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EVALUATION OF THE ANTI-ANEMIC PROPERTIES OF AN AQUEOUS EXTRACT OF LEAVES OF TECTONA GRANDIS (VERBENACEAE) IN SNTOUCHE WISTAR RATS

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SUMMARY

Tectona grandis (Verbenaceae) is a plant commonly used in traditional African medicine to treat several diseases including anemia. The present study was carried out to evaluate the pharmacological effects of an aqueous extract of dry leaves of Tectona grandis (EATg) on induced anemia in wistar rats in order to contribute to the valorization of the use of plants used in traditional medicine. for improving the health of populations. Hemolytic anemia was induced by oral administration of phenylhydrazine hydrochloride at a dose of 8 mg/kg body weight for 10 days. The hematological parameters for diagnosing anemia were measured in each batch of rats using an automatic hematological analyzer (URIT 3000 PLUS). Daily oral administration of EATg, at doses of 100, 500 and 1000 mg/Kg of BW and Ranferon® for 14 days to anemic rats, restores or restores the number of red blood cells, the hemoglobin level, the hematocrit, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin content (MCHC) deteriorated by phenylhydrazine. It appears from this study that EATg has anti-anemic properties similar to those of Ranferon®; which justifies its use in traditional medicine in the treatment of anemia.

KEYWORDS: Tectona grandis, Anemia, Antianemia, Phenylhydrazine, Hematological parameters.

INTRODUCTION

The health crisis caused by the coronavirus pandemic constitutes a tragic and dramatic experience for global society. This health situation marking the history of our century reveals that an adequate response from the man of the 21st century to the question of disease is the one which, inevitably, results from the articulation between sciences and traditions.

In Africa, traditional medicine and modern medicine still coexist very strongly. Indeed, in most black African countries, traditional medicine provides 70-80% of the health coverage of the population, with the same effectiveness as modern medicine.[1] However, everyone's duty would be to make themselves useful and, above all, to share their knowledge, research and experiences with others.[2] Also, despite the remarkable improvement in living conditions, anemia, classified among the ten most serious health problems in the world by the WHO, constitutes a truly major public health problem throughout the world.[3] Several therapeutic plants are used by traditional medicine in the treatment of anemia, including the leaves of Tectona grandis (Verbenaceae), commonly known as Teak.[4,5] Thus, the objective of our present study which focuses on the evaluation of the anti-anemic properties of Tectona grandis (verbenaceae) in rats of wistar strain is therefore to contribute to the valorization of the use of plants used in traditional medicine for the improvement of population health. Our research therefore aims to establish the scientific bases for a traditional and efficient use of the leaves of Tectona grandis (Verbenaceae) in the treatment of anemia.[6]

I- MATERIAL AND METHODS

I-1. Plant material

The plant material consists of leaves of Tectona grandis (Verbenaceae), collected in the commune of Yopougon in the district of Abidjan (Côte d'Ivoire) on July 24, 2021. The identification was made by Doctor YAO KONAN from the Laboratory of Botany thanks to the herbarium of the National Floristic Center (CNF) which is the herbarium of Côte d'Ivoire, on sample N014794 of 08-09-1979.

I-2. Animal material

Female mice of the species Mus Musculus (Muridae), of homogeneous Swiss parental strains, weighing between 20 and 27 g, were used for acute toxicity tests. Rattus norvegicus rats of the Wistar strain weighing between

150 and 260 g were used for pharmacological studies on anemia. These animals come from the vivarium of the Ecole Normale Supérieure (E.N.S). They are fed, ad libitum, with food provided by the company IVOGRAIN® of Abidjan, Ivory Coast and have free access to water.

I-3. Pharmacological substances

In this study, the pharmacodynamic substances used are Phenylhydrazine hydrochloride 99% (Sigma-Aldrich, Germany), a pharmacological substance used to induce anemia and Ranferon® (Sun Parmaceutical Ind. Ltd, India), an anti-anemic pharmaceutical substance used to treat anemia.

II. Methods

II-1. Preparation of the aqueous extract of the leaves of Tectona grandis (Verbenaceae)

The Tectona grandis leaves used are dried in the shade, at room temperature, before being ground into powder. One hundred (100) grams (g) of this leaf powder were boiled at 100°C in two (2) liters of distilled water for fifteen (15) minutes. The decoction obtained was filtered successively through hydrophilic cotton and "Wattman No. 2" filter paper. The filtrate obtained was dried in an oven at 60°C for 48 h. We obtain a perfectly water-soluble powder which is the aqueous extract of the dried leaves of Tectona grandis (EAqTg) used for the tests. The extraction process is summarized in Fig 1.

II-2. Pharmacological study

II-2-1. Induction of anemia

Anemia was induced by oral administration of phenylhydrazine hydrochloride at a dose of 8 mg/Kg BW for 10 days.[7]

II-2-2. Experimental protocol

Rats weighing between 150 and 260 g, without any prior treatment, were divided into six (06) batches of four (04) rats, five (05) of which were treated with phenylhydrazine hydrochloride. The aqueous extract of Tectona grandis was dissolved in distilled water to obtain the required concentrations administered by gavage. These six (06) batches of rats are then force-fed for fourteen (14) days as indicated below

Lot 1: normal rats (not anemic) which receive only distilled water (2ml).

Lot 2: anemic rats which receive only distilled water (2ml).

Lot 3: anemic rats treated with 1 ml of Ranferon® (reference anti-anemic substance) at a dose of 5 mg/Kg BW.

Lot 4: anemic rats treated with 100 mg/Kg BW of the aqueous extract of dry leaves of Tectona grandis.

Lot 5: anemic rats treated with 500 mg/Kg BW of the aqueous extract of dry leaves of Tectona grandis.

Lot 6: anemic rats treated with 1000 mg/Kg of body weight of the aqueous extract of the dry leaves of Tectona grandis.

II-2-3. Blood sampling

Approximately 2 ml of blood were collected in tubes containing an anticoagulant (EDTA) by puncture at the level of the retro-orbital sinus using a sterile Pasteur pipette after anesthetizing the rats with ether, before the administration of phenylhydrazine hydrochloride, then on days D0 (at the end of induction and start of gavage), D7 and D14 (after the administration of distilled water, the aqueous extract of Tectona grandis and the reference substance).

II-2-4. Assay of hematological parameters

The hematological parameters were measured in each batch of rats using an automatic hematological analyzer (URIT 3000 PLUS). A small quantity of blood contained in each EDTA tube is taken by the machine (URIT 3000 PLUS), then the different blood parameters are then automatically analyzed for 60 seconds.

II-3. Processing of results

The statistical analysis of the values and the graphical representation of the data were carried out using Graph Pad Prism 8.4 software (San Diego, California, USA). The statistical difference between the results was carried out using analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test, with the significance threshold $P<0.05$. All values are presented as mean ± SEM (Standard Error of the Mean).

III- RESULTS AND DISCUSSION

III-1. Results

III-1-1. Effects of phenylhydrazine hydrochloride on some hematological parameters for diagnosing anemia in rats Table I presents the mean values of the number of red blood cells (RBC), hemoglobin (Hb), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCHC) and mean corpuscular hemoglobin concentration (MCHC) of rats before the administration of phenylhydrazine hydrochloride (PHZ) and after the administration of phenylhydrazine hydrochloride (PHZ) at a dose of 8 mg/Kg BW.

The mean values of the number of red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (TCMH) and concentration mean corpuscular hemoglobin (CCMH) of rats before administration of phenylhydrazine hydrochloride (PHZ) are respectively.

6.98 x 1012 /L, 14.4g/dl, 42.7%, 58.8 fl, 20.4 pg and 33.3 g/dl.

The administration of phenylhydrazine hydrochloride (PHZ) resulted in a highly significant reduction ($p < 0.001$) in the number of red blood cells (3.80 x1012/L) corresponding to a percentage reduction of 45.56%. Furthermore, a very significant decrease ($p <0.01$) in the hemoglobin level (7.08g/dL), the hematocrit level (35.6%) and the CCMH (28 g/l) corresponding to respective decrease percentages of 57.77%, 16.62% and

15.74% were observed. On the other hand, a highly significant ($p < 0.001$) and very significant ($p < 0.01$) increase in respective MCV (93.0 fl) and TCMH (25.9 pg) corresponding to respective percentages of increase in 58.16% and 26.96% was observed.

Table I: Variation of some hematological parameters of rats after administration of phenylhydrazine.

	Avant PHZ		Après PHZ		PV	
	RN	**RT**	**RN**	**RT**	**RN**	**RT**
GR 10^{12}/L	7,11 ± 0,34	6,98 ± 0,20	7,08 ± 0,29	3,80 ± 0,30***	-0,42%	-45,56%***
Hb g/dL	13,6 ± 0,5	14,4 ±0,5	13,7 ± 0,5	7.08 ± 0,7**	+0,73%	-57,77%**
Ht %	39,8 ± 2,1	42,7 ± 2,1	38,9 ± 2,1	35,6 ± 2,1**	-2,34%	-16,62%**
VGM fL	56,1 ± 1,3	58,8 ± 4,1	57,1 ± 1,8	93,0 ± 3,5***	+1,79%	+58,16%***
TCMH pg	19,2 ± 0,2	20,4 ±0,5	19,1± 0,2	25,9± 0,6**	-0,13%	+26,96%**
CCMH g/dL	34,3 ± 0,6	33,3 ±0,6	34,1 ± 0,8	28 ± 0,7**	-0,44%	-15,74%**

GR: Red Blood Cells; Hb: hemoglobin; Ht: hematocrit; TCMH: Mean corpuscular hemoglobin content; CCMH: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; PHZ: Phenylhydrazine; PV: Percentage variation; RN: Normal Rats; RT: Treated Rats.

III-1-2. Effects of the aqueous extract of dry leaves of Tectona grandis on some hematological parameters of anemic rats.

III-1-2-1. Effects of aqueous extract of dry leaves of Tectona grandis on the number of red blood cells of anemic rats.

Fig 2 shows the effects of the aqueous extract of dry leaves of Tectona grandis (EATg) and Ranferon® on the number of red blood cells in anemic rats. On day 0, the number of red blood cells in anemic rats was 3.8 ± 0.30x1012/L, with a highly significant difference ($p < 0.001$) compared to that of the non-anemic control group. During the experiment, the number of red blood cells in the non-anemic control group (7.08 ± 0.31 x1012/L) did not vary significantly ($p > 0.05$).

Oral administration (gavage) of EATg resulted in a dose-dependent increase in the number of red blood cells in the treated batches on Day 7. Indeed, the number of red blood cells increased from 3.8 ± 0.30 x1012/L to, 5.09 ± 0.06 x1012/L, 5.95 ±0.36, 6.71 ±0.27 x1012 /L respectively for the batches treated with doses of 100, 500 and 1000 mg /Kg BW of EATg. The values of the batches treated with doses of 100 and 500 mg/Kg BW of EATg present a significant difference compared to the non-anemic control batch. On the other hand, that of the batch treated with 1000 mg/Kg BW of EATg presents a non-significant difference ($p > 0.05$) compared to the non-anemic control batch. For rats treated with Ranferon®, the number of red blood cells also increased with a non-significant difference ($p > 0.05$) compared to the non-anemic control group on the 7th day. It went from 3.8 x1012/L to 6.70 ± 0.40x1012/L. The number of red blood cells of untreated anemic rats on the 7th day (4.01 ± 0.03 x1012/L) presents a highly significant difference ($p < 0.001$) compared to that of the non-anemic control group.

On day 14, a dose-dependent increase in red blood cell counts was observed in all EATg-treated batches. Indeed, the number of red blood cells is 6.05 ± 0.04 x1012/L, 6.70 ± 0.40 x1012/L and 7.80 ±0.06 x1012/L respectively for the batches treated with the doses of 100, 500 and 1000 mg/Kg BW of EATg.

The values of the batches treated with 100 and 500 mg/Kg BW of EATg present a significant difference ($p < 0.05$) compared to the non-anemic control batch. On the other hand, that of the batch treated with 1000 mg/Kg BW of EATg presents a non-significant difference ($p > 0.05$) compared to the non-anemic control batch. For rats treated with Ranferon®, the number of red blood cells also increased with a non-significant difference ($p > 0.05$) compared to the non-anemic control group on the 14th day. It is 7.60 ± 0.07 x1012/L. The number of red blood cells of untreated anemic rats on the 14th day (5.78 ± 0.29 x1012/L) presents a very significant difference ($p < 0.01$) compared to that of the non-anemic control group.

The dose of 1000 mg/Kg BW of EATg and Ranferon® led to a recovery in the number of red blood cells in anemic rats at the end of the experiment. However, the greatest increase was obtained with the dose of 1000 mg/Kg BW of EATg.

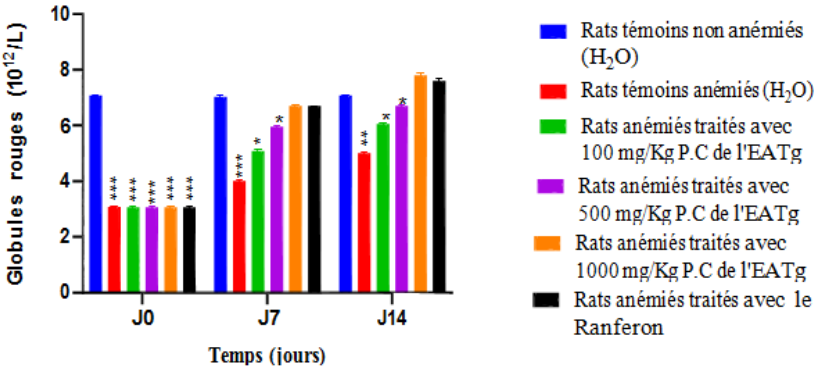

Figure 2: Variation in the number of red blood cells after treatment of anemic rats with aqueous extract of dry leaves of Tectona grandis and Ranferon.

The values expressed represent the mean ± SEM, with n=4; * $p < 0.05$; **$p<0.01$; *** $p < 0.001$ compared to non-anemic control rats, SEM: Standard Error of the Mean.

EATg leads to a dose-dependent increase in the number of RBCs during the treatment period. The greatest increase is obtained with rats from the group treated with the dose of 1000 mg/Kg.BW.

III-1-2-2. Effects of the aqueous extract of dry leaves of Tectona grandis on the hemoglobin level of anemic rats Fig 3 presents the effects of the aqueous extract of dry leaves of Tectona grandis (EATg) and Ranferon® on the hemoglobin level of anemic rats.

On day 0, the hemoglobin level of anemic rats was 7.08 ± 0.56 g/dl, with a highly significant difference ($p < 0.001$) compared to that of the non-anemic control group (13.70 ± 0.001). 5 g/dl). During the 14 days of experimentation, the hemoglobin level of the non-anemic control group did not vary significantly ($p > 0.05$).

Oral administration (gavage) of EATg resulted in a dose-dependent increase in the hemoglobin level of the treated batches on the 7th Day. Indeed, the hemoglobin level went from 7.08 ± 0.56 g/dl to 10.73 ±0.03 g/dl, 12.30 ±0.04 g/dl 13.50 ±0.05 g/dl respectively for doses of 100, 500 and 1000 mg/Kg BW of EATg. The values of the batches treated with 100 and 500 mg/Kg BW of EATg present a significant difference compared to the non-anemic control batch. On the other hand, that of the batch treated with 1000 mg/Kg BW of EATg presents a non-significant difference ($p > 0.05$) compared to the non-anemic control batch. For rats treated with Ranferon®, the number of red blood cells also increased with a non-significant difference ($p > 0.05$) compared to the non-anemic control group on the 7th day. It went from 7.08 ± 0.56 g/dl to 13.05 ± 0.05x1012/L. The number of red blood cells of untreated anemic rats on the 7th day (8.30 ± 0.05 x1012/L) presents a highly significant difference ($p < 0.001$) compared to that of the non-anemic control group.

On day 14, a non-significant dose-dependent increase ($p > 0.05$) in the hemoglobin level compared to that of the non-anemic control group is observed with all doses of EATg. Indeed, the hemoglobin level is 12.91 ±0.6 g/dl; 13.70 ±0.02 g/dl and 14.02 ±0.06 g/dl respectively for the batches treated with doses of EATg (100, 500 and 1000 mg/Kg BW). For rats treated with Ranferon®, the hemoglobin level also increased with a non-significant difference ($p > 0.05$) compared to the non-anemic control group on the 14th day. It is 13.65 ± 0.02 x1012/L. The hemoglobin level of untreated anemic rats on the 14th day (10.60 ± 0.05 x1012/L) presents a very significant difference ($p < 0.01$) compared to that of the non-anemic control group. EATg leads to a rapid and greater increase in hemoglobin levels compared to Ranferon®.

On day 14, a non-significant dose-dependent increase ($p > 0.05$) in the hemoglobin level compared to that of the non-anemic control group is observed with all doses of EATg. Indeed, the hemoglobin level is 12.91 ±0.6 g/dl; 13.70 ±0.02 g/dl and 14.02 ±0.06 g/dl respectively for the batches treated with doses of EATg (100, 500 and 1000 mg/Kg BW). For rats treated with Ranferon®, the hemoglobin level also increased with a non-significant difference ($p > 0.05$) compared to the non-anemic control group on the 14th day. It is 13.65 ± 0.02 x1012/L. The hemoglobin level of untreated anemic rats on the 14th day (10.60 ± 0.05 x1012/L) presents a very significant difference ($p < 0.01$) compared to that of the non-anemic control group. EATg leads to a rapid and greater increase in hemoglobin levels compared to Ranferon®.

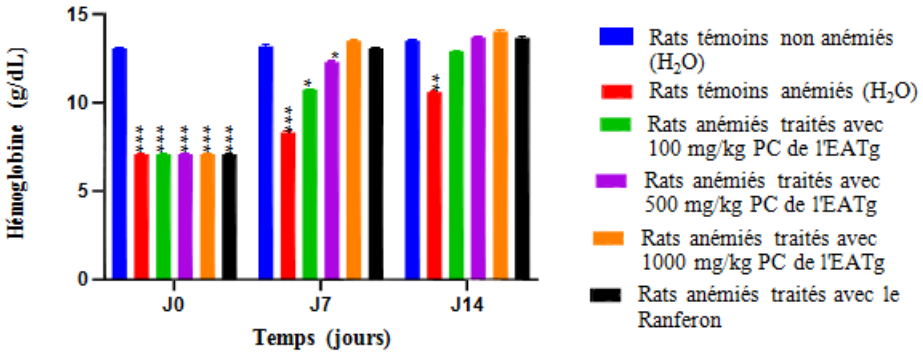

Figure 3: Variation in hemoglobin level after treatment of anemic rats with aqueous extract of dry leaves of Tectona gra ndis and Ranferon.

The values expressed represent the mean ± SEM, with n=4; * p < 0.05; **p<0.01; *** P < 0.001 compared to non-anemic control rats.

EATg causes a rapid and greater increase in hemoglobin levels compared to Ranferon.

III-1-2-3. Effects of aqueous extract of dry leaves ofTectona grandis on hematocrit level of anemic rats.

Figure 4 shows the effects of aqueous extract of dry leaves of Tectona grandis (EATg) and Ranferon on the hematocrit level of anemic rats.

On day 0, the hematocrit level of anemic rats was 35.6 ± 0.80%, with a significant difference (p < 0.05) compared to that of the non-anemic control group (38.90 ± 0.17%).

During the experiment, the hematocrit level of the non-anemic control group did not vary significantly (p > 0.05).

Oral administration of EATg resulted in a non-significant dose-dependent increase (p > 0.05) in the hematocrit level of the treated groups compared to the non-anemic control group on the 7th day. Indeed, the hematocrit rate increased from 35.60 ± 0.80%, to 37.23 ± 0.5%, 37.33 ± 0.64%, and 37.80 ± 0.63% respectively for batches treated with doses of 100, 500 and 1000 mg/Kg BW.

For rats treated with Ranferon®, the hematocrit level also increased with a non-significant difference (p > 0.05) compared to the anemic control group on the 7th day. It went from 35.6 ± 0.80% to 37.50 ± 0.25%. The hematocrit level of untreated anemic rats on the 7th day (36.50 ± 0.80 x1012/L) presents a significant difference (p < 0.05) compared to that of the non-anemic control group.

On day 14, a non-significant increase (P> 0.05) in the hematocrit level compared to that of the non-anemic control group is observed with all EATg doses and Ranferon®. In fact, the hematocrit rate is 38.23 ± 0.52%, 38.40 ± 0.27%, 39.90 ± 0.22% and 38.50 ± 0.68% respectively for the batches treated with doses of 100, 500 and 1000 mg/Kg BW of EATg and Ranferon. The hematocrit level of untreated anemic rats on the 14th day (37.60 ± 0.80) presents a non-significant difference (p > 0.05) compared to that of the non-anemic control group.

It appears from these results that doses of 100, 200 and 1000 mg/Kg BW of EATg and Ranferon® lead to an increase in the hematocrit level close to that of the level of the non-anemic control group. However, the dose of 1000 mg/Kg BW of EATg led to a complete restoration of the hematocrit level of anemic rats at the end of the experiment.

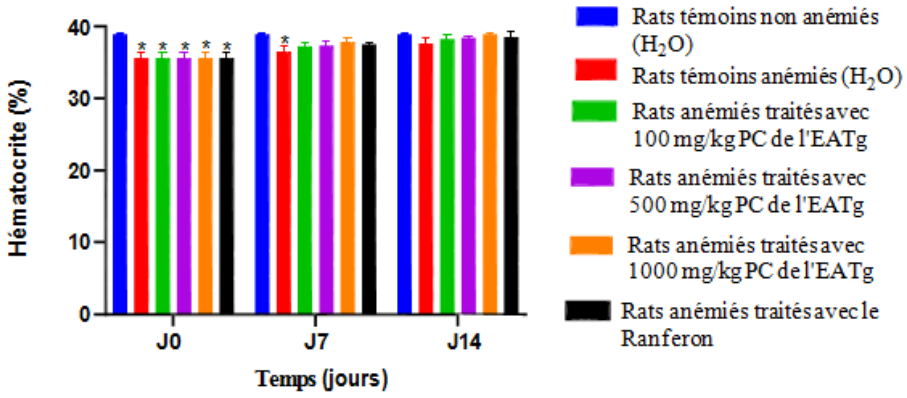

Figure 4: Variation in hematocrit level after treatment of anemic rats with aqueous extract of dry leaves of Tectona grandis and Ranferon.

The values expressed represent the mean ± SEM, with n=4; * p < 0.05 compared to non-anemic control rats.

EATg causes an increase in the hematocrit level until it normalizes on the 14th day with the dose 1000 mg/Kg BW.

III-1-2-4. Effects of aqueous extract of dry leaves of Tectona grandis on mean corpuscular volume (MCV) of anemic rats.

Figure 5 presents the effects of aqueous extract of dry leaves of Tectona grandis (EATg) and Ranferon® on the mean corpuscular volume (MCV) of anemic rats.

On day 0, the mean corpuscular volume (MCV) of anemic rats was 93.0 ± 3.5 fl, with a highly significant difference ($p < 0.001$) compared to that of the non-anemic control group (57.1 ± 1). 8 fl). During the experiment, the mean corpuscular volume of the non-anemic control group did not vary significantly ($p > 0.05$).

Oral administration (gavage) of EATg resulted in a dose-dependent reduction in the mean corpuscular volume of the treated groups compared to the non-anemic control group on the 7th day.

Indeed, the average globular volume increased from 93.0 ± 3.5 fl to 77.50 ± 1.32 fl, 65.93 ± 2.44, fl and 59.20 ± 0.67 fl respectively for the batches treated with doses of 100, 500 and 1000 mg/Kg BW of EATg. The values of the batches treated with 100 and 500 mg/Kg BW of EATg respectively present a very significant and significant difference compared to the non-anemic control batch. On the other hand, that of the batch treated with 1000 mg/Kg BW of EATg presents a non-significant difference ($p > 0.05$) compared to the non-anemic control batch. For rats treated with Ranferon®, the mean corpuscular volume also decreased with a non-significant difference ($p > 0.05$) compared to the non-anemic control group on the 7th day. It went from 93.0 ± 3.5 fl to 60.93 ± 1.08 fl. The mean corpuscular volume of untreated anemic rats on the 7th day (90.00 ± 0.25 fl) presents a highly significant difference ($p < 0.001$) compared to that of the non-anemic control group.

On day 14, a non-significant decrease ($P > 0.05$) in mean corpuscular volume compared to that of the non-anemic control group was observed with all doses of EATg. and Ranferon®. Indeed, the average globular volume is 49.43 ± 0.23 fl, 58.87 ± 0.15 fl, 57.09 ± 0.61 fl and 58 ± 0.28 fl respectively for the batches treated with the doses of 100, 500 and 1000 mg/Kg BW of EATg and Ranferon®. The mean globular volume of untreated anemic rats on the 14th day (78.00 ± 0.05fl) presents a very significant difference ($p < 0.01$) compared to that of the non-anemic control group.

It appears from these results that doses of 100, 200 and 1000 mg/Kg BW of EATg and Ranferon® lead to a reduction in the mean globular volume close to that of the non-anemic control group. However, the dose of 1000 mg/Kg BW of EATg led to a complete restoration of the mean corpuscular volume of anemic rats at the end of the experiment.

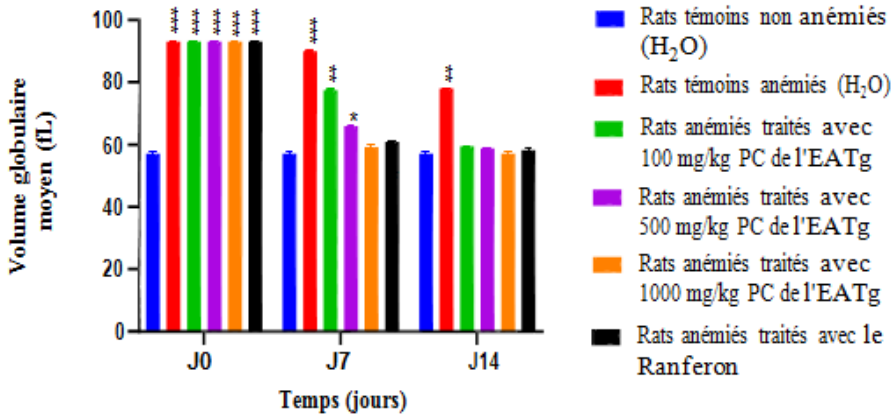

Figure 5: Variation in Mean Globular Volume (MCV) after treatment of anemic rats with aqueous extract of dry leaves of Tectona grandis and Ranferon.

The values expressed represent the mean ± SEM, with n=4; * p < 0.05; **p<0.01; *** P < 0.001 compared to non-anemic control rats.

EATg causes a reduction in MCV until it normalizes on the 14th day.

III-1-2-5. Effects of aqueous extract of dry leaves of Tectona grandis on mean corpuscular hemoglobin content (MCHC) of anemic rats.

Figure 6 presents the effects of aqueous extract of dry leaves of Tectona grandis (EATg) and Ranferon® on the mean corpuscular hemoglobin content (TCMH) of anemic rats.

On day 0, the mean corpuscular hemoglobin content (TCMH) of anemic rats was 25.9 ± 0.60 pg, with a highly significant difference ($p < 0.001$) compared to that of the non-anemic control group (19.1 ± 0.20 pg) During the experiment, the average corpuscular hemoglobin content of the non-anemic control group did not vary significantly ($p > 0.05$).

Oral administration (gavage) of EATg resulted in a dose-dependent reduction in the mean corpuscular hemoglobin content (TCMH) of the treated groups compared to the non-anemic control group on the 7th day. Indeed, the mean corpuscular hemoglobin content (TCMH) increased from 25.90 ± 0.60 pg to 23.10 ± 0.44 pg, 21.05 ± 0.70 pg and 20.33 ± 0.51 pg respectively for the batches treated with doses of 100, 500 and 1000 mg/Kg BW of EATg. The values of the batches treated with 100 and 500 mg/Kg BW of EATg present a significant difference ($p < 0.05$) compared to the non-anemic control batch. On the other hand, that of the batch treated with 1000 mg/Kg BW of EATg presents a non-significant difference ($p > 0.05$) compared to the non-anemic control batch. For rats treated with Ranferon®, the mean corpuscular hemoglobin content (TCMH) also decreased with a non-significant difference ($p > 0.05$) compared to the non-anemic control group on the 7th day. It went from 25.9 ± 0.60 pg to 20.10 ± 0.51 pg. The mean corpuscular hemoglobin content of untreated anemic rats on the 7th day (25.00 ± 0.59 pg) presents a highly significant difference ($p < 0.001$) compared to that of the non-anemic control group.

On day 14, a non-significant decrease ($P > 0.05$) in the mean corpuscular hemoglobin content (TCMH) compared to that of the non-anemic control group is observed with all doses of EATg. and Ranferon®. Indeed, the average globular volume is 19.83 ± 0.30 pg, 19.50 ± 0.46 pg, 19.10 ± 0.21 pg and 19.15 ± 0.44 pg respectively for the batches treated with doses of 100, 500 and 1000 mg/Kg BW of EATg and Ranferon®. The mean corpuscular hemoglobin content (TCMH) of untreated anemic rats on the 14th day (23.90 ± 0.60 pg) presents a very significant difference ($p < 0.01$) compared to that of the non-anemic control group. It appears from these results that doses of 100, 200 and 1000 mg/Kg BW of EATg and Ranferon® lead to a reduction in the mean corpuscular hemoglobin content (TCMH) close to that of the non-anemic control group. However, the dose of 1000 mg/Kg BW of EATg led to a complete restoration of the average corpuscular hemoglobin content of anemic rats at the end of the experiment.

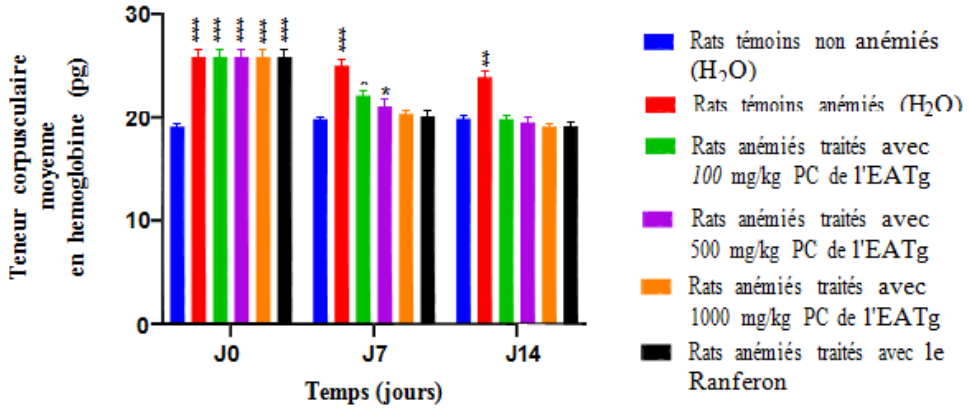

Figure 6: Variation in Mean Corpuscular Hemoglobin Content (TCMH) after treatment of anemic rats with aqueous extract of dry leaves of Tectona grandis and Ranferon.

The values expressed represent the mean ± SEM, with n=4; * $p < 0.05$; **$p<0.01$; *** $P < 0.001$ compared to non-anemic control rats.

EATg causes a reduction in TCMH until its normalization on the 14th day.

III-1-2-6. Effects of the aqueous extract of dry leaves of Tectona grandis on the mean corpuscular hemoglobin concentration (MCHC) of anemic rats.

Figure 7 presents the effects of the aqueous extract of dry leaves of Tectona grandis (EATg) and Ranferon® on the mean corpuscular hemoglobin concentration (MCHC) of anemic rats.

On day 0, the mean corpuscular hemoglobin concentration (MCHC) of anemic rats was 28.00 ± 0.70g/dl, with a highly significant difference ($p < 0.001$) compared to that of the non-anemic control group (34, 1 ± 0.8 g/dl) During the experiment, the average corpuscular hemoglobin concentration of the non-anemic control group did not vary significantly ($p > 0.05$).

Oral administration (gavage) of EATg resulted in a dose-dependent increase in the mean corpuscular hemoglobin concentration (MCHC) of the treated groups compared to the non-anemic control group on the 7th day. Indeed, the mean corpuscular hemoglobin concentration (MCHC) increased from 28 ± 0.70 g/dl to 29.90 ± 0.53 g/dl, 30.70 ± 0.34 g/dl and 32.43 ± 0.36 g/dl respectively for batches

treated with doses of 100, 500 and 1000 mg/Kg BW of EATg. The values of the batches treated with 100 and 500 mg/Kg BW of EATg present a very significant difference ($p < 0.01$) compared to the non-anemic control batch. On the other hand, that of the batch treated with 1000 mg/Kg BW of EATg presents a non-significant difference ($p > 0.05$) compared to the non-anemic control batch. For rats treated with Ranferon®, the mean corpuscular hemoglobin concentration (MCHC) also increased with a non-significant difference ($p > 0.05$) compared to the non-anemic control group on the 7th day. It went from 28 ± 0.70 g/dl to 32.10 ± 0.61 g/dl. The mean corpuscular hemoglobin concentration (MCHC) of untreated anemic rats on the 7th day (29.50 ± 0.68 g/dl) presents a very significant difference ($p< 0.01$) compared to that of the non-anemic control group.

On day 14, a non-significant increase ($P> 0.05$) in the mean corpuscular hemoglobin concentration (MCHC) compared to that of the non-anemic control group is observed with all doses of EATg. and Ranferon®. Indeed, the mean corpuscular hemoglobin concentration (MCHC) is 32.43 ± 0.42 g/dl, 34.00 ± 0.13 g/dl, 34.10 ± 0.23 g/dl and 34.08 ± 0.38 g/dl respectively for the batches treated with doses of 100, 500 and 1000 mg/Kg BW of EATg and Ranferon®. The mean corpuscular hemoglobin concentration (MCHC) of untreated anemic rats on the 14th day (29.50 ± 0.60g/dl) presents a significant difference ($p < 0.05$) compared to that of the non-anemic control group. It appears from these results that doses of 100, 200 and 1000 mg/Kg BW of EATg and Ranferon® lead to an increase in the mean corpuscular hemoglobin concentration (CCMH) close to that of the non-anemic control group. However, the dose of 1000 mg/Kg BW of EATg resulted in a complete restoration of the mean corpuscular hemoglobin concentration of anemic rats at the end of the experiment.

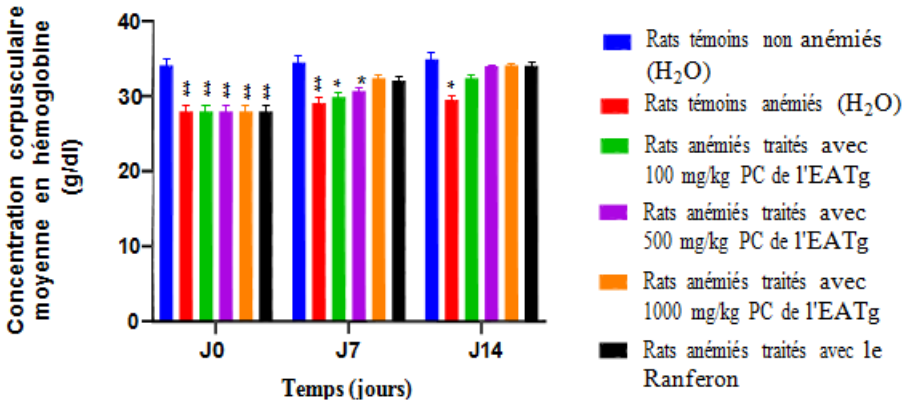

Figure 7: Variation in Mean Corpuscular Hemoglobin Concentration (MCHC) after treatment of anemic rats with aqueous extract of dry leaves of Tectona grandis and Ranferon.

The values expressed represent the mean ± SEM, with n=4; * $p < 0.05$; ** $p < 0.01$ compared to non-anemic control rats.

EATg leads to an increase in CCMH until its normalization on the 14th day.

III-2. DISCUSSION

The administration of phenylhydrazine hydrochloride (PHZ) caused disturbances or modifications in the hematological parameters of anemic rats. Indeed, a highly significant ($P<0.001$) decrease in the number of red blood cells and a very significant ($P<0.01$) decrease in the level of hemoglobin, hematocrit and CCMH was observed. On the other hand, a highly significant ($P<0.001$) and very significant ($P<0.01$) increase in respective MCV and TCMH was observed. Our results are consistent with those of.[4,8,9] who observed a decrease in the number of red blood cells, hemoglobin level and hematocrit in rats after administration of 40 mg/Kg BW of PHZ for 2 days and 4 mg/Kg BW of PHZ for 6 days.

According to the work of,[7] phenylhydrazine induces hemolytic anemia of toxic origin (anemia of peripheral cause or regenerative anemia), following the formation of methemoglobin, which results in liver, spleen and kidney damage.

The study of the effects of EATg on the hematological parameters of anemic rats shows that this extract, at doses of 100, 500 and 1000 mg/Kg BW, causes on the one hand a significant dose-dependent increase in the number of red blood cells, hemoglobin levels, hematocrit and CCMH and a significant decrease in MCV of TCMH on the other hand.

The effects of EATg on the hematological parameters of anemic rats are similar to those of Ranferon® (reference anti-anemic pharmaceutical substance). EATg, like Ranferon®, reduces the disturbances caused by PHZ and normalizes the hematological parameters of anemic rats. Indeed, the dose of 1000 mg/Kg BW of EATg causes a more rapid and total normalization of the parameters studied.

Similar results were obtained after the treatment of anemic rats by oral administration of aqueous extracts of leaves Justicia secunda (Acanthaceae) at doses of 1000 and 2000 mg/Kg BW,[4] fruits of Solanum torvum (Solanaceae) at a dose of 250 mg/Kg BW,[10] and Sorghum bicolor leaves (Poaceae) at doses of 1500 and 2000 mg/Kg BW.[9]

Furthermore, EATg caused variations in PHZ-impaired MCV, TCMH and CCMH values within the reference values described by.[11] EATg induced normalization of MCV, TCMH and CCMH. This means that EATg normalizes red blood cell size and hemoglobin content.

The potential anti-anemic effects of EATg linked to the increase in the number of red blood cells imply a possibility or capacity of this extract to activate erythropoiesis, hemoglobin production and hematocrit.

According to the work of,[12] there is a link between erythropoiesis and increased iron availability. Indeed, iron is involved in the formation of heme (porphyrin +iron+2), one of the constituents of hemoglobin. Furthermore, the work of.[13] demonstrated a high iron content (4.4%) in the leaves of Tectona grandis. Consequently, the effect of EATg on the number of red blood cells would be due to its richness in iron and therefore to its potential for activating erythropoiesis via erythropoietin, a hormone regulating the production of red blood cells. Indeed, erythropoietin produced by the kidneys increases the number of sensitive erythroblasts in the bone marrow which are converted into reticulocytes and later into mature erythrocytes.[14]

In this study, EATg was found to be effective in resolving phenylhydrazine-induced anemia. It is known that the pharmacological effects of plant extracts are linked to their phytochemical constituents. The bioactive molecules contained in the aqueous extract of Tectona grandis leaves would be able to repair the damage caused by phenylhydrazine.

CONCLUSION AND PERSPECTIVES

Administration by gavage of Phenylhydrazine (8 mg/Kg BW) caused lysis of red blood cells in rats resulting in hemolytic anemia characterized by a reduction in the number of red blood cells, hemoglobin level, hematocrit and mean corpuscular hemoglobin concentration (MCHC).

Oral administration (gavage) of EATg made it possible to reduce or correct the disturbances induced by phenylhydrazine and to normalize the hematological parameters of rats rendered anemic. This extract therefore has anti-anemic effects which are similar to those of Ranferon®, our reference anti-anemic substance. However, the dose of 1000 mg/kg BW of EATg has better anti-anemic activity. It was found to be more effective than Ranferon® in resolving anemia induced by phenylhydrazine.

These results confirm the bioactive potential of EATg, justify the therapeutic indication of Tectona grandis leaves (Verbenaceae) in traditional medicine in the treatment of anemia and provide a scientific basis for its use.

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