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Research Article
Assessment of Antianemic Activity of *Xanthosoma mafaffa* Leaves in the Wistar Rat

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Abstract
Background and Objective: *Xanthosoma mafaffa* is a plant often found in Africa whose leaves are used in Togo to treat anemia. The objective of this study was to evaluate the therapeutic efficacy of these leaves in the correction of anemia. **Materials and Methods:** Hemolytic anemia was induced in rats using phenylhydrazine (40 mg/kg) on Days 0 and 1. Rats were divided into five groups: Normal control, negative control, positive control (Vitafer® 10 mL/kg) and test groups receiving *X. mafaffa* extract (500 or 1000 mg/kg) from Days 2 to 15. Blood samples were collected on Days 0, 2, 7, 10 and 15 for erythrocytic analysis, with osmotic resistance assessed on Day 7. Results were expressed as mean±SEM and analyzed using ANOVA with Dunnett's test ($p<0.05$). **Results:** The results revealed that administration of the extract and Vitafer® by force-feeding to rats pretreated with phenylhydrazine resulted in normalization of hemoglobin level, red blood cell count, hematocrit, mean red blood cell volume and mean corpuscular hemoglobin concentration compared to the normal and negative control groups. Moreover, the osmotic resistance of the blood cells of the anemic rats treated with the extract was greater than rats from the normal and negative control groups. **Conclusion:** This study thus shows that the leaves of *X. mafaffa* have antianemic activity. It also justifies the therapeutic use of these leaves to correct anemia in Togo.

Key words: Anemia, *X. mafaffa*, erythropoiesis, osmotic resistance, antianemic activity, Wistar rat

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anemia is a major public health issue in most developing countries. It is characterized by a decrease in the amount of circulating hemoglobin below 13 g/dL in men and 12 g/dL in women[1]. According to the World Health Organization, more than two billion people around the world suffer from anemia. The causes that predispose to anemia are multiple as nutritional deficiencies, blood parasites such as plasmodium, trypanosomes and helminthic infections as well as pregnancy and breastfeeding[1-4]. It profoundly affects the physical and cognitive development of young children and impairs their learning ability and their subsequent social and economic integration[2]. Its global prevalence is around 25%, with more cases in Africa (67.6%) and Southeast Asia (65.5%)[3]. Despite this knowledge of the causes of anemia, its consequences and the efforts devoted to solving this health problem, the scale of the problem remains significant. To fight against this pathology, people in developing countries are increasingly using medicinal plants[4]. Medicinal plants have been a source to control many health problems and anemia is not an exception. These plants occupy an important place in the first-line treatment of various pathologies[5]. Indeed, ethnopharmacological studies have shown that the use of several medicinal plants for the correction of anemia was common[6]. The antianemic efficacy of some of these plants has been proven through previous studies. This is the case, for example, of the leaves of *Brillantaisia nitens* and the roots of *Cocos nucifera*[6-7]. The same is true for the bark of *Dialium guineense*, the roots of *Olax subscorpioidea* and *Uvaria chamae*, the leafy stems of *Salacia pallescens* and the leaves of *Sorindeia warneckei*[8] and the powdered leaves of *Moringa oleifera*[9]. In Togo, the antianemic efficacy of *Tectona grandis* was even tested by Diallo *et al.*[10]. *Xanthosoma mafaffa*, a food plant, that is one of these many species used in traditional medicine, is also involved in the correction of anemia in Togo. However, no study has yet investigated the antianemic activity of *X. mafaffa* leaves. Furthermore, in addition to the use of this species in the correction of anemia, its leaves and tubers are used in the diet of the Togolese population[11]. Wada *et al.*[12] reported that *X. mafaffa* leaves have pharmacological properties in the treatment of hepatitis, rheumatoid arthritis and joint pain. Some studies also reveal that these leaves have antibacterial properties and are involved in the treatment of colds[12]. In addition, Go-Maro *et al.*[13] reported that *X. mafaffa* leaves have high nutritional potential with a richness in proteins, carbohydrates, fats, calcium, magnesium, potassium and iron. Bammité *et al.*[14] also reported that the leaves of this species are used in Togo to correct anemia. It is thus necessary to carry out a pharmacological study on the effect of these leaves in the correction of anemia in the interest of contributing to the valorization of this species of the Togolese flora. A toxicity test is also necessary to orient the users of the leaves of *X. mafaffa*. This is what justifies the present study on the evaluation of the antianemic activity of these leaves. This study aims to evaluate the antianemic potential of *X. mafaffa* leaves in Wistar rats. The effects on hematological parameters analyzed to determine their efficacy in treating anemia.

MATERIALS AND METHODS

Materials

Plant material: Fresh leaves of *X. mafaffa* were collected in April, 2019 from Ayomé, a locality in the Amou prefecture (Togo). Botanical authentication was confirmed at the Laboratory of Botany and Vegetal Ecology, Faculty of Sciences, University of Lomé (Togo) and the voucher specimen was deposited at the herbarium (N°TOGO 15661). The leaves were washed, dried under shade and were coarsely powdered. The powder was stored in a sealed plastic container, labeled and stored away from light and moisture until use.

Animals: The 40 Healthy Wistar rats, male and female weighing between 129 and 200 g were used in this study. They are produced at the Animal Physiology Department of the University of Lomé (Togo). The rats were kept under normal standard environmental conditions of temperature (25-28°C) with a photoperiod of 12 hrs (12 hrs of light and 12 hrs of darkness). The rats were allowed free access to food and water.

Ethical consideration: The experimental usage of the animals was approved by the Bioethics Committee for Health Research (CBRS) of the Togolese Ministry of Health, Public Hygiene and Universal Access to Care (N°033/2022/CBRS). They were acclimatized for two weeks before the experiments and fed with a normal pellet diet and water *ad libitum*. The room has been regularly cleaned to ensure optimal development of the animals.

Methods

Extraction: The powder (300 g) was macerated at room temperature with 3 L of ethanol-water (50:50 v/v) for 72 hrs as described by Falleh *et al.*[15]. The macerate was filtered through a very fine-pored sieve and the filtrate was concentrated under vacuum at 45°C by a rotary evaporator AnEssOil 2 L (USA) with Motor Lift Lab Equipment Digital Heating Bathtub with Specification: Voltage (110V/60HZ), Speed (20-200RPM/Min), Motor Power (40W), High Borosilicate Glass

3.3, Temperature Control Accuracy (±1°C), Temperature Control Range: Ambient Temperature (0-210/0-572°F), Rotary Bottle Capacity (2 L), Vacuum Degree (0.098Mpa), Evaporation Capacity (20 mL/min) and Lifting Distance (0-150 mm). The yield of hydroethanolic extract of *X. mafaffa* was 19.29%. The recovered hydroethanolic extract was stored in a tightly closed glass vial and protected from light.

Preliminary phytochemical screening: Phytochemical analysis of the leaves of *X. mafaffa* was carried out to detect the existence of different classes of phytoconstituents using the methods described by Mahendran *et al.*[16]. Total carbohydrates were detected according to the protocol described by Elzagheid *et al.*[17].

Induction of anemia: In this work, the experiment was carried out on rats of both sexes to detect possible effects of gender on hematological parameters.

Anemia was induced in rats by administration of phenylhydrazine(Acros Organics, Geel (Belgium) by oral route (force-feeding) at a dose of 40 mg/kg for two days (Day 0 and Day 1) according to the method described by Lee *et al.*[18]. Five groups of eight rats each, with four female rats and four male rats per group, were formed:

- **Group 1:** Was a normal control and received only distilled water between day 0 and day 15
- **Group 2:** Was anemic control, having received phenylhydrazine at 40 mg/kg for two successive days (day 0 and day 1) and distilled water from day 2 to day 15
- **Group 3:** Positive control having received phenylhydrazine at 40 mg/kg for two successive days (day 0 and day 1) then 1 mL/kg of vitafer® from day 2 to day 15
- **Group 4:** Received phenylhydrazine at 40 mg/kg for two successive days (day 0 and day 1), then 500 mg/kg of hydroethanolic extract of *X. mafaffa* leaves from day 2 to day 15
- **Group 5:** Received phenylhydrazine at 40 mg/kg for two successive days (day 0 and day 1), then 1000 mg/kg of hydroethanolic extract of *X. mafaffa* leaves from day 2 to day 15 (Fig. 1)

Hematological analysis

Blood parameters: Blood (2 mL) was collected from retro-orbital sinus under ether-anesthesia on day 0 before phenylhydrazine administration and after phenylhydrazine administration on days 2, 7, 10 and 15. Blood was analyzed with a hematological machine (Hematology BC 3000 Plus) for parameters like red blood cells, hemoglobin, hematocrit levels, mean globular volume, mean corpuscular hemoglobin content and mean corpuscular hemoglobin concentration.

Osmotic resistance of red blood cells: The blood samples were also used to evaluate the osmotic resistance of red blood cells on day 7. The method for determining the osmotic resistance of red blood cells consisted of inducing their lysis in a hypotonic solution on the 7th day. he method of Amin *et al.*[19] was thus used. Three concentrations of saline solution of 0, 4.5 and 9 g/L were prepared. Then, 10 μL of blood from each rat was introduced into 2 mL of each of the saline solutions. The mixture is lightly shaken and incubated for 60 min. After incubation, the mixture is centrifuged at 1090 rpm for 10 min. The supernatant is collected and the absorbance is read at 550 nm with a spectrophotometer. The percentage of hemolysis is determined by the following formula:

$$\text{Hemolysis (\%)} = \frac{\text{Absorbance read with solution}}{\begin{array}{c}\text{Absorbance read at complete hemolysis}\\ \text{with distilled water (0 g/L)}\end{array}}$$

Acute toxicity test: Male and female Wistar rats were used in this study according to the guidelines of the Organization for Economic Cooperation and Development (OECD) for acute oral toxicity tests[20]. All animals were fasted overnight, but with free access to water and weighed before administration of the extract. Then, the animals were randomly divided into four groups according to their sex (n = 8; 4 males and 4 females per group):

- **Group 1 (Control):** Received distilled water orally
- **Group 2:** Received the extract at 1000 mg/kg
- **Group 3:** Received the extract at 2000 mg/kg body weight
- **Group 4:** Received the extract at 5000 mg/kg

The rats were fed and hydrated *ad libitum* and then weighed daily for 14 days. After the force-feeding, the rats of all the groups were observed minutely for 30 min and then after 1, 2, 3, 24 hrs and every day until the 14th day to note any changes related to eating habits and morbidity. The LD_{50} should be greater than 5000 mg/kg if the majority of rats survive. At the end of the experimental period, all animals were weighed, sacrificed and some organs were removed for observation.

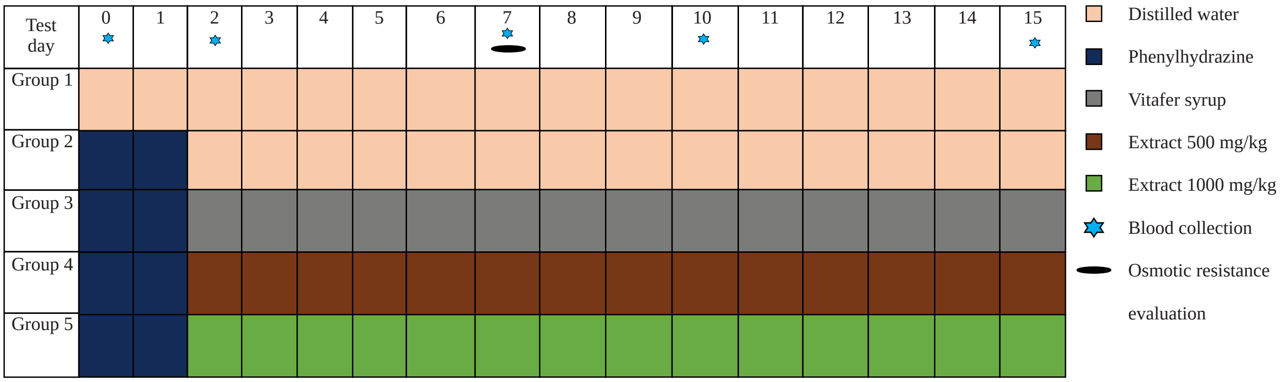

Fig. 1: Chronogram of the experiment

Statistical analysis: The results obtained are presented as the mean±standard error of the mean. They are processed using Graph Pad Prism 8.00 software (Graf Pad Software Inc. California), which was also used to construct the curves. Analysis of variance (ANOVA) followed by Dunnet's test was used to compare the means against the control. The differences were considered significant at the 5% level ($p<0.05$).

RESULTS

Hematimetric parameters of rats

Evolution of hemoglobin level: The mean hemoglobin level of the different groups of male rats on D_0 was between 13.15±0.05 and 14.05±0.95 g/dL with a non-significant difference (Fig. 2a). It was the same in the females, where this rate was between 13.15±0.05 and 14.25±0.25 g/dL (Fig. 2b). The administration of phenylhydrazine to the rats of the test groups caused a significant drop ($p<0.05$ and $p<0.01$) in the hemoglobin level of D_2 in these male and female rats compared to the normal control groups (Fig. 2a-b). From D_2, the hemoglobin level gradually increased in anemic rats treated with Vitafer® and *X. mafaffa* leaf extract at a dose of 1000 mg/kg. The hemoglobin contents of the two groups thus caught up with the normal control on D_7 and exceeded it on D_{10} with a significant difference ($p<0.05$) for the group treated with the extract of *X. mafaffa* leaves at the dose of 1000 mg/kg. On D_{15}, the hemoglobin levels of the rats treated with Vitafer® and those treated with *X. mafaffa* extract at a dose of 1000 mg/kg were significantly higher than those of the control group ($p<0.05$). The increment in hemoglobin was also observed with anemic rats treated with *X. mafaffa* extract at a dose of 500 mg/kg. However, this increment was slow and the average hemoglobin level of this group only exceeded that of the control group on D_{15} without significant difference (Fig. 2a). In female rats, the hemoglobin levels of the groups treated with Vitafer® and *X. mafaffa* extract at a dose of 1000 mg/kg did not significantly exceed ($p<0.05$) the control batch until D_{15}. As for the hemoglobin level observed in rats treated with *X. mafaffa* extract at a dose of 500 mg/kg, it did not reach the control group in female rats even on D_{15}. However, this rate was slightly higher than the anemic control lot (Fig. 2b). In the untreated anemic group, the hemoglobin level was significantly low ($p<0.05$ and $p<0.01$) compared to the control group up to D_{15}. In the non-anemic group, the mean hemoglobin level did not change significantly during the experiment (Fig. 2a-b).

Overall, the results show that the rats given the hydroethanolic extract of *X. mafaffa* leaves almost completely recovered on D_{15} ($p<0.01$) for both doses, with fast recovery for the dose of 1000 mg/kg compared to the control rats that received Vitafer®.

Evolution of red blood cell count: The mean number of red blood cells of the different groups of male rats on D_0 was between 6.83±0.17 and 8.33±0.66 g/L without significant difference. It was the same in the female rats, where this rate was between 6.74±0.44 and 8.03±0.35 g/L.

On D_2, in male rats, the number of red blood cells significantly decreased following hemolysis induced by phenylhydrazine and varied from 8.27±0.02 to 3.05±0.5 g/L in the anemic groups compared to the group control (Fig. 3a). On the other hand, on D_2, this variation was from 3.51±0.53 to 2.27±0.70 g/L in female rats (Fig. 3b). Indeed, the administration of phenylhydrazine caused on D_2 a significant decrease in the number of red blood cells in groups 2 and 3 ($p<0.01$) and in groups 4 and 5 ($p<0.05$) compared to control (Fig. 3a). From D_2 to D_{15}, the red blood cell level increased in the treated groups in both male and female rats (Fig. 3a-b).

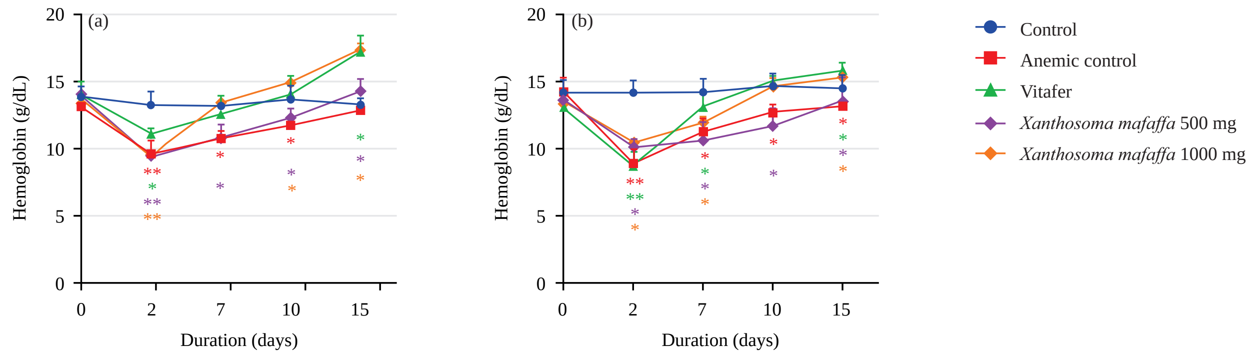

Fig. 2 : Effect of the extract on changes in hemoglobin levels in rats, (a) Male and (b) Female

Results are expressed as the mean±SEM (n = 4), Significantly different from the control: *p<0.05 and **p<0.01

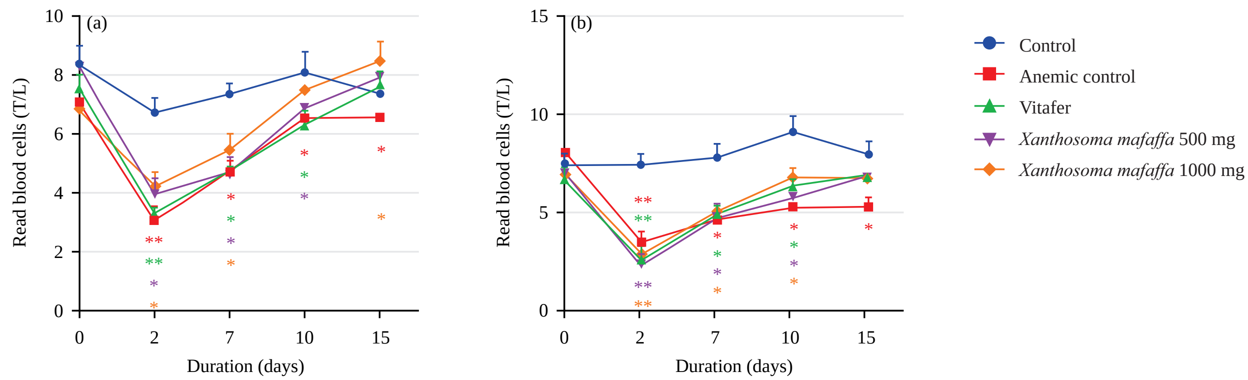

Fig. 3: Effect of the extract on the change in the number of red blood cells in rats, (a) Male and (b) Female

Results are expressed as the mean±SEM (n = 4). Significantly different from the control: *p<0.05 and **p<0.01

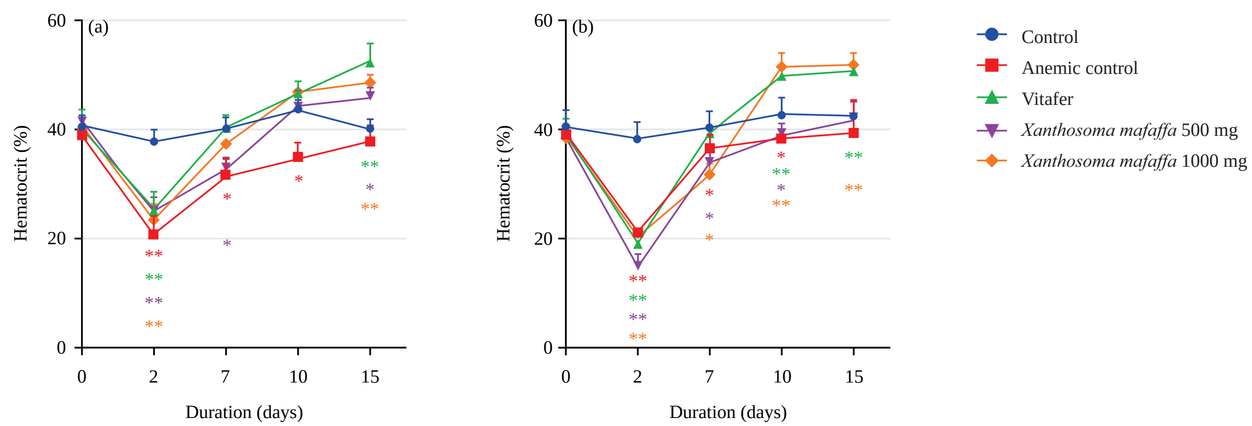

Fig. 4: Effect of the extract on the evolution of hematocrit in rats (a) Male and (b) Female

Results are expressed as the mean±SEM (n = 4), Significantly different from the control: *p<0.05 and **p<0.01

On D_{15}, the number of red blood cells of the rats in the treated groups caught up and exceeded the rats of the normal control group with a significant increment for the group treated with the extract at the dose of 1000 mg/kg (Fig. 3a).

In female rats, even on D_{15}, the number of red blood cells in the treated groups did not exceed the normal control group (Fig. 3b).

In the groups of untreated anemic rats, male and female, the increase in the number of red blood cells after D_2 was significantly low (p<0.05) compared to the normal control and to what was observed with the treated groups (Fig. 3a-b). In the non-anemic groups, the mean number of red blood cells did not undergo a significant variation during the test in both male and female rats compared to the normal control (Fig.3a-b).

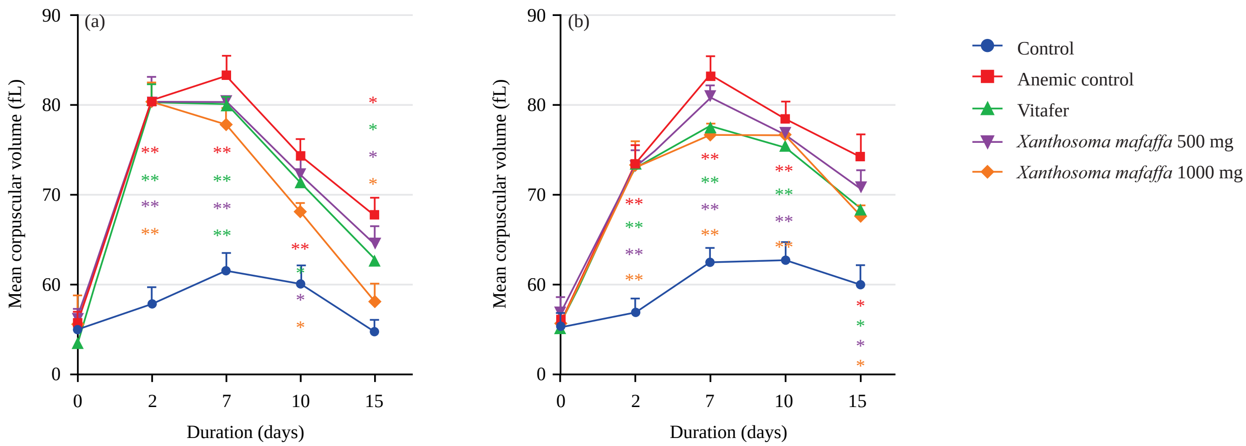

Fig. 5: Effect of the extract on the change in mean corpuscular volume in rats, (a) Male and (b) Female
Results are expressed as the mean±SEM (n = 4), Significantly different from the control: *$p<0.05$ and **$p<0.01$

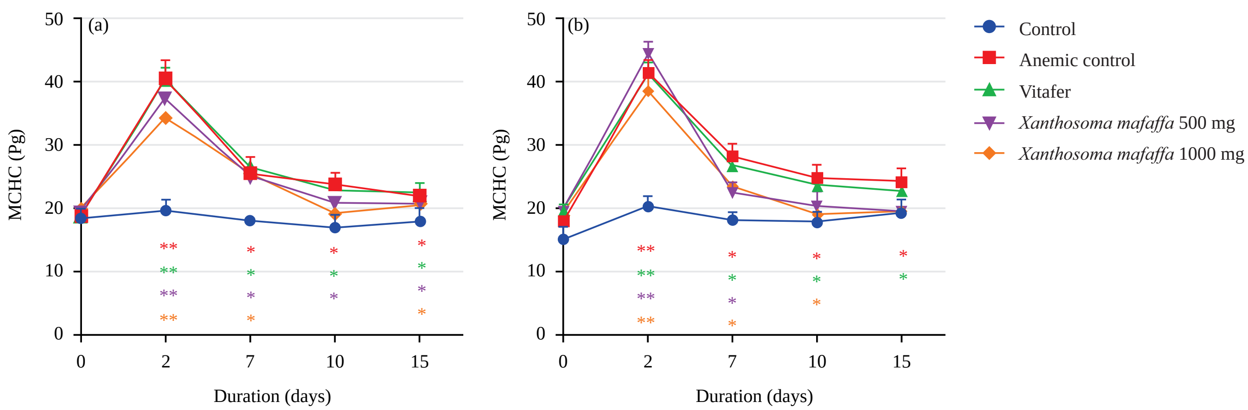

Fig. 6: Effect of the extract on the change in the mean corpuscular hemoglobin content in rats, (a) Male and (b) Female
Results are expressed as the mean±SEM (n = 4), Significantly different from the control: *$p<0.05$ and **$p<0.01$

Evolution of hematocrit: The mean hematocrit was between 39.25±0.05 and 41.55±0.95% in male rats and between 40.85±3.05 and 38.60±2.20 % in female rats in the different groups at D_0 without significant difference. The administration of phenylhydrazine significantly reduced the hematocrit on D_2 (Fig.4a-b). This resulted in D_2, a significant decrease ($p<0.01$) in the different groups compared to the normal control group (Fig. 4a-b). After treatment, on D_7, a significant increment in hematocrit was observed in the rats of the groups treated with the extract ($p<0.05$) (Fig. 4a-b). On D_{10}, in male rats, the difference was significant ($p<0.05$) only in the anemic control (Fig. 4a). On the other hand, on D_{10}, in female rats, the difference was significant in the groups treated with Vitafer® ($p<0.01$), at the dose of 500 mg/kg ($p<0.05$) of extract and 1000 mg/kg ($p<0.01$) compared to the control (Fig. 4b). On D_{15}, the difference compared to the control was significant ($p<0.01$) in the anemic groups treated with Vitafer® and the extract at a dose of 1000 mg/kg (Fig. 4a-b) and for the group treated with the extract at a dose of 500 mg/kg ($p<0.05$) (Fig. 4a). Results showed that rats treated with *X. mafaffa* extract returned to normal hematocrit levels compared to the control.

Evolution of the mean corpuscular volume (MCV): The variation in MCV was about 53.60±1.10 in male rats and between 56.65±2.01 and 55.30±1.60 fL in female rats in the different groups of rats on D_0 with a non-significant difference.

In male rats, the mean red blood cell volume increased significantly ($p<0.01$) in the different anemic groups and reached its peak on D_2 in group 5 and then in groups 2, 3 and 4 on D_7 compared to the control (Fig. 5a). On the other hand, in female rats, following the administration of phenylhydrazine, the MCV significantly increased ($p<0.01$) in the different groups and reached its peak on D_7 compared to the control (Fig. 5b). On D_{10}, in male rats, a significant decrease

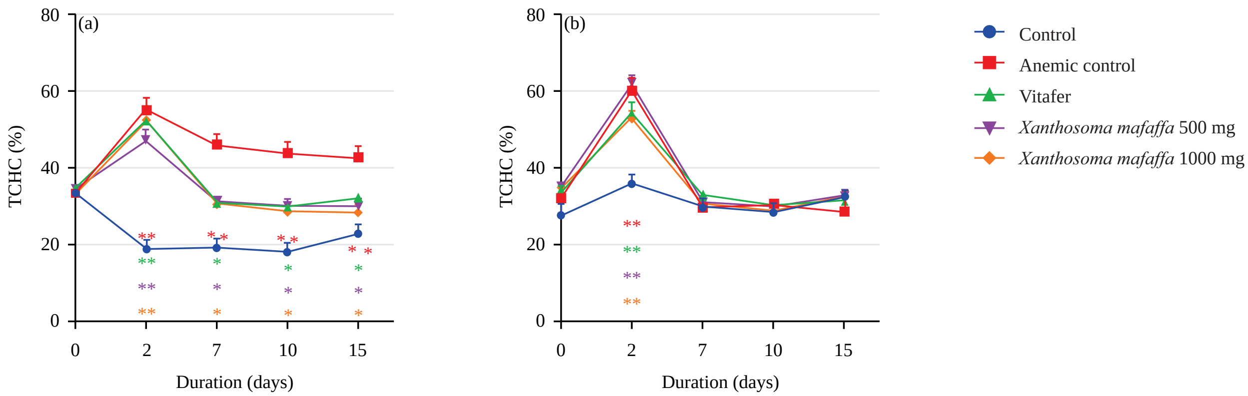

Fig. 7: Effect of the extract on the change in the mean corpuscular hemoglobin concentration in rats, (a) Male and (b) Female
Results are expressed as the mean±SEM (n = 4), Significantly different from the control: *p<0.05 and **p<0.01

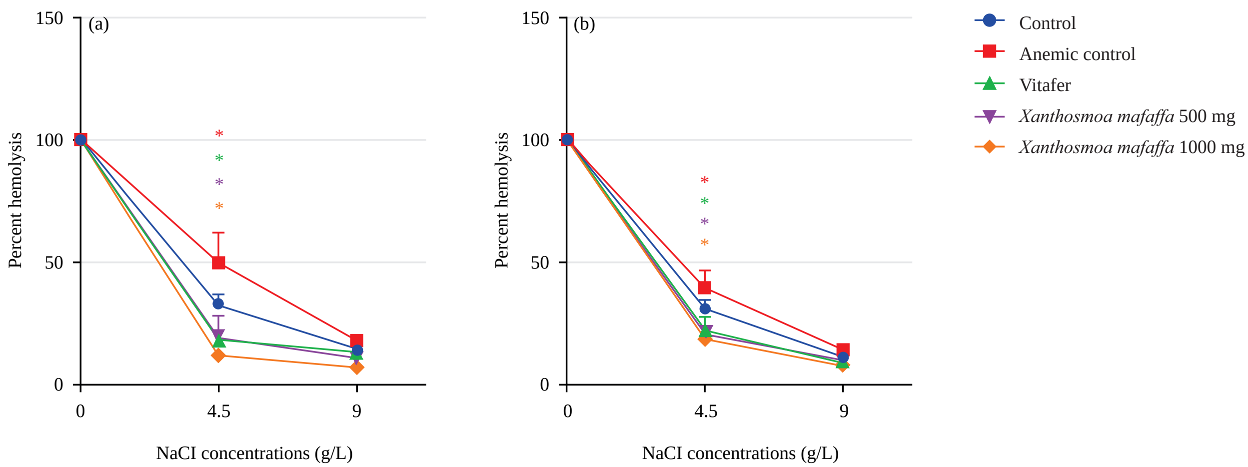

Fig. 8: Effect of the extract on the development of osmotic resistance of red blood cells in rats, (a) Male and (b) Female
Results are expressed as the mean±SEM (n = 4), Significantly different from the control: *p<0.05 and **p<0.01

in mean red blood cell volume was noted in groups 3, 4 and 5 (p<0.05) than in group 2 (p<0.01) compared to the control (Fig. 5a). On D_{10}, in female rats, a significant decrease (p<0.01) was noted in all the treated groups compared to the control (Fig. 5b). On D_{15}, the MCV decreased significantly (p<0.05) in the different anemic groups compared to the control with a greater decrease for the group treated with the extract of *X. mafaffa* at the dose of 1000 mg/kg followed by the group treated with Vitafer® (Fig. 5). In the control lot, the MCV changed very little during the test (Fig. 5a-b). Overall, the results showed that rats treated with hydroethanolic extract from the leaves of *X. mafaffa* saw their MCV return to normal compared to the control.

Evolution of the mean corpuscular hemoglobin content: The mean corpuscular hemoglobin content in males was between 18.35±0.15 and 19.85±0.05 pg and between 19.65±0.75 and 15.15±2 pg in females on D_0 in all groups of rats without significant difference. Following the administration of phenylhydrazine, it significantly increased (p<0.01) to reach its peak on D_2 in all the groups treated compared to the control (Fig. 6a-b). From D_2 to D_{15}, a decrease in the mean corpuscular hemoglobin content was observed in all the lots compared to the control group (Fig. 6a-b).

Evolution of the mean corpuscular hemoglobin concentration: On D_0, in all anemic groups, the variation in the mean corpuscular hemoglobin concentration was between 33.15±0.25 and 34.80±0.60 % in male rats and between 34.85±0.15 and 32±1.40 % in female rats. So, there was not a significant difference between these values on D_0. After administration of phenylhydrazine, it significantly

Table 1: Phytochemical screening of the hydroethanolic extract of the leaves of *X. mafaffa*

Chemical compound	Detection
Alkaloids	+
Catechetical tannins	+
Gallic tannins	+
Cardiac glycosides	+
Total carbohydrates	+
Saponins	+
Sterols and triterpenes	+
Flavonoids	+
Free quinones	+
Coumarins	+
Reducing sugar	+

Legend: +means presence

Table 2: Mass gain in rats at the end of the antianemic test

	Test	Non anemic control	Anemic control	Batch treated with Vitafer®	Extract at 500 mg/kg/day	Extract at 1000 mg/kg/day
Batch of male rats	Mass gain (g)	12.7±0.11	10.9±0.05	12.35±0.02	13.2±0.17	14.3±0.17
	% increase in mass	9.81±0.28	7.52±0.46	8.52±0.23	7.95±0.25	9.87±0.34
Batch of female rats	Mass gain (g)	14±0.11	11.8±0.01	13.3±0.01	14.2±0.01	14.5±0.01
	% increase in mass	13±0.28	11.13±0.28	14.99±0.34	11.65±0.17	13.67±0.40

Results are expressed as the mean±ESM (n = 4)

increased ($p<0.01$) in all anemic groups to reach its peak on D_2 compared to the control (Fig. 7a-b). From D_2 to D_{15}, it was noted in male rats, a significant decrease ($p<0.05$) in the groups treated with Vitafer® and extract compared to the control (Fig. 7a). During the same period, in female rats, mean corpuscular hemoglobin concentration also experienced a decrease without significant difference (Fig. 7b). In the anemic control the decrease was low ($p<0.01$) and the mean corpuscular hemoglobin concentration could not return to its initial value compared to the control (Fig. 7a).

The results show that the rats treated with the hydroethanolic extract of the leaves of *X. mafaffa* saw their mean corpuscular hemoglobin concentration return to normal compared to the control.

Evolution of the osmotic resistance of erythrocytes on day 7: The determination of the osmotic resistance showed that the hemolysis of red blood cells is complete in distilled water. In male rats, the concentrations of NaCl at 50% hemolysis are 4.5 g/L for anemic control rats, 4 g/L for normal rats, 3.75 for rats treated with Vitafer® and extract at 500 mg/kg then 3 g/L for the rats treated with the extract at a dose of 1000 mg/kg (Fig. 8a). On the other hand, in female rats, the concentrations of NaCl at 50% hemolysis are 4.3 g/L for the anemic control rats, 3.8 g/L for the normal rats, 3 g/L for the treated rats with Vitafer® and with the extract at a dose of 500 mg/kg then 2.9 g/L for the rats treated with the extract at 1000 mg/kg (Fig. 8b).

Preliminary phytochemical screening of the hydroethanolic extract of the leaves of *X. mafaffa:* The phytochemical screening of the hydroethanolic extract of the leaves of *X. mafaffa* revealed the presence of alkaloids, flavonoids, gallic tannins acid, catechetical tannins, saponins, total carbohydrates, coumarins, reducing compounds, triterpenes, sterols, cardiac glycosides and free quinones (Table 1).

Increase in mass during the antianemic test: Table 2 shows the gain and percent increase in mass of the rats at the end of the antianemic test. The results show that male and female rats treated with the extract at doses of 500 and 1000 mg/kg experienced an increment in mass with no significant difference from the control.

Assessment of the acute toxicity of the hydroethanolic extract of the leaves of *X. mafaffa*

Observations: Acute toxicity assessment indicated that at doses of 1000, 2000 and 5000 mg/kg, rats showed no signs of toxicity after administration of the hydroethanolic extract of the leaves of *X. mafaffa* during the 14 days of observation. In fact, during this period, no signs of morbidity related to coat, tremors, grooming, breathing, appearance of stools, mobility, eating habits and mass of rats. Moreover, no death has been recorded.

Relative weight of organs: Figure 9 and 10 shows the effects of the hydroethanolic extract of the leaves of *X. mafaffa* on target organs of toxicity in male and female rats, respectively.

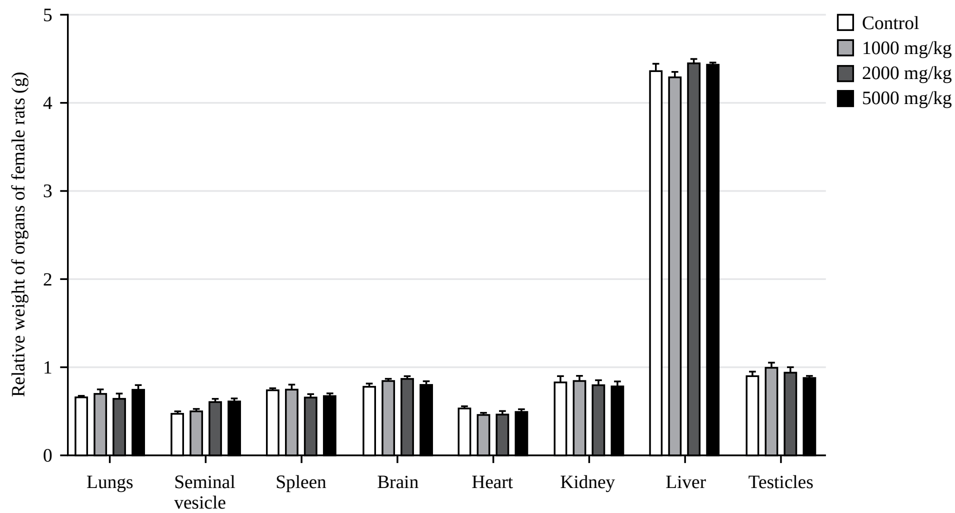

Fig. 9: Histograms showing the relative mass of organs from male rats collected at the end of the acute toxicity assay
Results are expressed as the mean±ESM (n = 4)

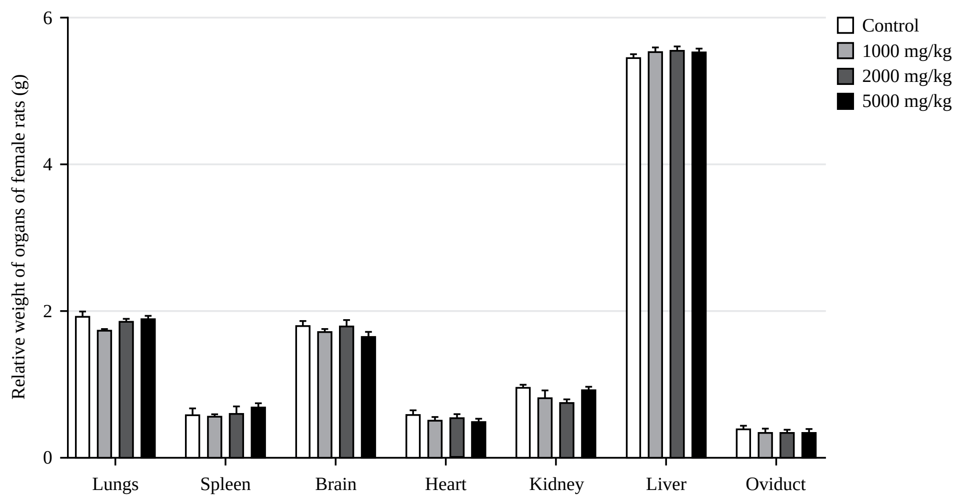

Fig. 10: Histograms showing the relative organ mass of female rats at the end of the acute toxicity assay
Results are expressed as the mean±ESM (n = 4)

The results show that the three doses of extracts did not significantly change the weight of the target organs of toxicity compared to control rats in both male and female rats.

DISCUSSION

This work aimed to evaluate the antianemic properties of the hydroethanolic extract of the leaves of *X. mafaffa*. The hemoglobin and red blood cell content and the hematocrit content obtained before the induction of anemia by phenylhydrazine varied relatively from one group to another while remaining within the regulatory limits (Fig. 2, 3 and 4).

Administration of phenylhydrazine caused a significant decrease in hemoglobin content, the main indicator of anemia, in all rats that underwent the treatment (Fig. 2a-b). This decrease is explained by the hemolysis induced by phenylhydrazine (Fig. 2a-b). results agree with those of Diallo *et al.*[10] who also observed a decrease in hemoglobin level with the administration of phenylhydrazine (40 mg/kg) in Wistar rats. In this study, from D_7 to D_{15}, the rats that received the hydroethanolic extract of *X. mafaffa* leaves had their hemoglobin content significantly increased compared to the anemic control group (Fig. 2a-b). Previous work[21,22] has also shown the reversibility of the anemia induced by phenylhydrazine on discontinuation of administration with the aqueous extract of the leaves of *Sorghum bicolor* and *Justicia secunda*, respectively. This reversibility of the anemia would be due to the stimulation of the synthesis of hemoglobin by the hydroethanolic extract of leaves of *X. mafaffa* concerning its phytochemical composition (Table 1) which indicates the presence of flavonoids, alkaloids, tannins, saponins[20].

Hemoglobin is a pigment in red blood cells for which any change in the content directly involves red blood cells. As in the case of hemoglobin, the number of red blood cells thus decreased on D_2 following the hemolysis induced by phenylhydrazine (Fig. 3a-b). But this decrease was quickly corrected with the extract of *X. mafaffa* from D_7 to D_{15} (Fig. 3a-b). This is explained by a stimulation of erythropoiesis by the extract as indicated by previous work[10-23], which also obtained an increment in the number of red blood cells following the administration of extracts from the leaves of *Tectona grandis*, *Sorghum bicolor*, *Justicia secunda* and fruits of *Solanum torvum,* respectively after administration of phenylhydrazine.

Hematocrit is the level of red blood cells per unit of blood volume. As in the case of red blood cells, phenylhydrazine caused a decrease in hematocrit on D_2 (Fig. 4a-b). However, this decrease was corrected by administration of the extract, indicating stimulation of erythropoiesis (Fig. 4a-b). The synthesis of young red blood cells and their release into the bloodstream therefore gradually increased the hematocrit from D_2 to D_{15} as observed by Boua *et al.*[23] with the extract of the fruits of *Solanum torvum*.

Current results showed that the hydroethanolic extract of leaves of *X. mafaffa* normalized the level of hemoglobin, red blood cells and hematocrit with activity relatively similar to that of Vitafer® at the dose of 1000 mg/kg.

Mean corpuscular volume (MCV) increased significantly on D_2 after administration of phenylhydrazine, indicating macrocytosis (Fig. 5a-b). This result is in agreement with that of Tchogou *et al.*[7], who also observed an increment in MCV following the administration of phenylhydrazine in their study on the vetting of anemia with aqueous extract from the roots of *Cocos nucifera*. This increment in MCV reflects a release of immature erythrocytes into the bloodstream according to Tchogou *et al.*[7] However, from D_7, a progressive decrease in MCV was observed, which evolved towards what was observed with the control group (Fig. 5a-b). This significant decrease is explained by normocytosis characterized by the marked differentiation of erythrocytes[23].

The mean corpuscular hemoglobin content, for its part, underwent a development similar to the mean corpuscular volume (Fig. 6a-b). In fact, on D_2, the mean corpuscular hemoglobin content significantly increased following the administration of phenylhydrazine. This is explained by the macrocytosis induced by phenylhydrazine. However, from D_7 onwards, a gradual decrease in the mean corpuscular hemoglobin content was observed until $D_{15,}$ when it was close to what was observed with the control. This variation is also explained by normocytosis due to an increasingly marked maturation and differentiation of erythrocytes. The erythropoietic mechanism of the hydroethanolic extract of the leaves of *X. mafaffa* corrected anemia, as in the case of the aqueous extract of the leaves of *Parquetina nigrescens*[24].

The mean corpuscular hemoglobin concentration also experienced a similar evolution to the mean corpuscular volume with a significant increase due to the macrocytosis induced by the administration of phenylhydrazine (Fig. 7a-b). However, from D_7 onwards, the mean corpuscular hemoglobin concentration gradually decreased until $D_{15,}$ when it was close to the witness. The variation in this parameter is explained by normocytosis due to increasingly marked maturation and differentiation of erythrocytes[21-24].

The hydroethanolic extract of the leaves of *X. mafaffa* induced normalization of the mean corpuscular hemoglobin content, the mean corpuscular hemoglobin concentration and the mean corpuscular volume. The extract therefore normalized erythrocyte size and hemoglobin content. These results show that the hydroethanolic extract of the leaves of *X. mafaffa* stimulated hematopoiesis via erythropoietin. Erythropoietin is a hormone that regulates the production of red blood cells. This hormone therefore increases the number of sensitive erythroblasts in the bone marrow which are transformed into young reticulocytes and later into mature erythrocytes[23].

The erythropoietic mechanism of the hydroethanolic extract of the leaves of *X. mafaffa* thus corrected macrocytosis as well as anemia. This was confirmed by the osmotic resistance of red blood cells.

Indeed, this osmotic resistance was proportional to the level of red blood cells in the bloodstream. It was evaluated on D_7 according to the Redondo protocol[19]. The results showed that the hemolysis of the red blood cells of the rats given by force-feeding the phenylhydrazine and treated with the extract was lower than that of the normal control group's rats (Fig. 8a-b). On the other hand, their osmotic resistance was higher. This observation is in agreement with that of Sènou *et al.*[21] and Adewole *et al.*[22]. The 50% hemolysis in normal rats and untreated anemic rats being greater than that observed in anemic rats treated with the extract of *X. mafaffa*, there are more hemolysis resistant cells in extract treated rats than in untreated rats. This increment in the osmotic resistance of rat erythrocytes is due to the presence of young cells in the blood[25].

Administration of *X. mafaffa* extract, therefore, increased the production of young red blood cells by stimulating erythropoiesis.

The antianemic activity observed is linked to the phytochemical constituents contained in the leaves of *X. mafaffa*, which was revealed by the preliminary screening. Indeed, this preliminary screening revealed the presence of major phytochemical groups such as alkaloids, gallic tannins, catechin tannins, flavonoids, saponins and alkaloids (Table 1). These compounds have been shown to have antioxidant power, promoting tissue regeneration, reducing the permeability of blood capillaries and reinforcing their resistance to hemolysis[23-26]. Flavonoids, for example, can directly stimulate hematopoiesis, probably via erythropoietin[23]. The reversibility of phenylhydrazine-induced anemia is, therefore, attributable to the richness of *X. mafaffa* in these phytochemical compounds. These results are in agreement with those obtained with extracts from the leaves of *Tectona grandis*[10], *Mangifera indica*, *Amaranthus hybridus* and *Telfairia occidentalis*[27,] which significantly increased the hemoglobin concentration and the red blood cell number after induction of anemia with phenylhydrazine.

The hemolysis induced by phenylhydrazine is attributable to oxidative stress according to previous studies. Given the presence of reducing compounds in the leaves of *X. mafaffa*, one could then think about protection against membrane lipoperoxidation, which can induce lysis of erythrocytes and significantly reduce bone marrow activity[28-29]. However, this mechanism of action remains to be confirmed concerning the current study.

The acute toxicity assay was carried out to guide users of this vegetable. The administration of doses of extract of 1000, 2000 and 5000 mg/kg to healthy rats showed no signs of toxicity linked to the coat, tremors, appearance of the stools, eating habit and mobility. The extract would, therefore have a lethal dose 50 (LD_{50}) greater than 5000 mg/kg compared to OECD standards[20]. Furthermore, the extract did not significantly modify the relative weight of the organs removed (Fig. 9 and 10) as well as the weight of the animals (Table 2), thus revealing its harmlessness. Although this study provides an initial scientific approach to validate the use of *X. mafaffa* for anemia, it merits further investigation. To strengthen the reliability of the results, it is therefore relevant to include biochemical analysis, histopathological studies and comparative groups with other standard treatments. Furthermore, mechanistic studies on iron absorption and red blood cell production would provide a better understanding of *X. mafaffa*'s mode of action.

CONCLUSION

This work has shown the antianemic efficacy of *X. mafaffa* leaves. Administration of phenylhydrazine, by force-feeding, to rats produced hemolytic anemia characterized by a decrease in hemoglobin content, in hematocrit and red blood cell number. Administration of *X. mafaffa* extract by force-feeding at doses of 500 and 1000 mg/kg corrected the anemia caused by phenylhydrazine in male and female rats. The hydro-ethanolic extract of *X. mafaffa* leaves is, therefore, effective against anemia. The extract, due to the properties of the major phytochemical groups it contains, has, through the regeneration of young cells, improved the resistance of blood cells to hemolysis.

This extract appears to enhance erythropoiesis and facilitates the early compensatory response to anemia by promoting the release of immature red blood cells into circulation. Although the precise mechanism underlying this effect remains to be determined, it may involve protection against oxidative stress induced by phenylhydrazine. Consequently, *X. mafaffa* could be considered a potential candidate for development into improved traditional medicine, provided its biological tolerance is assessed and validated through appropriate clinical trials.

SIGNIFICANCE STATEMENT

Anemia is a major health issue, requiring effective and accessible therapeutic alternatives. The leaves of *Xanthosoma mafaffa* are traditionally used for their medicinal properties, but their effectiveness against anemia has not been scientifically validated. This study evaluated their anti-anemic potential based solely on hematological parameters and the osmotic resistance of red blood cells. The hydroethanolic extracts of the leaves corrected hemolytic anemia induced in Wistar rats by phenylhydrazine through the restoration of hemoglobin levels and erythrocyte osmotic resistance.

This research opens new perspectives for phytotherapy and the fight against anemia by combining traditional knowledge with scientific validation. From a scientific standpoint, it provides new data on the pharmacological properties of *X. mafaffa*, a plant potentially used in traditional medicine and confirms its effectiveness in treating anemia. This paves the way for further research in pharmacology and natural medicine.

The identification of this species as an effective natural alternative offers an accessible and affordable therapeutic solution for populations in the management of anemia. Beyond the mechanism of action, additional studies will be necessary to identify the bioactive compounds responsible for the anti-anemic effect. Furthermore, clinical trials in humans could be considered to confirm the efficacy and safety of *X. mafaffa* in the treatment of anemia.

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