

The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X
JPHYTO 2019; 8(5): 216-219
September- October
Received: 24-08-2019
Accepted: 11-10-2019
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doi: 10.31254/phyto.2019.8502

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Study of antianemic properties of *Parquetina nigrescens* (*Apocynaceae*) in wistar rats

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ABSTRACT

Stability *Parquetina nigrescens* is a plant of the family *Apocynaceae*. She is commonly called "baba niama" in Boulé, and she is used in Côte d'Ivoire in the treatment of anemia in a traditional environment. Our study aims to know the different chemical components of the plant and test the antianemic properties of the total aqueous Extract on Wistar rats, after induction of anemia by phenylhydrazine hydrochloride. Phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids, polyphenols, and polyterpenes and iron. The administration of the aqueous extract by gavage to the anemic rats at doses of 2000 mg/kg/day and 2500 mg/kg/day resulted in an increase in hemoglobin, in the number of red blood cells and a decrease in reticulocyte levels. Hemolysis of *Parquetina nigrescens* extract-treated rats was lower than that of untreated rats, while their osmotic resistance was high. This indicates the presence of young red cell cells in the treated rats than in the control rats. These results confirm and validate the therapeutic indication of *Parquetina nigrescens* in the fight against anemia in traditional medicine.

Keywords: *Parquetina nigrescens*, anemia, hemoglobin, reticulocytes, osmotic resistance.

INTRODUCTION

The use of plants for therapeutic purposes is a centuries-old practice. In this [1] affirmed: "Very early, during the evolution, the men, to take care, used the resources present in their natural environment". Several works have been carried out on the biological properties of extracts of certain plants and have allowed the discovery of many active ingredients used in modern medicine. In the rich and diverse flora of Benin and Cote d'Ivoire, plants such as *Hibiscus sabdariffa*, *Tectona grandis*, *Justicia secunda*, *Alchornea cordifolia*, *Justicia secunda Vahl* and many other plants are known to have resorption effects on the skin anemia [2, 3]. Anemia, which results in lower hemoglobin levels below 12 g/dL, is common in tropical environments; 10 to 20 % of the population has less than 10 µL hemoglobin [4]. These plants could be used in place of pharmaceuticals becoming increasingly expensive for the indigenous population [5]. In this work, we propose to study the antianemic properties of *Parquetina nigrescens* Afzel (*Apocynaceae*), a plant that contains chemicals that may have antianemic properties.

MATERIAL AND METHOD

Animal material

The animal material consists of male and female rats, Wistar strain of average body weight 184.47 g. These animals come from the animal laboratory of the Laboratory of Animal Physiology of the Training and Research Unit (UFR) Biosciences, Felix University Houphouet-Boigny of Abidjan. They are kept in plastic cages with stainless steel covers containing litter of wood chips renewed every other day. The animals are fed regularly and received drinking water as tap water in stainless steel bottles.

Plant material

It consists of a total aqueous extract of the leaves of *Parquetina nigrescens* Afzel harvested in Godjiboué, in a village of Sassandra (Central West of Côte d'Ivoire), in June 2010. The plant is identified and authenticated at the National Center of Floristry (CNF) Felix Houphouet Boigny University of Cocody Cote d'Ivoire by the number of the following Herbarium: AKE Assi 15031 Gautier 586.

Preparation of the extract

The preparatory phase consisted first of all in the preparation of the total aqueous extract, then to the chemical analysis of the plant, based on differential reactions of coloration and precipitation, according to the method of [6].

Experimental protocol

Four batches of 4 rats were constituted: white control rats, negative controls, anemic rats receiving 2000 mg of extract per kg of body weight (lot D1) and rats receiving 2500 mg of extract per kg of weight per day (lot D2).

During the experimental phase, we first took the blood from the rats by puncturing the tail veins, induced the anemia by the intraperitoneal administration of 20 mg/kg/day of the phenylhydrazine hydrochloride [7] for five days (D1 to D5). We also administered rats D1 and D2, 2000 mg/kg and 2500 mg/kg of the total aqueous extract of *Parquetina nigrescens* Afzel by gavage for seven (7) days. The haematological parameters and bilirubin [8, 9, 10] were assayed at the end of the seven days at the five batches of rats: Lot T (healthy control), Lot Ta (Anemic rats control), ANT lot (untreated anemic rats), D1 lot (2000 mg/kg.pc dose) and D2 lot (2500 mg/kg.pc) using an automated blood cell counter (Sysmex KX 21) and the variations have been determined. Finally, we studied the osmotic resistance of the five batches of rats constituted: lot Ts, lot Ta, lot ANT, lot D1 and lot D2, according to the protocol of [11] at day 7.

Statistical analysis

The graphical representation of the data was performed using the Graph Pad Prism 5.0. The mean value is accompanied by the standard error of the mean (mean ± SEM). The difference between the two values is considered significant when $P < 0.001$. The statistical analysis of the results was performed using analysis of variance (ANOVA).

RESULTS

Effects on hematological parameters

Hemoglobin

Administration of phenylhydrazine caused a significant ($p < 0.001$) decrease in hemoglobin level in rats at day 5. After treatment a gradual recovery is obtained the following days (Figure 1). The results show that, on the one hand, rats receiving the total aqueous extract of *Parquetina nigrescens* Afzel almost completely recovered on day D7 for both doses and, on the other hand, that the dose of 2500 mg/kg/bw allows faster recovery. In untreated anemic rats (ANT), the decrease in hemoglobin is highly significant ($p < 0.001$), ie 13.15 % ± 0.078.

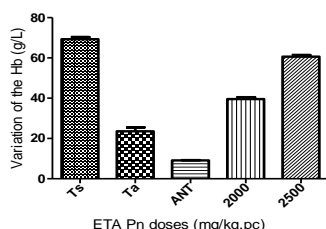


Figure 1: Change in hemoglobin levels of rats during treatment of anemia versus doses of ETA from Pn

Ts: Healthy witness; Ta: Anemic witness; ANT: Untreated anemia; Hb: hemoglobin; ETA: Aqueous total extract; Pn: *Parquetina nigrescens*

Red blood cells

After injection of the phenylhydrazine, there was a decrease in red blood cells in the three lots (22.92 % ± 2.35) on day D5. An increase in the number of red blood cells is observed after treatment on the

following days. The results show that the D1 lot rats almost recovered on day 7 (64.91 % recovery) and that those in batch D2 completely recovered on day 7 (99.73 % recovery) (Figure 2). On the other hand, the recovery rate is only 0.93 % in untreated anemic control rats. The results also show that the 2,500 mg/kg.bw extract gives better recovery compared to the 2000 mg/kg bw dose.

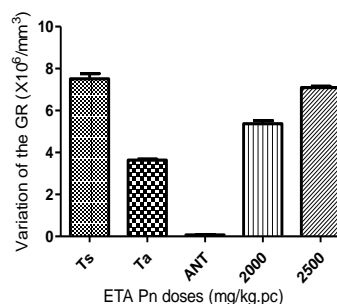


Figure 2: Variation in red blood cell levels of rats during treatment of anemia versus doses of ETA from Pn

Ts: Healthy witness; Ta: Anemic witness; ANT: Untreated anemia GR: Red blood cells; ETA: Aqueous total extract; Pn: *Parquetina nigrescens*

Reticulocytes

Administration of phenylhydrazine increased the level of reticulocytes on day 5. This increase is 47.55 % compared to control rats (15.52 %). After treatment, the decrease in reticulocytes at day 7 is 52.91 % in the D1 lot and 77.30 % in the D2 lot (Figure 3).

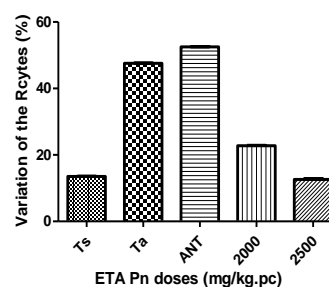


Figure 3: Variation of reticulocyte levels of rats during anemia treatment versus ETA doses from Pn

Ts: Healthy witness; Ta: Anemic witness; ANT: Untreated anemia ETA: Aqueous total extract; Pn: *Parquetina nigrescens*; Rcytes: Reticulocytes

Effect of Bilirubin

The administration of phenylhydrazine increased the level of bilirubin on day 5 in the different batches of rats. After administration of the different doses of the extract to lots D1 and D2, bilirubin decreased. This decrease is much better with the dose D2. However, in untreated anemic rats (ANT), bilirubin remains very high (Figure 4).

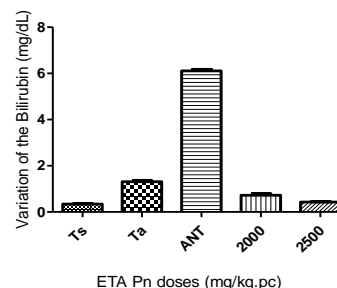


Figure 4: Variation in the amount of Bilirubin in rats during treatment of anemia with different doses of ETA from Pn

Ts: Healthy witness; Ta: Anemic witness; ANT: Untreated anemia ETA: Aqueous total extract; Pn: *Parquetina nigrescens*

Osmotic resistance

The determination of the osmotic resistance made it possible to note with the concentrations NaCl at 0.9 %, 0.6 % and 0.3 %, the percentage of hemolysis of the red blood cells is very low respectively for the rats

of lots D1 and lot D2 (0.003 %), compared to the ANT lot where the percentage of hemolysis is high (0.02 %) (Table 1). The red cell membrane of rats treated with D2 has better resistance than batch D1 in all three concentrations.

Tableau 1: Osmotic resistance of red blood cells during the treatment of anemia with the total aqueous extract of *Parquetina nigrescens* Afzel (ETA Pn)

% Nacl	Ts	Ta	ANT	D1	D2
0.9 %	0.010 ± 0.005	0.016 ± 0.006	0.020 ± 0.005	0.003 ± 0.003	0.0035 ± 0.003
0.6 %	30.97 ± 0.864	36.40 ± 0.75***	52.92 ± 0.13***	11.76 ± 0.630***	8.89 ± 0.335***
0.3 %	33.77 ± 1.744	42.85 ± 0.263***	63.16 ± 16***	8.303 ± 0.569***	6.800 ± 0.383***

Ts: Healthy witness; Ta: Anemic witness; ANT: Untreated anemia; ETA: Aqueous total extract; Nacl: Sodium chloride; ns: Not significant difference (p > 0.05); ***: Highly significant difference (p < 0.001)

DISCUSSION

The objective of this work is to evaluate the antianemic properties of the aqueous extract of *Parquetina nigrescens* in rats. Before administration of the phenylhydrazine hydrochloride at day 0, we obtained average amounts of red blood cells, hemoglobin, and reticulocyte in agreement with those obtained by [12] and [13] with rabbits. These results demonstrate that blood cells can vary within the same population, or from one medium to another while respecting the reference values. According to [14], the number of red blood cells is between 3.8x10⁶/mm³ and 7.9x10⁶/mm³.

Administration of phenylhydrazine caused a significant mean decrease in hemoglobin and red blood cell counts. Our results are close to those of [15] who observed a decrease in the number of globules and hematocrit (respectively 50 % and 55 %) with a phenylhydrazine administration of 40 mg/kg/j for 4 days in Sprague Drawley rats.

Indeed, in our study, on day 7, the rats receiving the extract almost completely recovered (p < 0.001) whereas this is not the case in untreated anemic control rats. Gbenou *and al.* [3], Ryu *and Yook* [15] then Redondo *and al.* [16] also observed this reversibility of phenylhydrazine-induced anemia upon discontinuation of administration.

The results show that the rats in lot D2 totally recovered with respect to the number of red blood cells on day D7 [3]. Hemolysis of red blood cells at day 7 of the phenylhydrazine hydrochloride rats was lower than in normal rats. On the other hand, their osmotic resistance is superior. Our results are comparable to those of [16]; Gbenou *and al.* (2006) [3].

Our results show a significant decrease in the mean values of total bilirubin in batches of rats treated with the doses of the extract. For the 2000 mg/kg bw dose, bilirubin increased from 1.31 ± 0.04 g/L on day 0 to 0.82 ± 0.03 mg/dL on day D7, and then for the dose of 2500 mg/kg bw bilirubin increased from 1.31 ± 0.04 mg/dL to 0.33 ± 0.03 mg/dL. Bilirubin comes from the degradation of hemoglobin (free bilirubin). Then it is captured by the liver (conjugated or direct bilirubin) and degraded. The level of total or free bilirubin is increased in cases of major haemolysis, especially congenital or acquired haemolytic anemias, drug, toxic or infectious hemolysis, transfusional accidents. These results demonstrate that *Parquetina nigrescens* does not induce any harmful effects that can lead to haemolysis. On the other hand, they corroborate those of [17] who showed that *Spirulina platensis* restores the number of red blood cells and the hemoglobin level in rabbits experimentally anemic by phenylhydrazine hydrochloride.

Hemolysis at 50 % of normal rats and untreated anemic rats being higher than those of anemic rats treated with *Parquetina nigrescens* Afzel extract, we can say that there are more cells resistant to hemolysis in rats. Treated only in untreated rats. This increase in the osmotic resistance of red blood cells of rats is due to the presence of young cells

in the blood. The administration of *Parquetina nigrescens* Afzel extract therefore increased the production of young red blood cells.

The phytohimic analysis revealed the presence of the big chemical groups that are: alkaloids, tannins, flavonoids, polyterpenes, polyphenols and iron. They have an antioxidant capacity, promote the regeneration of tissues, decrease the permeability of blood capillaries and strengthen their resistance to hemolysis [18]. The presence of these large chemical groups by their properties, justifies the resistance of the red blood cells of rats treated with the extract.

Anemias are common in tropical environments; 10 to 20 % of the population has less than 10 g/dL of hemoglobin [19]. Pregnant women and children constitute high-risk groups. Malaria, parasitosis and folic acid deficiency are the main causes. *Parquetina nigrescens* Afzel could be advised to fight against anemia.

CONCLUSION

Injection of phenylhydrazine hydrochloride caused haemolytic anemia characterized by decreased haematological parameters. The administration of the aqueous *Parquetina nigrescens* Afzel extract by gavage at doses of 2000 mg/g/bw and 2500 mg/kg/bw significantly increased the hemoglobin and red blood cell count at day 7. The dose of 2500 mg/kg/bw allowed a complete recovery (99.73 %) of the number of red blood cells of the rats at day 7. The extract, because of the properties of the large chemical groups that are present, allowed, because of the properties of the large chemical groups that are present, allowed because of the regeneration of young cells better resistance of blood cells to hemolysis.

Our results confirm and validate the traditional therapeutic indication of *Parquetina nigrescens* Afzel leaves in the treatment of anemia. This plant could therefore be recommended to fight against anemia.

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HOW TO CITE THIS ARTICLE

Angèle GP, Calixte B, Richard KK, Justine TW, Marcellin GR, Nazaire D, Adama C. Study of antianemic properties of *Parquetina nigrescens* (*Apocynaceae*) in wistar rats. J Phytopharmacol 2019; 8(5):216-219.