SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 6.
Issue: 5.
Page N. 103-109
Year: 2022

Original Article www.wjahr.com

IMMUNOREGULATORY EFFECTS OF THE AQUEOUS EXTRACT OF THE BARK *DISTEMONANTHUS BENTHAMIANUS BAILL*. (CAESALPINIACEAE: LEGUMINOSAE - CAESALPINIOIDEAE) ON ANEMIA IN RATS WISTAR INDUCED BY PHENYHYDRAZIN

Kouadio K. J.*[1], Ouattara-Soro F. S.[1], Yao K. B.[1] and Abizi G.

Laboratory of Biology and Health, UFR Biosciences, University Felix Houphouet Boigny, Abidjan, Côte d'Ivoire.

Received date: 11 March 2022 **Revised date:** 01 April 2022 **Accepted date:** 22 April 2022

***Corresponding Author: Kouadio K. J.**

Laboratory of Biology and Health, UFR Biosciences, University Felix Houphouet Boigny, Abidjan, Côte d'Ivoire.

ABSTRACT

Background: *Distemonanthus benthamianus* is a plant commonly used in traditional African pharmacopoeia to treat several inflammatory problems, and pains. It is also used in cases of blood disorders during infections. The objective of the present work is to evaluate, on white rats of wistar strain, the anti- anemic property of the aqueous extract of the barks of *D. benthamianus*. **Material and Methods**: Laboratory animals were randomly grouped into six groups of five rats each. Phenylhydrazin (PHZ) was administered twice at 40 mg/kg/bw to induce hemolytic anemia. Blood samples were taken on day D0, D2, D7, D14, D21 to follow the evolution of blood parameters. **Results**: Administration of PHZ to the animals significantly decreased the red blood cell count, hemoglobin concentration and hematocrit level (a reduction of about 35% of the parameters). Administration of the plant extract to PHZ-induced anemic rats induced the increase of red blood cell count (14.3%), hemoglobin (37.12) and hematocrit (66.16%) on the seventh day of treatment. A significant decrease ($p < 0.05$) in the body weight of the rats was observed, however an increase in the relative weights of the kidneys, liver, spleen, thymus ofthe untreated anemic rats was observed compared to the healthy rats and anemic rats treated with the extract there. **Conclusion**: This study shows that Distemonanthus benthamianus effectively improves the hematological parameters of anemic rats. It would therefore be a powerful remedy against anemia, however additional studies must be done to find the ideal fraction or the molecule responsible for this activity.

KEYWORDS: Distemonanthus benthamianus, anti-anemic, Phenylhydrazin, red blood cell, hemoglobin, hematocrit.

INTRODUCTION

Anemia, a real public health problem, is characterized by the reduction in the number of red blood cells or hemoglobin concentration, thus reducing the capacity of the blood to carry oxygen. It also affects the mental and physical ability of individuals, affecting the economic development of countries. It affects a significant proportion of the population at different ages. Approximately 35% of the world's population suffer from anemia. It is much more frequent in developing countries where about 47% of the population is affected, whereas indeveloped countries, the prevalence is around 10%.[1] In Côte d'Ivoire, anemia is present in 40% to 50% of children and adult women and more than half of the cases are due to iron deficiency.

Infectious diseases, in particular malaria, helminths, in particular helminthiasis (hookworm and schistosomiasis), but also tuberculosis and HIV/AIDS infection contribute substantially to the high prevalence of anemia in Côte d'Ivoire.[2,3] In Côte d'Ivoire, anemia is an important cause of morbidity and mortality, particularly among children and pregnant women. Traditional medicine offers itspatients various plants are an important source of natural antioxidants capable of correcting the problems generatedand recipes to alleviate the health problems. Medecinal plants are an important source of natural antioxidants capable of correcting the problems generated by radicals on red blood cells and their stability.

It is in this context that our attention has focused on the bark of *Distemonanthus benthamianus*, a medicinal plant known in West Africa for its therapeutic virtues.

This plant is used against skin infections, oral and urogenital problems. It is also used in the treatment of malaria, tuberculosis, inflammatory diseases such as hepatitis, rheumatism, pain, bronchitis and blood disorders.[4,5,6]

METHOD INDUCTION OF ANEMIA

This experimental study was conducted on 30 male and female Wistar rats weighing between 140 and 150 g.

During the experiments, the temperature of the animal room was adjusted to 25 ± 3°C under a 12 h dark/light cycle. During the experimental phase, it was performed a first blood sampling of rats by tail incision (D0), then inducing anemia, to all groups, except the negative healthy control, 40 mg/kg/d of phenylhydrazine at two different times (days 0 and 1) were injected intraperitoneally.[7] On the second of PHZ induction, a second sampling of the rats was performed by tail incision. After the blood determination on day 2, rats with hemoglobin concentration <10 g/dl were considered anemic and were used for the study.

Animal treatment: Six groups of rats5 were formed and treated daily for days 21 as follows:

Healthy Control Group: The normal control received 10 ml / kg of distilled water from day D2 to D21.

Anemic group: The anemic control received 10 ml / kgof distilled water from day D2 to D21.

VIT B group: Treated with vitamin B 12 syrup (Vit B12) (1 ml /kg day) from D2 to D21. Group.

EADB1: Treated with aqueous extract of *D. benthamianus* (200 mg / kg) from day D2 to D21.

Group EADB2: Treated with aqueous extract of *D. benthamianus* (400 mg / kg) from day D2 to D21.

Group EADB3: Treated with aqueous extract *D. benthamianus* (800 mg / kg) from day D0 to D21.

During the experiment, blood samples were taken from the rats of the different batches for the evaluation of hematological parameters on days 7, 14 and 21.

Hematological parameters were measured on days 0, 2, 7,14 and 21 in the six batches of rats using an automatic blood cell counter at the Institut Pasteur in Abidjan. Boodsamples were taken by the tail incision method of anesthetized rats.[8] Variations in the mean values of the hematological parameters were calculated with respect to the mean values of D0 and D2.

Thus, the red blood cell (RBC) count, hemoglobin (Hb) concentration, and hematocrit of rats were determined by the following mathematical relationships:

$$A = \frac{D2-D0}{D0} * 100$$

$$B = \frac{DT-D2}{D2} * 100$$

A: percent change from day 0;
B: percent change from day 2.

The weights were monitored during the experiment. At theend of the experiment, the kidney, spleen, and thymus of the rats were removed to determine the relative weight.

STATISTICAL ANALYSIS

Data analysis was performed using Graphpad Prism 7.0. All results were expressed as means ± standard deviations and subjected to Analysis of Variance (ANOVA). Where significant differences were found, pairs of samples were compared using Tukey's test as a post hoc test at $p < 0.05$.

ETHICAL CONSIDERATION

The manipulations were made in the rules of ethics and the strict respect of the experimental protocols with regard tothe work on the animals in particular the wistars rats raised within the laboratory under the supervision of Professor Ouattara-Soro F.S. The animals were handled with all theprecautions to avoid any stress on the animal.

RESULTS

Effects of the extract on hematological parameters Effects of the extract on the red blood cell count

After injection of phenylhydrazine into the rats, a decrease in red blood cell count of 35.38% was observed on thesecond day of the experiment (Table I). A gradual increase in red blood cell count was observed during treatment when blood was collected on days 7, 14 and 21. The results show that the rats of the batches treated with the extract at the doses of 200, 400 and 800 mg/kg already recovered from the second week of treatment with red blood cell levels of 6.7±0.2 106/µL, 6.71±0.19 106/µL and 6.88±0.45106/µL respectively, corresponding to percentages of increase compared to the second day(44.71% 49.41% and 59.19%).

The red blood cell count of the rats in the batch treated with vitamin B also showed a strong increase. The results showed that the aqueous extract of D. benthamianus at the dose of 800 mg/kg/bw provided better recovery comparedto the reference molecule.

Effects of the extract on the hemoglobin level

Administration of phenylhydrazine caused a highly significant ($p<0.0001$) decrease in hemoglobin levels inrats compared to healthy control rats (Table II). There was a progressive increase in hemoglobin level of 32.95%, 21.01%, 37.06% and 53.29% respectively for batchesreceiving vitamin B and extract at doses of 200, 400 and 800 mg/kg/bw from the first week of treatment. The hemoglobin level (9.9±0.29 g/dL) of the anemic controllot not receiving treatment was significantly lower

($p<0.01$) than that of the healthy control lot (13.65±0.24g/dL) at the same period.

At the end of the experiment, the treated rats recovered completely with percentages of increase in hemoglobin of 63.57%, 51.38%, 59.29% and 73.29% respectively for the batches receiving the vitamin B and the extract at doses of 200, 400 and 800 mg/kg/bw.

Effects of the extract on the hematocrit level

The hematocrit levels of rats in the different batches are recorded in Table III. Phenylhydrazine induced a significant decrease in the hematocrit level at day D2. The percentages of hematocrit recorded were 38.48%, 43.53%, 39.12%, 36.9% and 40.26% respectively for the anemic batches, Vitamin B_{12} batches and batches of EADB1, EADB2 and EADB3 (Distemonanthus administration at 200, 400 and 800 mg/kg/bw respectively).

From the first week, a progressive increase of the hematocrit rate was observed in the batches treated with Distemonanthus extract and vitamin B_{12} (56.74%, 65.72%, 75.55% and 65.24% respectively). At the end of the experiment, the rats recovered completely in all the batches treated with the extract at the doses of 200, 400 and 800 mg/kg/bw; the values were 46.63±2.72%, 46.63±2.72% and 49.8±1.09% corresponding to percentages of increase with respect to day D2 of 78.66%, 83.58% and 98.08% respectively. Rats receiving vitamin B12 also showed a very good recovery with a hematocrit level of 42.08±1.27% corresponding to a percentage increase of 90.22%. Concerning the untreated anemic rats with a hematocrit level of 39.35±2.67% (i.e. a percentage increase of 54.94%); this recovery was significantly ($p<0.05$) poor compared to the treated batches.

Effects of the extract on the rate of white blood cells and platelets

The blood platelet levels of anemic rats are recorded in Table IV. The white blood cell levels of PHZ-treated rats were significantly ($p<0.001$) elevated compared to the white blood cell levels of non-anemic rats. This rate decreased during the different treatments; from the second week of treatment, the values returned to normal in all batches. Platelet levels in anemic rats remained significantly higher than in non-anemic rats until the end of the second week. At the third week, there was no significant difference ($p>0.05$) in the platelet levels of the treated lots compared to the non-anemic rats.

However, the blood platelet levels of the untreated anemic rats until the third week were significantly higher than those of the non-anemic controls.

Effects of the extract on body weight and relative organ weights of rats

Effects of the extract on the body weight of rats The weights of rats before and after administration of phenylhydrazine are recorded in Table V.

It was observed in the first week of the experiment, a very significant decrease ($p<0.0001$) in the weight of the rats having received the phenylhydrazine compared to healthy control rats. From the first week of treatment, a clear weight recovery was observed in anemic rats treated with the extract at different doses (2.49%, 4.02% and 3.69%, percentage increase in weight respectively) and vitamin B (1.19%). At the end of the treatment, the rats of the treated batches practically recovered however the percentages of increase (8.69%, 10.71%, 12.1%, 11.87% and 14.3% respectively for the anemic control batches, VIT B, EADB1, EADB2, EADB3) remained low compared to the healthy control batches (20.71%).

Effects of extract on relative organ weights of rats

The relative organ weights of the rats at the end of the experiment are recorded in table VI below. No significant difference was observed in the relative organ weights of the anemic rats treated with the extract and vitamin B compared to the non-anemic rats. In untreated anemic rats, an increase in weights was observed relative of organ (heart, liver, spleen, kidney, thymus) compared to non- anemic rats; this increase was significant in kidney ($P<0.01$), liver and spleen ($p<0.05$).

Table I: Variation in red blood cell count of rats with anemia.

Red blood cells (10^6 /µL)						
	Healthy control	**Lot anemic**	**Vit B**	**EADB1**	**EADB2**	**EADB3**
J0	6.83±035	6.95±0.34	6.70±0.6	6.73±0.4	6.89±0.54	6.98±0.21
J2	6.69±0.34	4.44±0.14	4.45±0.18	4.68±0.25	4.62±0.09	4.58±0.21
		-35.96A	-33.65A	-32.97A	-32.78A	-34.2A
J7	6.87±0.34	4.59±0.12	5.03±0.26	4.92±0.31	5.39±0.31	5,54±0.17
		3.68 B	14.12 B	5.69 B	17.12 B	21.9 B
J14	6.91±0.35	5.08±0.22	6.77±0.26	6.7±0.2	6.71±0.19	6.88±0.45
		14.74 B	52.88 B	44.71 B	45.65 B	51.19 B
J21	6.93±0.36	5.57±0.26	7.10±0.44	6.84±0.48	6.89±0.47	7.34±0.69
		25.99 B	59.87 B	47.17 B	49.41 B	62.24 B

Table II: Variation in hemoglobin levels in anemic rats.

HEMOGLOBIN (g/dL)						
DATES	**Healthy control**	**Lot anemic**	**Vit B**	**EADB1**	**EADB2**	**EADB3**
J0	13.75±0.38	13.68±0.54	14±0.34	13.63±0.49	14±0.14	13.9±0.39
J2	13.83±0.34	8.6±0.25[d] -34.51 A	8.82±0.24[d] -36.72 A	8.85±0.41 [d] -32.49 A	8.75±0.25 [d] -37.53 A	8.26±0.39 [d] -40.45 A
J7	13.65±0.24	9.9±0.29[b] 15.5 B	11.7±0.72 32.95 B	10.98±0.67 21.01 B	11.9±0.87 37.06	12.65±0.33 53.29 B
J14	13.93±0.23	11.73±0.96 36.48 B	13.9±0.26 58.12 B	13.08±0.31 44.56 B	135±0.28 55.06	13.530.17 63.9 B
J21	14.05±0.16	11.98±0.31[a] 39,51 B	14.43±0.37 63.57 B	13.7±0.25 51.38 B	13.9±0.34 59.29 B	14.3±0.2 73.29[a] B

Table III: Variation in hematocrit level of rats with anemia.

HEMATOCRIT (%)						
	Healthy control	**Lot anemic**	**Vit B**	**EADB1**	**EADB2**	**EADB3**
JO	44.93±5.4	41.9±2.28	44.63±2.9	43.23±2.2	42.28±4.6	43.9±4.8
J2	44.33±5.07	25.53±0.51	24.75±0.96	26.2±0.86	25.85±0.53	25.3±0.96
		-38.48A	**-43.53A**	**-39.12A**	**-36.9A**	**-40.26A**
J7	44.18±3.85	32.5±1.45	40.7±2.12	40.8±1.97	42.68±2.32	44.13±0.86
		26.94 B	**65.24 B**	**56.74 B**	**65.72 B**	**75.55 B**
J14	44.18±5.11	36.28±0.94	45.48±2.29	45.6±1.2	45.95±2.27	49.15±1.64
		42.09 B	**83.39[b] B**	**74.99[a] B**	**77.97[b] B**	**95.26[b] B**
J21	44.18±5.13	39.35±2.67	42.08±1.27	46.63±2.72	46.63±2.72	49.8±1.09
		54.94 B	**90.22 B**	**78.66 B**	**83.58 B**	**98.08 B**

Table IV: Variation in white blood cell and platelet counts of rats with anemia.

White blood cells and platelets (10^9/L)							
		Health control	**lot anemic**	**VIT B**	**EADB1**	**EADB2**	**EADB3**
J2	GB	8.52±0.8	41.01±3.1[c]	39.8±8 [c]	41.96±11 [c]	42.22±7.7 [c]	41.48±11 [c]
	PL	861.3±5.8	1030±19.2[a]	914±11.6	923±14.2 [a]	1011±10.33 [a]	958.8±13.1 [a]
J7	GB	-	21.69±2.4 [b]	19.13±0.5 [a]	17.44±0.9 [a]	18.05±2.11 [a]	16.82±1.8 [a]
	PL	-	1007±6.23 [a]	900.8±5.19	914.3±9.82	977.5±21.8	993.5±10.1 [a]
J14	GB	-	14.16±2.3	13.43±1.02	12.28±1.8	12.25±2.2	11.11±0.9
	PL	-	1059±12.24	922±5.4 [a]	968.8±5.68[a]	970.8±9.6 [a]	970.5±6.07 [a]
J21	GB	-	12.41±1.2	11.18±0.5	10.53±1.4	9.74±0.89	9.60±0.7
	PL	-	959±6.47 [a]	922±5.4	936.8±5.61	920.8±6.18	920.5±5.97

Table V: Effect of treatments on weights of anemic rats.

	Healthy control	**Lot anemic**	**Vit B**	**EADB1**	**EADB2**	**EADB3**
JO	147.3±2.01	145.8±3.47	147.8±1.70	152.8±2.65	148.5±3.92	148.8±1.7
J2	155.3±5.27 5.35	138.8±4.13 -4.83 [d]	140±0.41 -5.21 [d]	146.5±2.3 -4.07 [d]	140.8±5.6 -5.79 [d]	140.5±0.9 -5.53 [d]
J7	163.5±6.56 9.456	148.3±4.56 [a] 0.011 [d]	150.5±1.2 [a] 1.91 [d]	156.5±2.3 2.49[c]	152.5±5.5 4.02 [b]	153.5±2.2 3.69[b]
J14	181.5±7.71 18.87	158.3±4.07 [a] 6.93 [d]	160.3±1.4 8.52 [d]	167±1.08 9.44 [d]	163.8±2.9 9.83 [d]	165±4.42 12.13 [c]
J21	182.8±8.14 20.71	160.3±3.49 8.69 [d]	163.5±1.6 10.71 [d]	171±1.83 12.1 [d]	166.8±2.3 11.87 [d]	167.5±4.9 14.3 [c]

Table VI: Effects of treatments on relative organ weights in anemic rats.

Organes	Healthy control	Lot anemic	VIT B	EADB1	EADB2	EADB3
heart	0.43±0.005	0.50±0.004	0.45±0.01	0.44±0.05	0.44±0.01	0.44±0.0
kidney	0.41±0.04	0.69±0.03 [b]	0.49±0.03	0.53±0.01	0.49±0.04	0.43±0.01
liver	2.51±0.15	3.9±0.14	3.04±0.39	3.20±0.32	3.09±0.37	3.07±0.83
Thymus	0.16±0.00	0.48±0.22 [a]	0.2±0.06	0.19±0.011	0.17±0.02	0.162±0.02
Rate	0.30±0.04	0.49±0.03 [a]	0.33±0.02	0.38±0.02	0.35±0.02	0.35±0.00

*: a; **: b; ****: d Values are means ± standard errors (n=4/lot). *p<0.05; **p<0.01;****p<0.001: significantly different from healthy controls. A: Percent change from day 0; B: Percent change from day 2. EADB: Distemonanthus benthamianus aqueous extract, EADB1: dose 200mg/kg/pc, EADB2: dose 400mg/kg/pc, EADB3: dose 800mg/kg/pc, TM: control, Vit: vitamin

DISCUSSION

Distemonanthus benthamianus barks have been reported to have anti-inflammatory properties[6] and are involved in the treatment of blood disorders. In this study, phenylhydrazine was used to induce hemolytic anemia characterized by a decrease in red blood cells, hemoglobin and hematocrit.[9] Indeed, phenylhydrazine administered to rats causesoxidative stress in red blood cells resulting in radical production and lipidperoxidation in these cells[10,11,12] ROS reacts with hemoglobin and leads to the conversion of oxyhemoglobin to methemoglobin and then to hemichrome as well as other hemoglobin degradation products such as Hens body. PHZ generates superoxide anion and hydrogen peroxide to cause the formation of lipid peroxidation resulting in oxidative degradation of the cell membrane spectrum and lysis of red blood cells.[13,14,15] Intraperitoneal administration of 40 mg/kg/d of phenylhydrazine for two days (D0 and D1) in Wistar rats resulted in a significant decrease in red blood cell count, hemoglobin and hematocrit during blood sampling on the second day of the experiment. These results corroborate those of O'Riordan and al.[16] and Criswell and al.[17] who showed that intraperitoneal injection of phenylhydrazine decreased the concentrations of red blood cells, hemoglobin and hematocrit. According to Berger[18], animals are considered anemic when there is a decrease in hemoglobin, red blood cell, and hematocrit levels as well as altered erythrocytes. Yenon and al.[19] showed in the work that administration of phenylhydrazin for two successive days induced anemia in rats with a decrease in hematological parameters. The anemia resulting from the destruction of red blood cells was naturally reversed from the following week by the regeneration of blood cells due to the increase of reticulocytes. Administration of theaqueous extract showed an increase in hemoglobin, red blood cell and hematocrit levels compared to untreated anemic controls after the first week of treatment. Also, vitamin B showed an increase in hemoglobin, hematocrit and red blood cell content comparable to that of the extract. The extract could stimulate the erythropoiesis process by increasing the number of young red blood cells which explains the high osmotic resistance of red blood cells in rats treated with the extract. At the end of the study, the animals recovered completely. The red blood cell, hemoglobin and hematocrit levels of the batches of B12 vitamins and the extract at the dose of 800 mg/kg/pc were increased compared to the healthy control rats. However, the hemoglobin level of the anemic rats remained significantly ($p<0.05$) low compared to the healthy control rats. The extract inhibited phenylhydrazine-induced anemia in rats which model is similar to that induced by plasmodium falciparum responsible for malaria. These results confirm the traditional use of the plant in thetreatment of blood disorders and malaria. The increase in the number of red blood cells could be attributed to the phenolic compounds present in the aqueous extract of the bark of the plant. The metabolites present in plants have an antioxidant power, promote tissue regeneration, reduce the permeability of blood capillaries and increase their resistance to hemolysis.[20] The presence of polyphenols, flavonoids, tannins, alkaloids and saponins by their properties justifies the resistance of red blood cells of rats treated with the extract. Indeed, flavonoids, saponins and alkaloids have shown very interesting anti-anemic properties.[21] Flavonoids have antioxidant properties by neutralizing free radicals, which attack most of the cells of the body, thus neutralizing diseases, tonify the veins and protect the blood capillaries.[20,22] Alkaloids improve erythropoiesis by stimulating protein phosphorylation andsynthesis.[23] Saponins, due to their properties, inhibit platelet aggregation and thrombosis. They are used in the form of sedative tonic formulas, to revitalize blood circulation.[24,25] However, saponins can be the active agents by licking the membrane of red blood cells and cells. To overcome this effect, saponins produce a glycosidic enzyme that cleaves some of its terminal sugars resulting in its detoxification.[26] This detoxification of the saponins reinforces the good use of the iron contained in the plant. The plant is believed to contain substancesconstituting hematopoietic factors thus enabling the synthesis of heme/hemoglobin for the production of new red blood cells in the bone marrow, leading to improved blood parameters.[19] Antioxidants in bark could play an important role in reducing phenylhydrazine-induced oxidative stress by scavenging released free radicals and degradation products to maintain homeostasis. Indeed, the released radicals interfere with red blood cell membranes through fatty acid peroxidation[27,28] to induce anemia. The antioxidant

activity of the plant would have reversed the adverse effects of phenylhydrazine on blood cells. Diallo and al.[29] and Ogbe and al.[30] also showed that administration to rats, extracts of *Tectona grandis, M. indica, A. hybridus* and *T. occidentalis* increased the concentration of hemoglobin, red blood cells after phenylhydrazine induced anemia in rats. Blood cell and platelet levels in anemic rats were significantly higher than in nonanemic rats. Indeed, studies have shown that during the installation of PHZ-induced anemia, the immune system is activated.

Anemia could be considered by the immune system as an infection or pathology, therefore, the system intervenes through the production of white blood cells and defense platelets to fight against the pathogen.[31] Furthermore, the number of white blood cells and platelets increases rapidlyafter a foreign attack on the system by pathogens to provide a normal physiological response to the system forthe stimulation of the body's defense mechanisms.[7,32] This justifies the sharp increase in these parameters after the first few weeks of induction. Any organs such as the liver, kidney, bone, thymus and spleen are involved in the production of red blood cells. PHZ can cross the red bloodcells and bind to circulating autologous antibodies. This antigen-antibody complex is recognized by macrophage receptors that trigger phagocytosis in the spleen and liver.In addition to blood storage and immune competence, the spleen also acts as the major erythrophagocytic organ in rodents with PHZ-induced hemolytic anemia. Studies have indicated that phenylhydrazine, by increasing hemolysis, causes liver hypertrophy and chronic failure by hypertrophy of liver cells, spleen hypertrophy, and chronic and acute renal failure by destruction of structures such as proximal and distal renal cells and glomeruli.[11,33,34] In addition, phenylhydrazine has been reported to decrease body weight and improve the relative weights of the liver, spleen, kidney, and thymus. In this study, there was a significant increase in kidney, thymus, spleen weights in anemic controls compared to non-anemic controls. According to Jakabovsky and al.[35], the spleen serves to clean up old particles from PHZ degradation of red blood cells; this suggests an increase in relative spleen weight. Regarding the body weight of rats, there was a decrease in body weight after induction of anemia by phenylhydrazine. Yenon et al.[19] also showed that administration of phenylhydrazine to rats resulted in a decrease in body weight. Indeed, loss of body weight is one of the symptoms of anemia, which would be due to a lack of appetite in anemic rats.

It could also be explained by a reduction of disaccharide activities in anemic rats.[36,37]

CONCLUSION

This study is part of the research of new therapeutic sources from plant resources. This study confirms the anti-anemic virtue of the aqueous extract of the leaves of *Distemonanthus benthamianus* since it significantly regulate the erythrocyte concentration in the rat used induced anemic by PHZ. Further analysis will allow to quantify this cell blood regulation activity by establishinga precise dose-effect relationship and then to look for a stable galenic formulation that can be used to support thisactivity in order to make it a medicine against problem ofanemic.

ACKNOWLEDGEMENT

Our gratitude goes to the UFR Biosciences of University Felix Houphouët Boigny and National Superior School ofAbidjan, Côte d'Ivoire, for the premises and equipment made available to us.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Khan, K.S., Wojdyla, D, Say L., Gulmezoglu, A.M., and Van Look, P.F.,. WHO analysis of causes of maternal death: a systematic review. *Lancet*, 2006; 376(9516): 1066-1074.
2. Staubli A.F. Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalence of malaria and other infection: a study in Côte d'Ivoire. *American Journal of Clinical Nutrition*, 2001; 74: 776-786.
3. Van den Broek N.R. & Letsky E.A. Etiology of anemia in pregnancy in south Malawi. *TheAmerican Journal of Clinical Nutrition*, 2000; 72(1): 247S-256S.
4. Nguelefack E.M.P., Ngu K.P., Atchade A., Dimo T., Tsabang N. & Mbafor J.T. Phytochemical composition and in vitro effects of the ethyl acetate bark extract of Distemonanthus benthamianus Baill. (Caesalpiniaceae) on Staphylococcus aureus and Streptococcus agalactiae in Cameroon. *Journal of Experimental Biology*, 2005; 1: 50-53.
5. Adeniyi B.A., Obasi O.J. & Lawal T.O. In-vitro antifungal activity of Distemonanthus benthamianus stem. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2011; 3(4): 52-56.
6. Kouadio K.J., Ouattara-Soro F.S., Abizi G., Zouglou N.E, Kouakou K.R., Begbin K.E, Kplé T.K.M., Kablan K.J.M, Koffi S., Activité Anti-inflammatoire et étude phytochimiques de l'extrait aqueux de *Distemonanthus benthamianus* Baill. (Caesalpiniaceae: Leguminosae- Caesalpinioideae*), European Scientific jounal*, 2021; 17(7): 74.
7. B.A. Naughton, B.S. Dornfest, and D.M. Lapin. Immune activation is associated with phenylhydrazine-induced anemia in the rat, *J. Lab. Clin. Med*., 1990; 116: 498-507.
8. Leonard R. & Ruben Z. Hematology reference value for periphera blood of laboratory rat. *Laboratory Animal Sciences*, 1986; 36(30): 277-281.
9. Unami A, Nishina N, Terai T, Sato S, Tamura T, Noda K and Mine Y. Effect of cisplatin on erythropoietin production in rats. *Journal of*

Toxicological Sciences, 1996; 21(3): 157-158.
10. Giffin H and Allen E. The control and complete remission of polycythemia vera following the prolonged administration of phenylhydrazine hydrochloride. *American Journal of Medical Sciences*, 1993; 185: 1-13.
11. Ferrali M, Signorini C, Sugherini L, Pompella A, Maura L, Caciotti B, Ciccoli L and Comporti M. Release of free redoxactive iron in the liver and DNA oxidative damage following phenylhydarzine intoxication. *Biochemical Pharmacology*, 1997; 53(11): 1743-1751.
12. Cighetti G, Debiasis S, Paroni R, and Allevi. Free and total malondialdehyde assessment in biological matrices by GC/MS. What is needed for an accurate detection? *Anaytical Chemistry*, 1999; 266(2): 222-229.
13. Rifkind, R.A. Heinz body anemia: an ultrastructural study. II. Red cell sequestration and destruction. *Blood*, 1965; 26(4): 433-448.
14. Jain SK and Hochstein P. Generation of superoxide radicals by hydrazine: Its role in phenylhydarzine - induced Hemolytic anaemia. *Biochemica et Biophysica Acta (BBA)*, 1979; 586: 128-136.
15. Horn S, Gopas J and Bashan N. A lectin like receptor on murine macrophage is involved in the recognition and phagocytosis of human red cells oxidized by phenylhydrazine. *Biochemical Pharmacology*; 1990; 39(4): 775-780.
16. O'Riordan DK, Sharp P, Sykes RM, Srai SK, Epstein O, Debnam ES. *Eur J Clin Invest*, 1995; 25: 722.
17. Criswell K, Sulhanen A, Hochbaum AF, Bleavins MR. *J Appl Toxicol*, 2002; 20: 25.
18. Berger, J. Review: Phenylhydrazine haematotoxicity. *J. Appl. Biomed*, 2007; 5: 125-130. J.
19. Yenon Achielie1, Yapi Houphouet F *1, Gnahoue Goueh1, Yapo Adou F1, Nguessan Jean D1 and Djaman Allico J 1,. Anti-anaemic activity of aqueous and ethanolic extracts of Entandrophragma angolense bark on phenylhydrazine- induced anaemic rats. Asian *Journal of Biochemical and Pharmaceutical Research*. Issue, 2015; 3(5): 2231-2560.
20. J. Bruneton, TEC & DOC et médic internat, ed. 3rd, Paris, France, 1999; 1120.
21. Falcone, P. Musto, P. Rasario, M. Rosella, Compounds and methods for treatment of chemotherapy-induced Anaemia. *Europ J of Haematol*, 1997; 58: 34-319.
22. Ekanayake, S., Jansz, E. R., Nair, B. M. Literature review of an underutilized legume: Canavalia gladiata L. *Plant Foods Hum. Nutr.*, 2000; 55(4): 305-321.
23. Magnani M., Stocchi V., Cucchiarini L., Chiarantini L. & Fornaini G. Red blood cell phagocytosis and lysis following oxidative damage by phenylhydrazine. *Cell Biochemistry and Function*, 1986; 4(4): 263–269.
24. Shi J., Arunasalam K., Yeung D., et al. Saponins from edible legumes: chemistry, processing, and health benefits. Journal of medicinal food, 2004; 7(1): 67-78.25.
25. J. Wang, J. Xu, J.B. Zhong, Effect of Radix notoginseng saponins on platelet activatingmolecule expression and aggregation in patients with blood hyperviscosity syndrome *Alternat Med Review*, 2004; 24: 312-316.
26. Pathirana C., Gibney M. & Taylor T. Effect of soy protein and saponins on serum and liver cholesterolin rats. *Atherosclerosis*, 1990; 36: 595-599.
27. Bjelakovic, G., Nikolova, D., Simonetti, R. G., & Gluud, C. Antioxidant supplements for preventionof gastrointestinal cancers: a systematic review and meta-analysis. *The Lancet*, 2004; 364(9441): 1219-1228.
28. Onyeabo C., Achi N K, Ekeleme-Egedigw CA, Ebere C U., Okoro C K. haematological and biochemical studies on justicia carnea leaves extract in phenylhydrazine induced-anemia in albino rats. *Acta Sci. Pol. Technol. Aliment*, 2017; 16(2): 217-230.
29. Diallo, A., Gbeassor, M., Vovor, A., Eklu-Gadegbeku, K., Aklikokou, K. Agbonon, A. Effectof Tectona grandis on phenylhydrazine induced anemia in rats. *Fitoterapia*, 2008; 79: 332-336.
30. Ogbe, R. J., Adoga, G. I., Abu, A. H. Antianaemic potentials of some plant extracts on phenyl hydrazineinduced anemia in rabbits. *J. Med. Plants Res*., 2010; 4(8): 680-684.
31. Okonkwo, C. C., Agu, C. V., Njoku, O. U., Abonyi, U., Apeh, V., Anaduaka, E.G., ..., Odo, C. E. Hypoglycaemic and haematinic properties of ethanol leaf extract of Artocarpus heterophyllus in alloxan induced diabetic rats. *Afr. J. Tradit. Comp. Altern. Med*., 2015; 12(2): 144-148.
32. Eyong, E. U., Umoh, I. B., Ebong, P. E., Eteng, M. U. Antai, A. B., Akoa, A. O. Heamotoxic effects following ingestion of Nigerian crude oil and crude oil polluted shellfish by rats. *Nig J. Physiol Sci*., 2004; 19(1-2): 1-6.
33. Beaven, G., white, J. Oxidation of Phenyl-Hydrazines in the Presence of Oxyhæmoglobin.
34. Goldberg B and Stern A. The generation of superoxide by the interaction of the hemolytic agent phenylhydrazine with human hemoglobin. *Journal of Biological Chemistry*, 1975; 251: 3045-3051.
35. Jakubovský, J., Brozman, M., Ruzicková, M., Surmíková, E., Belko, I., Líska, J., ..., Belosovic, M. Structural basis of the spleen in rats. *Bratisl. Lek. Listy*, 1990; 91(6).
36. Gudmand-Høyer, E. et Skovbjerg, H. Disaccharide digestion and maldigestion. Scandinavian Journal of Gastroenterology, 1996; 31(216): 111-121.
37. Hariom, Kushwaha, Joshi, Ankur, Malviya, Sapna, et al. Anti-Anemic activity of Hydro-alcoholic extract of Calotropis procera flower on phenylhydrazine-induced anaemic rats. Int. J. Comprehensive and Adv. Pharmacol, 2017; 2(1): 6-10.