

## **Comparative Study of the Effect of Aqueous Extract of *Entandrophragma angolense* and Vitamin B12 on Induced Hemolytic Anemia and Inflammation in the Wistar Rat**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Author YAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AFM and VBFJ managed the analyses of the study. Author ADPB managed the literature searches. All authors read and approved the final manuscript.

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### **ABSTRACT**

*Entandrophragma angolense* is a plant of the family Meliaceae of African pharmacopoeia. It is used in traditional medicine as an analgesic and anti-inflammatory in cases of abdominal or hips pain. The aim of this study is to compare the effect of oral administration of aqueous extract of *E. angolense* and vitamin B12 on hemolytic induced anemia and inflammation in the rat. Anemia was induced by

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intraperitoneal administration of phenylhydrazine at the dose of 40 mg / kg / day for two days in 24 Wistar rats, followed by aqueous extract of *E. angolense* and vitamin B12 chosen as reference molecule administered orally to rats once a day for 28 days. A biological assessment on blood count, CRP and the study of secondary metabolites of the aqueous extract of *E. angolense* have been realized. The results obtained showed the existence of anemia characterized by a significant decrease ( $p < 0.01$ ) of the hemoglobin level of  $42.80\% \pm 3.09$  and red blood cells of  $44.21\% \pm 3.16$  in rats, and inflammation revealed by the significantly elevated CRP assay ( $p < 0.05$ ). The aqueous extract of *E. angolense* at the dose of 200 mg / kg increased significantly ( $p < 0.001$ ) the number of red blood cells and hemoglobin at the 4th week of treatment compared to the vitamin B12. In conclusion, the aqueous extract of the plant has an anti-inflammatory and anti-anemic effect. This double physiological action could come from the phytochemicals (flavonoids, saponins and alkaloids) contained in the extract.

**Keywords:** *Entandrophragma angolense*; Vitamin B12; anemia; inflammation; haematological parameters; CRP.

## 1. INTRODUCTION

Anemia is a very common biological symptom in developing countries and etiologies are multifactorial [1]. In the case of haemolytic anemia, the red blood cells are destroyed or eliminated from the bloodstream before their normal life span of 120 days. Anemia will occur if the bone marrow fails to compensate [2]. Hemolytic anemia is usually classified as being caused by a red blood cell that is itself abnormal (intracorporeal), or a factor that is outside the red blood cell (extracorporeal). Hereditary and acquired haemolytic anemias are also distinguished [3]. Acquired haemolytic anemia may be due to a reaction of the immune system (autoimmune or allergic), the presence of toxic agents in the blood (benzene, phenylhydrazine, antibiotics, snake venom) or infections. Infectious diseases, particularly malaria and helminthiasis, but also tuberculosis and HIV / AIDS, contribute substantially to the high prevalence of anemia seen in many places [4,5].

In addition, inflammatory anemia, also known as chronic anemia, is a type of anemia that affects people with conditions that cause inflammation, such as infections, autoimmune diseases, cancer, and chronic kidney disease. Inflammatory anemia is the second most common type of anemia after iron deficiency anemia [6].

The study of biological and chemical properties has shown that Ivorian flora has a real therapeutic and nutritional potential that can be used to treat or prevent many diseases [7,8]. *Entandrophragma angolense* one of the Ivorian plants is used in traditional medicine to treat

fever and as an analgesic against stomach upset and peptic ulcers, earache and hip pain [9]. Several studies have been carried out on the biological properties of bark extracts of this plant such as antiplasmodial activity [10], antiulcer activity [11]. A species of this genus, *Entandrophragma utile* has been studied for its antisickling property [12]. This study therefore proposes to compare the administration of the aqueous extract of *Entandrophragma angolense* and vitamin B12 on the hemolytic anemia induced by the administration of phenylhydrazine which would trigger inflammation in the rat.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The plant material consists of *E. angolense* stem bark. Our sample was collected in the area of Abidjan (southern Ivory Coast). It was identified at the National Floristic Centre of University Felix Houphouët-Boigny where a herbarium specimen of the plant was deposited. The bark was cut out then dried in the shade, at the room temperature for two weeks. Then, it was pulverized using an electric crusher in order to obtain a powder.

### 2.2 Experimental Animals

Female and male Rats of Wistar strain of body weight ranging between 150-230 g were used for this study. The animals were housed in cages and acclimatized for two weeks in the animal house of the Higher Teacher Training School. They had been maintained under standard conditions (room temperature  $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$ , humidity 35 to 60%, light and dark period 12/12

hours). All animals had regular supply of clean drinking water and food.

### 2.3 Preparation of the Aqueous Extract by Decoction

One hundred grams (100 g) of plant powder was boiled for 20 minutes in 2 liters of distilled water. The cooled juice was filtered thrice off through a cotton plug and once with a Whatman No.3 filter paper. The obtained filtrate was concentrated under reduced pressure at temperature below 50°C through a Büchi rotary evaporator and gave the dry aqueous extract of *E. angolense*.

### 2.4 Phytochemical Screening

The different groups of compounds (sterols, polyterpenes, alkaloids, tannins, polyphenols, flavonoids, quinones, saponins and cardiac glycosides) have been researched in the extract of *E. angolense* according to the methods [13,14,15].

### 2.5 Induction of Anemia

Anaemia was induced in rats by intraperitoneal administration of 40 mg / kg /day of phenylhydrazine (PHZ) for two days (D0 and D1) [16,17]. The treated rats with phenylhydrazine whose haemoglobin concentration <13 g / dl were considered as anemic and included for the study.

### 2.6 Treatment of Animals

Four groups of 6 rats were formed and treated daily for 4 weeks as follows:

- Group I (G1) - Normal control received 10 ml / kg of distilled water from day D2 to D28.
- Group II (G2) -Anaemic control received 10 ml / kg of distilled water from day D2 to D28.
- Group III (G3) - Treated with Vitamin B12 syrup (1 ml /day) from day D2 to D28.
- Group IV (G4) - Treated with aqueous extract of *E. angolense* (200 mg / kg) from day D2 to D28.

### 2.7 Analysis of Hematological Parameters and Determination of CRP

Blood samples were collected from the rats by tail incision before induction of anaemia (D0), after induction of anemia with PHZ (D2) and at 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> weeks of treatment [18,19].

The red blood cell number (RBC), haemoglobin concentration (Hb) and haematocrit were determined at days D0, D2, D7, D14, D21 and D28 using an automatic blood cell counter (Sysmex KX 21) and the variations of average values of hematological parameters were calculated relative to the mean values of D0 and D2.

The determination of CRP was made from blood serum of the animals by the technique of immunoturbidimetry on Cobas C311 (Hitachi) on days D0, D2, D7, D14, D21 and D28. The principle is based on the photometric measurement of the disorder brought by the antigen-antibody reaction in the final point method at 340 nm [20].

### 2.8 Statistical Analysis

The values were expressed as mean accompanied by the standards errors on the mean (Mean  $\pm$  SEM). The graphic representation of data was carried out starting from the software Graph Pad PRISM 5.0 (Microsoft U.S.A). The statistical analysis of the results was made by using one way ANOVA followed by Dunnett's test to verify the significant difference.  $p < 0.05$  was considered significant.

## 3. RESULTS

### 3.1 Phytochemical Screening

Phytochemical analysis of aqueous extract of *E. angolense* bark revealed the presence of large chemical groups that are: alkaloids, polyphenols, sterols, terpenes, tannins, flavonoids, leucoanthocyanins, quinones, saponins and cardiac glycosides.

### 3.2 Effects of Aqueous Extract of *E. angolense* Bark and Vitamin B12 on Hematological Parameters

**Red cells:** After injection of phenylhydrazine to rats of the three groups except the normal group, there was a significant decrease ( $p < 0.01$ ) of red blood cells ( $44.21\% \pm 3.16$ ) at day D2. An increase in the number of red blood cells is observed after treatment on the following days. The results show that the untreated anemic control rats (G2) and those of G3 group treated with vitamin B12 recover with difficulty. In contrast, the anemic rats treated with the aqueous extract of *E. angolense* (G4) completely

recovered at the 4th week (Table 1). The results also show that the aqueous extract at the dose of 200 mg / kg / day allows better recovery compared to that of the vitamin B12.

**Hemoglobin:** The administration of phenylhydrazine caused a significant decrease ( $p < 0.01$ ) in hemoglobin level at day 2 in rats of groups G2, G3 and G4 by  $42.80\% \pm 3.09$ . After treatment, a gradual recovery is obtained the following days (Table 2). The results show that rats receiving Vit B12 had hemoglobin levels than lower those receiving the aqueous extract of *E. angolense* every 4 weeks ( $p < 0.001$ ). Therefore, the aqueous extract at the dose of 200 mg / kg / day allows a faster recovery.

**Hematocrit:** The administration of phenylhydrazine also decreased hematocrit at day D2. This decrease is 17.58%, 20.28% and 21.36 respectively in untreated rats G2, the rats of group G3 and G4. After treatment, the hematocrit increase at day D7 (1<sup>st</sup> week) was 41.17% in the untreated anemic rats, 41.93% and 40.45 respectively in the G3 and G4 rats. At the fourth week (D28), it was 42.88% in rats of group G2, 45.17% in rats of group G3 and 43.97% in rats of group G4 (Table 3).

### 3.3 Serum Concentration of CRP after Injection of Phenylhydrazine

CRP concentration was significantly elevated ( $p < 0.05$ ) at day 2 in the rats of G2, G3 and G4. Administration of the aqueous extract decreased the concentration of CRP the following days. On

the other hand, the rats treated with vitamin B12 always have a high CRP concentration (Fig. 1).

## 4. DISCUSSION

The objectives of this work were to carry out a comparative study of the administration of the aqueous extract of *Entandrophragma angolense* bark and vitamin B12 on inflammation and anemia and to highlight the anti-inflammatory activity of this aqueous extract. The bark of the stem, in decoction, is used for the treatment of fever or malaria in Cameroon and Ivory Coast. In Congo, it is used as an analgesic and anti-inflammatory [21].

However, phenylhydrazine, which is toxic to the animal, induces haemolytic anemia following the formation of methemoglobin, which results in liver, splenic and renal lesions [22]. It represents a pathogen that triggers an acute inflammation in animal. The rat CRP assay in our study revealed a high concentration of CRP; which translates the presence of acute inflammation. Inflammation associated with anemia may have an impact on reticulocytes production. Any inflammation, especially the decrease in serum iron that it causes, will limit the regeneration capacities of erythropoiesis. This is the case of inflammation occurring, for example, during treatment of Biermer's disease with vitamin B12, infections in dialysis patients on erythropoietin [23,24]. This is in accordance with our results, which showed lower hemoglobin levels in Vit B12 treated rats than those receiving the aqueous extract of *E. angolense*.

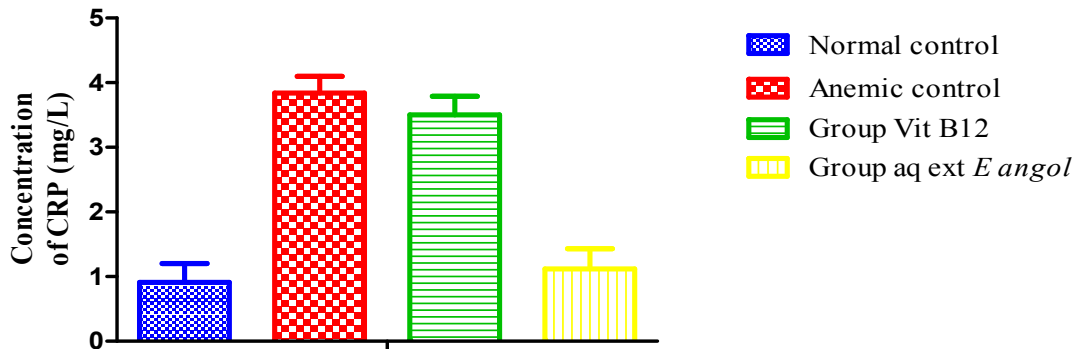


Fig. 1. Effect of the aqueous extract of *E. angolense* on the serum concentration of CRP after injection of phenylhydrazine on D2

**Table 1. Effect of the aqueous extract of the bark of *E. angolense* on the number of red blood cells during and after induction of anemia by phenylhydrazine in the rat**

Groups	RBC (10 <sup>6</sup> cells/ $\mu$ l)					
	D0	D2	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
Normal control G1 (DW 10 ml/kg)	7.08 $\pm$ 0.07	7.40 $\pm$ 0.10	7.46 $\pm$ 0.24	7.39 $\pm$ 0.04	6.46 $\pm$ 0.19	7.14 $\pm$ 0.26
Anemic control G2 (DW 10 ml/kg)	7.33 $\pm$ 0.15	4.32 $\pm$ 0.16–41.06 <sup><math>\alpha</math>***</sup>	5.77 $\pm$ 0.10+33.56 <sup><math>\beta</math></sup>	6.69 $\pm$ 0.15+54.86 <sup><math>\beta</math></sup>	6.97 $\pm$ 0.07+61.34 <sup><math>\beta</math></sup>	7.33 $\pm$ 0.27+69.67 <sup><math>\beta</math>**</sup>
G3 (Vit B12 syrup 1 ml/day)	7.43 $\pm$ 0.14	4.38 $\pm$ 0.10–41.04 <sup><math>\alpha</math>**</sup>	5.53 $\pm$ 0.18+26.25 <sup><math>\beta</math></sup>	6.81 $\pm$ 0.10+55.47 <sup><math>\beta</math></sup>	7.17 $\pm$ 0.04+63.69 <sup><math>\beta</math>*</sup>	7.52 $\pm$ 0.08+71.68 <sup><math>\beta</math>**</sup>
G4 (Aqu ext of <i>E. angolense</i> 200 mg/kg)	7.30 $\pm$ 0.18	3.61 $\pm$ 0.09–50.54 <sup><math>\alpha</math>**</sup>	5.42 $\pm$ 0.08+50.13 <sup><math>\beta</math></sup>	6.10 $\pm$ 0.20+68.97 <sup><math>\beta</math></sup>	6.25 $\pm$ 0.18+73.13 <sup><math>\beta</math>*</sup>	7.16 $\pm$ 0.07+98.33 <sup><math>\beta</math>***</sup>

Values are expressed as Mean  $\pm$  SEM (n = 6). \*P < 0.05, \*\*P < 0.01 & \*\*\*P < 0.001  
a: Percentage variation compared to day D0, b: Percentage variation compared to day D2

**Table 2. Effect of the aqueous extract of *E. angolense* bark on the hemoglobin level during and after induction of anemia by phenylhydrazine in the rat**

Groups	Hemoglobin (g/dl)					
	D0	D2	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
Normal control G1 (DW 10 ml/kg)	13.10 $\pm$ 0.11	13.25 $\pm$ 0.15	13.37 $\pm$ 0.17	13.20 $\pm$ 0.20	12.3 $\pm$ 0.40	13.30 $\pm$ 0.5
Anemic control G2 (DW 10 ml/kg)	13.18 $\pm$ 0.10	8.3 $\pm$ 0.10–37.02 <sup><math>\alpha</math>***</sup>	12.33 $\pm$ 0.70+48.55 <sup><math>\beta</math></sup>	12.80 $\pm$ 0.10+54.21 <sup><math>\beta</math></sup>	13.10 $\pm$ 0.40+57.83 <sup><math>\beta</math>*</sup>	13.40 $\pm$ 0.40 +61.44 <sup><math>\beta</math>**</sup>
G3 (Vit B12 syrup 1 ml/day)	13.78 $\pm$ 0.14	7.75 $\pm$ 0.15–43.75 <sup><math>\alpha</math>**</sup>	13.00 $\pm$ 0.24+67.74 <sup><math>\beta</math>**</sup>	13.40 $\pm$ 0.10+72.90 <sup><math>\beta</math>**</sup>	13.47 $\pm$ 0.08+73.80 <sup><math>\beta</math>**</sup>	13.63 $\pm$ 0.08 +75.87 <sup><math>\beta</math>**</sup>
G4 (Aqu ext of <i>E. angolense</i> 200 mg/kg)	13.08 $\pm$ 0.48	6.85 $\pm$ 0.15–47.62 <sup><math>\alpha</math>***</sup>	12.95 $\pm$ 0.65+89.05 <sup><math>\beta</math>***</sup>	13.35 $\pm$ 0.15+94.89 <sup><math>\beta</math>***</sup>	13.40 $\pm$ 0.20+95.62 <sup><math>\beta</math>***</sup>	13.60 $\pm$ 0.10 +98.54 <sup><math>\beta</math>***</sup>

Values are expressed as Mean  $\pm$  SEM (n = 6). \*P < 0.05, \*\*P < 0.01 & \*\*\*P < 0.001  
a: Percentage variation compared to day D0, b: Percentage variation compared to day D2

**Table 3. Effect of the aqueous extract of *E. angolense* bark on hematocrit during and after induction of anemia by phenylhydrazine in rats**

Groups	Hematocrit (%)					
	D0	D2	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
Normal control G1 (DW 10 ml/kg)	42.83 ± 0.24	43.23±0.54	43.65± 0.06	43.97±0.13	42.13±0.03	42.95±0.18
Anemic control G2 (DW 10 ml/kg)	42.55 ± 0.06	24.97 ± 0.27-41.31 <sup>α***</sup>	41.17± 0.33 +64.87 <sup>β***</sup>	41.84± 0.25 +67.56 <sup>β***</sup>	42.25±0.23+69.20 <sup>β***</sup>	42.88±0.15+71.72 <sup>β***</sup>
G3 (Vit B12 syrup 1ml/day)	45.16 ± 0.19	24.88 ± 0.27-44.90 <sup>α***</sup>	41.93± 0.22 +68.52 <sup>β***</sup>	42.35± 0.15 +70.21 <sup>β***</sup>	44.05±0.16+77.04 <sup>β***</sup>	45.17±0.14+81.55 <sup>β***</sup>
G4 (Aqu ext of <i>E angolense</i> 200 mg/kg)	43.86 ± 0.16	22.50 ± 0.10-48.70 <sup>α***</sup>	40.45± 0.05 +79.77 <sup>β***</sup>	41.90± 0.05 +86.22 <sup>β***</sup>	42.90±0.10+90.66 <sup>β***</sup>	43.97±0.15+95.42 <sup>β***</sup>

Values are expressed as Mean ± SEM (n = 6). \*\*\*P<0.001

a: Percentage variation compared to day D0, b: Percentage variation compared to day D2

Phytochemical screening carried out on bark of *E. angolense* revealed the presence of alkaloids, polyphenols, sterols, terpenes, tannins, flavonoids, leucoanthocyanins, quinones, saponins and cardiotoxic glycosides. The presence of these secondary metabolites in the plant could justify, on the one hand, the anti-inflammatory activity and on the other hand the antianemic activity observed. However, studies have showed that the anti-inflammatory effect of flavonoids would be due to an inhibition of prostaglandin synthesis and polyphenols could act on the enzymatic activities of arachidonic acid metabolism [25]. As for the saponins and alkaloids, studies have shown their antianemic properties [26]. Alkaloids inhibit cyclic adenosine monophosphate phosphodiesterase (cAMP) thus accumulating cAMP. This effect stimulates protein phosphorylation and protein synthesis, which improves erythropoiesis [27]. Saponin containing in herbs have been used successfully in the treatment of inflammation of liver, as tonic, sedative formulas to promote and vitalize blood circulation [28,29]. Due to its dual anti-inflammatory action, the aqueous extract of *E. angolense* would treat the inflammation caused by the injection of phenylhydrazine as a pathogen and antianemic that would correct phenylhydrazine induced anemia in the rat. As for vitamin B12, its action would have been interfere with inflammation.

## 5. CONCLUSION

Injection of phenylhydrazine to rats caused haemolytic anemia characterized by decreased haematological parameters. However, the phenylhydrazine that is toxic to the animal has triggered inflammation in the rat. This inflammation was revealed by the CRP assay. Administration of the aqueous extract of *Entandrophragma angolense* by gavage at the dose of 200 mg / kg / day to rats significantly increased the hemoglobin level at the first week of treatment. The antianemic effect of the aqueous extract was more pronounced than that of vitamin B12 (hemoglobin level 98.54% against 75.87% at the 4th week). This shows that the action of vitamin B12 would have been interfering with the inflammation. The antianemic and anti-inflammatory potentials of the plant could come from phytochemicals and also from its possible vitamin and mineral constituents.

For medical care of anemia regardless of the type and severity of anemia, it is best to determine the cause, treat and monitor the correction of the deficiency and anemia.

## ETHICAL APPROVAL

As per university standard guideline ethical approval has been collected and preserved by the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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