic acid equivalent per g of extract (mg GAE/g extract). The values were determined using the standard curve for gallic acid, defined as y = 0.005903x, R2 = 0.9780. The total phenol content of this extract was estimated at 0.169 ± 0.015 mg GAE/g extract.

**3.2 Acute toxicity**

Oral administration of aqueous root bark extract of *Morinda lucida* at a single dose of 2000 mg/kg b.w. did not cause any deaths in mice after 24 h and 14 days. No signs of agitation, convulsion, torsion, diarrhea or reduced mobility were observed in these animals during this period. These results indicate that the lethal dose 50 (LD50) of aqueous root bark extract of *Morinda lucida* is greater than 2000 mg/kg b.w.

**3.3 Anti-anemic activity**

Six hematological parameters were determined during the induction of anemia and its treatment with the aqueous root bark extract of *Morinda lucida* and the standard anti-anemia drug. These parameters are hemoglobin level, red blood cell (RBC) count, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin content (MCHC).

**3.3.1 Effect of *Morinda lucida* extract on hemoglobin level**

The effect of aqueous root bark extract of *Morinda lucida* and Ranferon® on hemoglobin level in anemic rats is shown in Fig. 1. The results show that Phenylhydrazine caused a significant (*P* < .0001) reduction in hemoglobin level on day 2 (D2) in intoxicated rats compared to that in normal control rats (13.33 ± 0.15 g/dL). The hemoglobin level in these rats, ranging from 13.30 ± 0.4 to 13.70 ± 0.21 g/dL before induction of anemia (D0), decreased to values ranging from 8.33 ± 0.42 to 9.53 ± 0.09 g/dL at D2.

Daily administration of *M. lucida* extract and Ranferon® to anemic rats for 7 days significantly increased hemoglobin level (D8) in all treated groups compared to anemic control rats (9.5 ± 0.32 g/dL). In rats treated with aqueous extract of *M. lucida*, hemoglobin level increased significantly (*P* < .001 and *P* < .05), from 9.53 ± 0.09 to 11.27± 0.34 g/dL and from 8.48 ± 0.73 to 11.26 ± 0.021 g/dL, respectively at doses of 100 and 200 mg/kg b.w. With the reference drug (Ranferon®), hemoglobin level increased significantly (*P* < .0001) from 9.17 ± 0.23 to 11.77 ± 0.34 g/dL compared to anemic control rats. After two weeks of treatment (D15), the hemoglobin level of rats treated with Ranferon® (12.93 ± 0.145 g/dL) and those treated with the aqueous extract of *M. lucida* at 100 mg/kg b.w. (13.27 ± 0.26 g/dL) were restored to the level of normal control rats (13.17 ± 0.03 g/dL). The aqueous root bark extract of *M. lucida* at 200 mg/kg b.w. also restored hemoglobin level at the end of treatment (D22).

**Fig. 1. Hemoglobin level variation in anemic rats treated with aqueous root bark extract of *Morinda lucida* and Ranferon®**

*AEMl: aqueous extract of Morinda lucida. Values are expressed as mean ± SEM, with n=5. Symbols (# and \*) indicate statistical significance. ####P < .0001: significant difference between normal control group and other groups. \*P < .05; \*\*P < .01; \*\*\*P < .001; \*\*\*\*P < .0001: significant difference between anemic control group and other groups.*

**3.3.2 Effect of *Morinda lucida* extract on red blood cell count**

The changes in red blood cell count during treatment of anemic rats with aqueous root bark extract of *Morinda lucida* and Ranferon® are shown in Fig. 2. A significant (*P* < .0001) decrease in red blood cell count was observed in all phenylhydrazine-intoxicated rats compared with normal control rats (8.35 ± 0.04×1012/L) (D2). The red blood cell count, estimated from 7.94 ± 0.37×1012/L to 8.44 ± 0.69×1012/L at D0, decreased to values ranging from 3.19 ± 0.17×1012/L to 4.3 ± 0.23×1012/L at D2.

After one week of treatment (D8), a significant increase (*P* < .05) in red blood cell count was observed in rats treated with the aqueous extract of *Morinda lucida* at 100 mg/kg bw and in those treated with Ranferon® compared with that of anemic control rats (4.57 ± 0.09×1012/L). Only the extract at 200 mg/kg b.w. did not cause any significant variation. At the second week of treatment (D15), the red blood cell count increased very significantly (*P* < .0001) in all groups of rats treated with plant extract and Ranferon® compared with anemic control rats (5.52 ± 0.37×1012/L). The RBC count in rats treated with *M. lucida* extract at 100 mg/kg bw (8.18 ± 0.1×1012/L) was comparable to that in normal control rats (8.36 ± 0.3×1012/L) and significantly higher than that in rats treated with Ranferon® (7.92 ± 0.35×1012/L). After three weeks of treatment (D22), the RBC count of all groups of treated rats were reduced to those of normal control rats (8.36 ± 0.3×1012/L). The values recorded were 8.60 ± 0.06×1012/L; 8.25 ± 0.08×1012/L and 8.19 ± 0.09×1012/L in rats treated with aqueous root bark extract of *M. lucida* at doses of 100 and 200 mg/kg and Ranferon®, respectively.

**Fig. 2. Red blood cell count variation in anemic rats treated with aqueous root bark extract of *Morinda lucida* and Ranferon®**

*AEMl: aqueous extract of Morinda lucida. Values are expressed as mean ±SEM, with n=5. Symbols (# and \*) indicate statistical significance. ####P < .0001: significant difference between normal control group and other groups. \*P < .05; \*\*\*P < .001; \*\*\*\*P < .0001: significant difference between anemic control group and other groups.*

**3.3.3 Effect of *Morinda lucida* extract on hematocrit**

Fig. 3 shows the effect of aqueous root bark extract of *Morinda lucida* and Ranferon® on hematocrit in anemic rats. Phenylhydrazine-induced anemia was evidenced by a significant (*P* < .0001) decrease in hematocrit on day 2 (D2) in rats of groups 2, 3, 4 and 5 compared with that in normal control rats (47.67 ± 0.88 %). The hematocrit in these rats, estimated from 46.93 ± 1.06 to 47.47 ± 1.01 % before induction of anemia (D0), decreased to values ranging from 23.10 ± 0.23 to 25.43 ± 0.59 % at D2.

Treatment of anemic rats with aqueous extract of *M. lucida* and Ranferon® for 7 days (D8) resulted in a significant increase in hematocrit in all treated groups compared to anemic control rats (27.83 ± 0.44 %). The hematocrit values recorded in treated rat groups ranged from 30.58 ± 0.36 % to 31.10 ± 0.49 % and were lower than those of normal control rats (46.67±0.67%). On the 14th day, the hematocrit of rats treated with *M. lucida* extract at 100 mg/kg bw (46.60±1.14%) and Ranferon® (45.87±1.62%) was statistically identical to that of normal controls (46.07 ± 0.58%). After 21 days of treatment (D22), this hematological parameter was also normalized in rats treated with the plant extract at 200 mg/kg b.w. Values obtained were 47 ± 1.1, 47.37 ± 0.63 % and 46.20 ± 0.92 % in animals treated with Ranferon® and *M. lucida* extract at 100 and 200 mg/kg b.w. respectively. The value for normal control rats was 46.27 ± 0.41%.

**Fig. 3. Hematocrit variation in anemic rats treated with aqueous root bark extract of *Morinda lucida* and Ranferon®**

*AEMl: aqueous extract of Morinda lucida. Values are expressed as mean ±SEM, with n=5. Symbols (# and \*) indicate statistical significance. ####P < .0001: significant difference between normal control group and other groups. \*\*P < .01; \*\*\*P < .001; \*\*\*\*P < .0001: significant difference between anemic control group and other groups.*

**3.3.4 Effect of *Morinda lucida* extract on mean corpuscular volume**

The effect of aqueous root bark extract of *Morinda lucida* and Ranferon® on mean corpuscular volume (MCV) in anemic rats is shown in Fig. 4. A significant (*P* < .05) increase in mean corpuscular volume was observed in all phenylhydrazine-intoxicated rats compared with normal control rats (63.77 ± 0.39 fL) (D2). The MCV in intoxicated rats, ranging from 60.63 ± 0.95 to 61.83 ± 1.68 fL before phenylhydrazine administration (D0), increased to values ranging from 79.27 ± 0.59 to 83.6 ± 1 fL at D2.

Treatment of rats for 7 days (D8) with the aqueous extract of *M. lucida* and Ranferon® resulted in a significant reduction in the MCV of the treated rat groups compared with that of the anemic control rats (73.67 ± 0.33 fL). However, the value of the normal control rats (62.57 ± 0.3 fL) was not reached. Two weeks after treatment (D15), MCV in rats receiving *M. lucida* extract at 100 mg/kg b.w. (60.70 ± 0.35 fL) and 200 mg/kg b.w. (61.87 ± 0.94) reached normal control levels (62 ± 0.51 fL). After 21 days of treatment (D22), MCV was restored in all treated rats. A better effect was observed for *M. lucida* extract at 100 mg/kg b.w. (58.53 ± 0.53 fL), followed by the extract at 200 mg/kg b.w. (60 ± 0.58 fL) and Ranferon® (62 ± 1 fL). The MCV of normal control rats did not change significantly during the experiment.



**Fig. 4. Mean corpuscular volume variation in anemic rats treated with aqueous root bark extract of *Morinda lucida* and Ranferon®**

*AEMl: aqueous extract of Morinda lucida. Values are expressed as mean ±SEM, with n=5. Symbols (# and \*) indicate statistical significance. #P < .05; ##P < .01: significant difference between normal control group and other groups. \*P < .05; \*\*P < .01; \*\*\*P < .001; \*\*\*\*P < .0001: significant difference between anemic control group and other groups.*

**3.3.5 Effect of *Morinda lucida* extract on mean corpuscular hemoglobin**

The changes in the mean corpuscular hemoglobin (MCH) of anemic rats treated with aqueous root bark extract of *Morida lucida* and Ranferon® are shown in Fig. 5. Administration of phenylhydrazine for two consecutive days (D0 and D1) resulted in a significant increase (P < .0001) in MCH in intoxicated animals compared to normal controls (18.50 ± 0.26 pg). Rats given phenylhydrazine showed MCH levels from 28.33 ± 1.76 to 32.13 ± 0.61 pg at D2. Treatment with the aqueous extract of *M. lucida* and Ranferon® for seven days did not result in any significant change (P ˃ .05) in the MCH of these rats. The effect of plant extract and Ranferon® was only noticeable in anemic rats after 2 weeks of treatment (D15), where MCH was significantly reduced in all groups treated compared to anemic control rats (21.33 ± 0.33 pg). Values of 17.3 ± 0.4 pg, 18.23 ± 0.22 and 19.40 ± 0.31 obtained in animals treated with Ranferon® and *M. lucida* extract at 100 and 200 mg/kg b.w. respectively, reached those of normal control rats (18.63 ± 0.38 pg). At the end of treatment (D22), MCH was significantly reduced in rats treated with Ranferon® and *M. lucida* extract at 200 mg/kg b.w. compared to normal control rats (18.43±0.9 pg). MCH levels were 16.47 ± 0.27 pg, 17.87 ± 0.19 pg and 17.23 ± 0.15 pg in rats treated with Ranferon® and M. lucida extract at 100 and 200 mg/kg b.w.

**Fig. 5. Mean corpuscular hemoglobin variation in anemic rats treated with aqueous root bark extract of *Morinda lucida* and Ranferon®**

*AEMl: aqueous extract of Morinda lucida. Values are expressed as mean ±SEM, with n=5. Symbols (# and \*) indicate statistical significance. ##P < .05; ###P < .01; ####P < .01: significant difference between normal control group and other groups. \*P < .05; \*\*P < .01; \*\*\*P < .001; \*\*\*\*P < .0001: significant difference between anemic control group and other groups.*

**3.3.6 Effect of *Morinda lucida* extract on mean corpuscular hemoglobin concentration**

Fig. 6 shows the changes in the mean corpuscular hemoglobin concentration (MCHC) during the treatment of anemic rats with aqueous root bark extract of *Morinda lucida* and Ranferon®. A significant (*P* < .05) reduction in MCHC was observed on day 2 (D2) after phenylhydrazine administration for two days (D0 and D1) to rats in groups 2, 3, 4 and 5 compared to normal control rats (45,1 ± 1,43 g/dL). With initial values ranging from 41.07 ± 0.24 to 43.33 ± 1.22, the MCHC decreased in these rats to values ranging from 35.40±0.31 to 37.5 ± 0.46 g/dL at D2. One week after treatment (D8), aqueous extract of *M. lucida* at a dose of 100 mg/kg b.w. and Ranferon® induced a significant increase (*P* < .05) in MCHC of treated rats compared to that of anemic control rats (36.53 ± 0.29 g/dL). On the 14th day of treatment (D15), all treated rats show a significantly higher MCHC than the anemic control rats (38 ± 0.23 g/dL). At the end of treatment (D22), MCHC levels were 41.47 ± 0.32, 42.53 ± 0.74 and 43.1 ± 0.49 g/dL in rats treated with Ranferon® and *M. lucida* extract at 100 and 200 mg/kg b.w. respectively. Treatment of anemic rats did not reach normal control MCHC level (45.77 ± 1.48 g/dL), but the effect of the extract was better than that of Ranferon®.

**Fig. 6. Mean corpuscular hemoglobin concentration variation in anemic rats treated with aqueous root bark extract of *Morinda lucida* and Ranferon®**

*AEMl: aqueous extract of Morinda lucida. Values are expressed as mean ±SEM, with n=5. Symbols (# and \*) indicate statistical significance. #P < .05; ##P < .01: significant difference between normal control group and other groups. \*P < .05; \*\*P < .01; \*\*\*P < .001; \*\*\*\*P < .0001: significant difference between anemic control group and other groups.*

4. DISCUSSION

In this study, the aqueous root bark extract of *Morinda lucida* was tested for its efficacy to reverse phenylhydrazine-induced anemia in rats. The total phenol content of this extract was first determined. Phenolic compounds in plants have redox properties, and these properties allow them to act as antioxidants (Soobrattee et al., 2005), reducing free radical generation and alleviating diseases caused by oxidative stress (Ballard and Junior, 2019). The results showed the presence of these compounds at an estimated level of 0.169 ± 0.015 mg GAE/g extract. The phenolic content of *Morinda lucida* leaf, stem and root bark extracts were reported to be 4.84, 4.84 and 1.05 mg/100 g extract respectively (Adeleye et al., 2018). This shows that the phytochemical content of the same plant can vary according to its parts and terroir.

In the acute oral toxicity study of the aqueous root bark extract of *M. lucida*, behavioral observations of the experimental animals did not show any sign of toxicity and any mortality. The lethal dose 50 (LD50) was greater than 2000 mg/kg b.w. This extract is therefore assigned to category 5 of the Globally Harmonised System of Classification of Chemicals, the category of substances with low toxicity (OECD, 2001). This is in agreement with the findings of Joppa et al. (2008) who showed that the hydroethanolic extract of *M. lucida* leaves with a LD50 above 5000 mg/kg b.w. is almost non-toxic.

For the anti-anemic activity test, hematological parameters were measured before and after inducing anemia and while treating rats with the aqueous extract of *Morinda lucida*. Intraperitoneal administration of phenylhydrazine (40 mg/kg b.w. for two days) to rats resulted in significant changes in hematological parameters. A very marked decrease in red blood cell (RBC) count, hemoglobin level and hematocrit (HCT) was observed, as well as a significant decrease in mean corpuscular hemoglobin concentration (MCHC). On the other hand, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) increased significantly. This very significant decrease in RBC count, hemoglobin level and hematocrit indicates anemia, probably caused by increased destruction of erythrocytes or impairment in their production.

Phenylhydrazine was mainly used for experimental induction of anemia in animals. It is known to cause hemolytic anemia by damaging the membranes of red blood cells, leading to their premature destruction (Berger, 2007). Its interaction with red blood cells leads to the formation of free radicals. These initiate the peroxidation of membrane lipids, leading to the lysis of red blood cells (Cohen and Hochstein, 1964; Jain and Hochstein, 1979). This results in a marked decrease in the number of red blood cells, hemoglobin levels and hematocrit, as observed in all rats given phenyhydrazine. The significant decrease in MCHC, which represents the concentration of hemoglobin in red blood cells, may indicate a relative dilatation of these blood cells or a reduction in hemoglobin production in anemic rats (Hoffbrand et al., 2006). In contrast, the increases in MCV and MCH observed in rats given phenylhydrazine may reflect the body's compensation for the reduced number of red blood cells. Higher MCV may indicate macrocytosis, where red blood cells are larger in response to anemia (Dacie and Lewis, 1991). The increase in MCH may suggest that the remaining red blood cells contain more hemoglobin, which may be an attempt by the body to maintain oxygen transport despite the reduction in the total number of red blood cells. Phenylhydrazine-induced anemia was therefore manifested by a significant decrease in red blood cell (RBC) count, hemoglobin level, hematocrit and mean corpuscular hemoglobin concentration (MCHC), and an increase in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH).

Treatment with aqueous root bark extract of *Morinda lucida* at doses of 100 and 200 mg/kg b.w. and Ranferon®, the standard anti-anemic drug at 50 mg/kg b.w., improved RBC count, hemoglobin level, hematocrit, MCV, MCH and MCHC in anemic rats. Hemoglobin levels, a key parameter in anemia, increased progressively during treatment of anemic rats. After two weeks of treatment (D15), this parameter was corrected by Ranferon® and *M. lucida* extract at 100 mg/kg b.w. At the end of treatment (D22), the hemoglobin level of all treated rats were restored to the level of normal control rats. There was also a progressive increase in RBC count during the treatment of anemic rats with Ranferon® and *M. lucida* extract. The results show that RBC count of all groups of treated rats were reduced to those of normal control rats after three weeks of treatment (D22). Red blood cells contain hemoglobin, so their production is associated with an increase in hemoglobin levels. The hematocrit reached that of normal control rats in rats treated with *M. lucida* extract at 100 mg/kg bw and Ranferon® on the 14th day of treatment (D15). After 21 days of treatment (D22), the plant extract at both doses (100 and 200 mg/kg b.w.) and Ranferon® normalized this parameter, i.e. the values observed in treated rats reached those of normal control rats. MCHC levels increased during the treatment of anemic rats, but did not reach those of normal control rats in rats treated with *M. lucida* extract at the end of treatment, with a better effect than Ranferon®. Finally, MCV and MCH decreased during treatment until they reached the values of normal control rats at the end of treatment. This was the case for rats treated with 100 mg/kg b.w. extract as well as for those treated with Ranferon®. The partial or complete recovery of hematological parameters in anemic rats during 3 weeks of treatment suggests that *M. lucida* extract restores erythrocyte size and hemoglobin content, synonymous with normal cell proliferation and hemoglobin synthesis. These observations show that the aqueous root bark extract of *M. lucida* is effective in repairing phenylhydrazine-induced damage to red blood cells and therefore in the treatment of anemia. The results are in agreement with those of Oladiji et al (2007) who showed that *M. lucida* leaf extracts were able to restore hemoglobin levels in anemic rats. Since phenylhydrazine-induced hemolysis is due to free radical peroxidation of erythrocyte membrane lipids, the effect of *M. lucida* extract may be due to the presence of phenolic compounds, notably flavonoids within it. Flavonoids are powerful antioxidants that can prevent and repair free radical/reactive oxygen species-induced cellular oxidative damage (Shen et al., 2022). Sheth et al. (2021) reported that most anti-anemic compounds are known to scavenge free radicals and can improve anemia. Furthermore, the hematological parameters of the anemic control rats showed a progressive improvement during the treatment period of the other rats. This may be due to the body's ability to regenerate after phenylhydrazine-induced damage.

5. Conclusion

The aqueous root bark extract of *Morinda lucida* was evaluated for its anti-anemic potential in this study. The results showed that this extract is effective in repairing phenylhydrazine-induced erythrocyte damage and therefore in treating anemia by improving and correcting hematological parameters after two to three weeks of treatment. These findings suggest that this plant could provide a therapeutic alternative to conventional treatments for anemia.

Ethical approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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