Journal of Global Biosciences
Peer Reviewed, Refereed, Open-Access Journal
ISSN 2320-1355
Volume 9, Number 9, 2020, pp. 7912-7927
Website: www.mutagens.co.in
URL: www.mutagens.co.in/jgb/vol.09/09/090903.pdf

SCAN ME

Research Paper

EVALUATION OF ANTIANEMIC EFFECTS OF AQUEOUS EXTRACT OF *Spinacia oleracea* LEAVES (CHENOPODIACEAE) IN MOUSE

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Abstract

Spinacia oleracea (spinach) is a leaf vegetable belonging to the family Chenopodiaceae, used in the treatment of several diseases such as hemolytic anemia. The aim of ths study is to evaluate the anti-anemic potential of aqueous extract of *Spinacia oleracea* leaves in mouse after induction of anemia by phenylhydrazine hydrochloride. Phytochemical analysis revealed the presence of sterols, polyphenols, flavonoids, alkaloids and saponosides. The aqueous extract of *Spinacia oleracea* showed no evidence of single dose toxicity (2000 mg/kg) in studying acute toxicity. Induction of anemia by injection of phenylhydrazine hydrochloride resulted in decreased red blood cell, hemoglobin and hematocrit levels in mouse compared to control treated only with distilled water. Treatment of animals for 7 days after induction of anemia showed a significant increase erythrocyte parameters (red blood cells, hemoglobins and hematocrit) of mouse treated at a dose of 200 mg/ kg B.W. *Spinacia oleracea* (spinach) has anti-anemic potential which supports its traditional use in the treatment of anemia.

Key words: *Spinacia oleracea*, anémia, phénylhydrazine hydrochloride, mouse.

INTRODUCTION

Anemia is a public health problem around the world and especially in developing countries. According to World Health Organization (WHO**)**, 1.62 billion people are affected by anemia in the world [1]. WHO also estimates that around 50% of cases are due to iron deficiency and that high-risk populations are infants, children and women of childbearing age [2]. Several scientific studies have shown that a healthy diet rich in fruits and vegetables has a positive impact on health and reduces the negative impact of

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the risk of several chronic diseases such as cancer, cardiovascular disease, diabetes and anemia.

Vegetables have beneficial effects on physiological functions by increasing well-being and reducing the risks of various pathologies [3]. In addition, vegetables are very rich in minerals, vitamins and dietary fiber. Leaf vegetables provide more minerals than other vegetables. In addition to their nutritional importance, leaf vegetables are of economic and social interest due to their relatively low cost, ease of access and the speed of their preparation [4].

Spinacia orelacea, vernacular name spinach is a leafy vegetable from the chenopodiaceae family, it is a vegetable plant, which is cultivated in all temperate regions for its nutritional qualities [5].

The aim of the study is to evaluate the antianemic potential of the aqueous extract of *Spinacia orelacea* (spinach) in mouse.

MATERIAL AND METHODS

Plant material

The fresh leaves of *Spinacia oleracea* have been harvested in August in AKEIKOI village in the municipality of Abobo (Abidjan- Ivory Coast) in a vegetable garden. This plant has been authenticated at the Botanical Laboratory of the University Félix Houphouët Boigny (Abidjan - Ivory Coast).

Extraction method

The harvested leaves of Spinacia oleracea were washed with distilled water. 200 g of leaves were crushed then macerated in 1/2 L of distilled water using a Blender-moulinex for 9 minutes. The macerated is filtered through a poplin cloth, then on Whatmann 1 filter paper. The filtrate was placed in a Memmert brand oven at 50 ° C until a dry extract was obtained.

Phytochemical study

The qualitative phytochemical study was performed to identify the main phytoconstituents present in the leaves of *Spinacia oleracea.*

Animal Material

SWISS albino mouse four weeks old and weighing between 21 and 36 g were used. The animals were acclimatized for a month in the vivarium from l'ENS (Ecole Normale Supérieure, Abidjan –Côte d'Ivoire). They were raised at ambient temperature of 22 ± 3 °C with 40 to 60% moisture and a photoperiod of 12 hours light and 12 hours darkness. They were fed pellets and had free access to water.

Acute toxicity study

This study is conducted in accordance with guideline 423 of l'Organization for Economic Cooperation and Development (OECD) for the assessment of acute toxicity of medicinal plants [6]. Two groups (Control and group test) of three healthy female mice were administered orally at the limit dose of 2000 mg/kg B.W of aqueous extract of *Spinacia oleracea*. The observations focused on the fur, eye color, mucous membrane, salivation, lethargy, the sleep, coma, convulsion, tremor, diarrhea, mortality, heartbeat, food intake and water intake. The animals were observed the first hour and monitored each day for 14 days.

Study of anti-anemic effect of the aqueous extract of *Spinacia oleracea*

For this study 30 healthy mouse were used. Among these animals, 6 mouse constituting the negative control were force-fed only with distilled water (1ml/day) throughout the experience. Induction of anemia was done in 24 mouse for 2 days by intraperitoneal injection of 60 mg/kg de phenylhydrazine hydrochloride. After induction of anemia over 2 days by injection of phenylhydrazine hydrochloride in 24 mouse, animals were divided into 4 groups of 6 mice each and distributed as follows :

- Positive control batch treated with distilled water
- B12 batch treated with vitamin B12 (1ml/Kg)
- Aqueous extract of *Spinacia oleracea* batch treated with 100 mg/kg of BW ($AESO_{100}$)
- Aqueous extract of *Spinacia oleracea* batch treated with 200 mg/kg of BW ($AESO_{200}$)

Animals (including the batch of negative controls) were treated for 7 days after induction of anemiaThe blood of the animals was collected by puncturing the tail veins

in the tubes (EDTA), on the first and last day of treatment after induction of anemia by phenylhydrazine hydrochloride for the analysis of haematological parameters. The animals were weighed every day during the experiment in order to study the evolution of body weight.

Statistical analysis

The software used are: EXCEL and Graph Pad. All data are expressed on average ± SD. Analysis of variances (ANOVA) was applied to the different results. The Tukey and T-student test was used to compare the different columns. The value $p < 0.05$ is considered significant.

RESULTS

Phytochemical study

The results of the phytochemical study of the aqueous extract of *Spinacia oleracea* revealed presence of sterols, polyphenols, flavonoids, alkaloids and saponosides (**Table I**).

Acute toxicity

The acute toxicity results showed no evidence of toxicity of the aqueous extract of *Spinacia oleracea* in animals administered at the 2000 mg/kg dose limit. Any toxic symptoms et changement de comportement were observed on animals treated (**Table 2**).

Induction of anemia over 2 days

- ***Effects of phenylhydrazine hydrochloride on erythrocyte lineage parameters***

Results after administration of phenylhydrazine hydrochloride (PHZ) for 2 days in mouse showed a very significant reduction ($p<0.001$) the value of red blood cells in mouse who received phenylhydrazine hydrochloride which is $6,29±0,64.10^6/\mu L$ compared to control having received only distilled water which is $8,84±0,16.10^6/\mu L$ **(Figure 1).** The hemoglobin value was significantly reduced in the mouse who received the phenylhydrazine hydrochloride which is 10,87±0,28g/dL compared to control which is 14,15±0,25g/dL **(Figure 2)**. On the hematocrit level, the results showed a very significant reduction ($p<0.001$) in mouse who received phenylhydrazine hydrochloride which is 31,73±5,622 % compared to control which is 50,05±0,15 % **(Figure 3).**

- ***Effects of phenylhydrazine hydrochloride on white blood cells***

On white blood cells value, the results showed a significant increase (P< 0.05) in mouse who received phenylhydrazine hydrochloride which is 44,26±4,13.10^3/µL compared to control who only received distilled water which is 10,37.10^3/µL **(Figure 4).**

- **Effects of phenylhydrazine hydrochloride on platelets**

The results relating to the value of platelets showed a significant decrease in mouse who received phenylhydrazine hydrochloride which is 624,7±114,2.10^3/dL compared to control which is 850,3±0.577.10^3/dL (**Figure 5**).

Table I : Phytochemical screening of aqueous extract of *Spinacia oleracea*

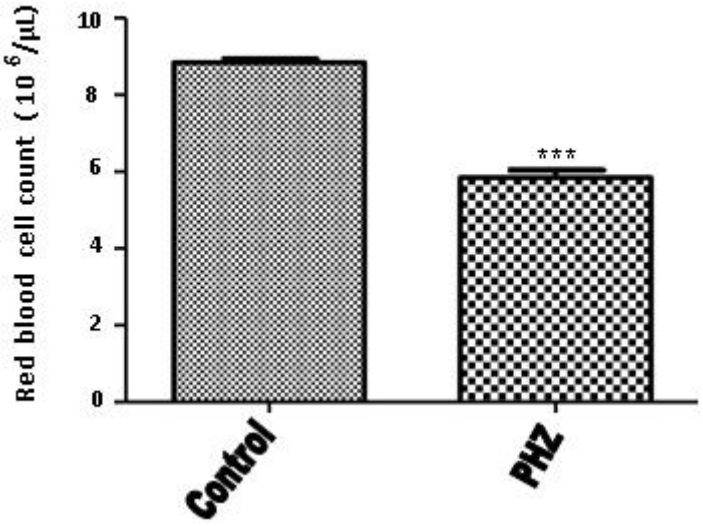
Phytochemical Compounds	Sterols-polyterpenes	Polyphenols	Flavonoids	Tannins		Quinones	Alkaloids			Saponosides
				Gal	Cat		D	B		
Reactions	**+**	**+**	**+**	**-**	**-**	**-**	**+**	**+**	**+**	

(+) : Presence ; **(-)** : Absence
Gal : gallic; **Cat** : Catechin; **D** : DRAGENDORFF **; B** : BOUCHARDAT

Table II : Clinical signs observed during 14 days of the acute toxicity test

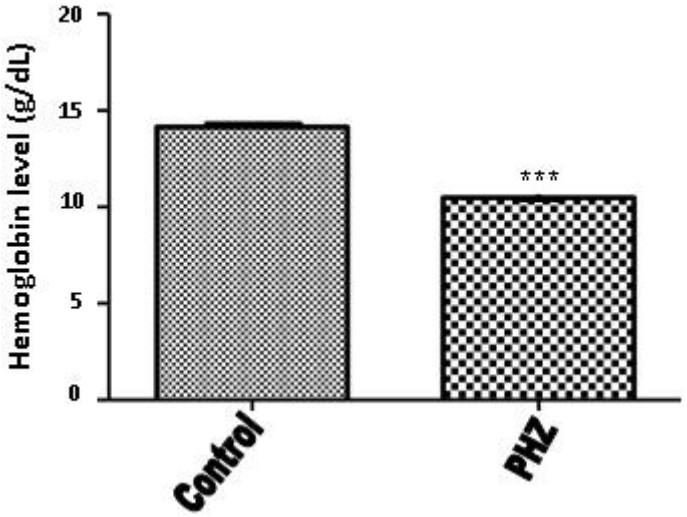
Signes cliniques	**Animals**	
	Control (distilled water)	Aqueous extract of *S. oleracea* (2000mg/Kg de PC)
Eye color	**-**	**-**
Mucous membrane	**-**	**-**
Salivation	**-**	**-**
Lethargy	**-**	**-**
The sleep	**-**	**-**
Coma	**-**	**-**
Convulsion	**-**	**-**
Tremor	**-**	**-**
Diarrhea	**-**	**-**
Morbidity	**-**	**-**
Mortality	**-**	**-**
Heartbeat	**-**	**-**
Food intake	**-**	**-**
Water intake	**-**	**-**
Fur	**-**	**-**

(-) : No change of sign
(+) : Presence of sign change



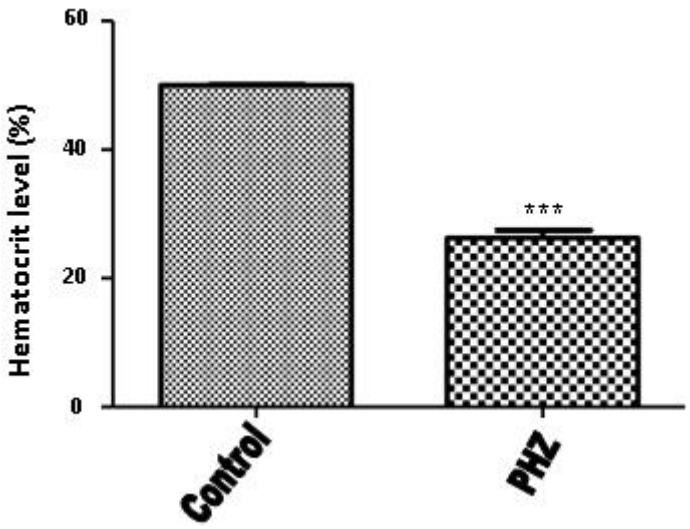
PHZ : phénylhydrazine chlorhydrate ; * : significant difference compared to control

Figure 1: Effect of injection of Phenylhydrazine hydrochloride on red blood cell count



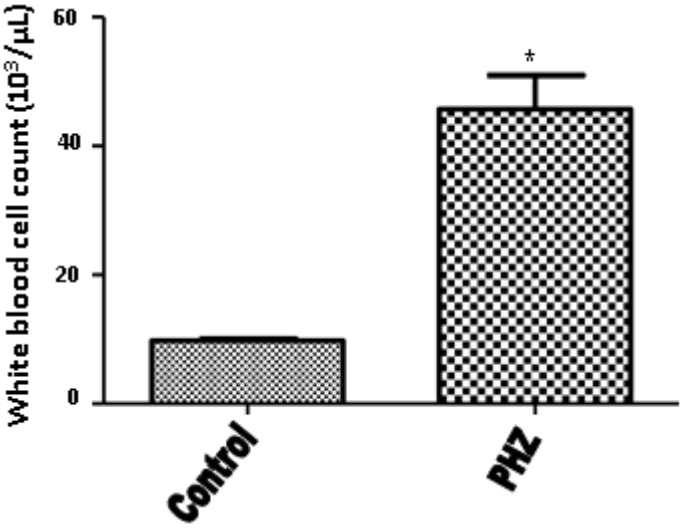
PHZ : phénylhydrazine chlorhydrate ; * : significant difference compared to control

Figure 2: Effect of injection of Phenylhydrazine hydrochloride on hemoglobin level



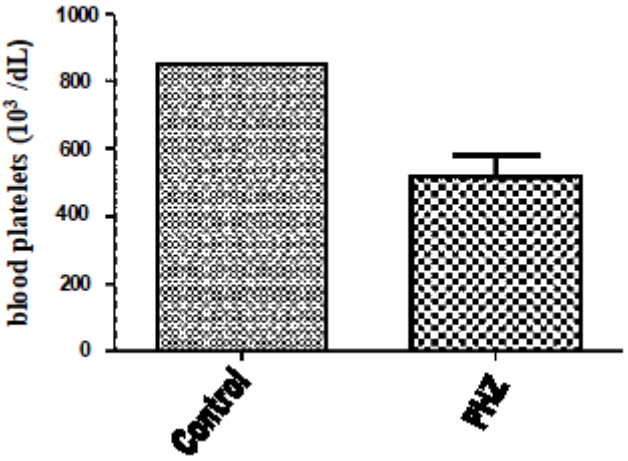
PHZ : phénylhydrazine chlorhydrate ; * : significant difference compared to control

Figure 3: Effect of Phenylhydrazine hydrochloride injection on hematocrit level



PHZ : phénylhydrazine chlorhydrate ; * : significant difference compared to control

Figure 4: Effect of injection of Phenylhydrazine hydrochloride on white blood cells



PHZ : phénylhydrazine chlorhydrate ; ***** : significant difference compared to control

Figure 5: Effect of injection of Phenylhydrazine hydrochloride on platelets

Effects of the aqueous extract *Spinacia oleracea* on haematological parameters

- ***Effect of aqueous extract of Spinacia oleracea on body weight of mouse***

Results for body weights of mouse over 7 days of treatment revealed variations in body weight compared to control (negative and positive control). The body weight of mouse treated with aqueous extract of *Spinacia oleracea* at a dose of 200 mg/kg of BW increased very significantly. ($p<0.001$) compared to control whose anemia was induced by phenylhydrazine hydrochloride (positive control). Likewise, compared to control whose anemia was not induced (negative control), the body weight of the mouse treated at the dose of 200 mg/kg significantly increased ($p<0.05$). The body weights of animals treated at dose of 100 mg/kg BW and treated with vitamin B12 increased very significantly ($p<0.01$) compared to positive control. No significant change ($p>0.05$) in animals treated with 100 mg/kg and those treated with vitamin B12 compared to negative control has been recored **(Figure 6).**

- ***Effects of EASO on erythrocyte lineage parameters***

On red blood cell, the administration of the aqueous extract of Spinacia oleracea at dose of 100 mg/kg BW ($AESO_{100}$) which is $5,347±1,06.10^6$/µL did not record any significant change compared to positive control which is $5,95±1,16.10^6$/µL. Compared to the negative control group with the value of $9±0,22.10^6$/µL, administration of $AESO_{100}$ decreased very significantly ($p<0.001$) red blood cell count. Results relating to the effect

of vitamin B12 on red blood cells showed no significant change compared to the positive control group. However, significant decreases were recorded compared to the negative control group, following the administration of vitamin B12 on the level of red blood cells. No significant change in red blood cell count was recorded between the negative control group and $AESO_{200}$ (aqueous extract of *Spinacia oleracea* at dose 200 mg/kg of P.C), with respective value $9\pm0,22.10^6/\mu L$ and $8,03\pm0,82.10^6/\mu L$. Compared to the positive control group, a significant increase ($p<0.05$), resulting by a increase of 34,95% the red blood cell count is recorded following the administration of $AESO_{200}$ **(figure 7)**.

On hemoglobin level, the results showed very significant reductions ($p<0.001$) following the administration of $AESO_{100}$ and vitamin B12 compared to negative controls. No significant changes were observed in animals treated with vitamin B12 and animals treated with $EASO_{100}$ compared to positive control. Administration of $AESO_{200}$, on hemoglobin level, showed no significant change ($p>0.05$) compared to the negative control. The results showed a significant increase in hemoglobin level, resulting in an increase of 44.50% in animals treated with $AESO_{200}$ compared to positive control **(Figure 8).**

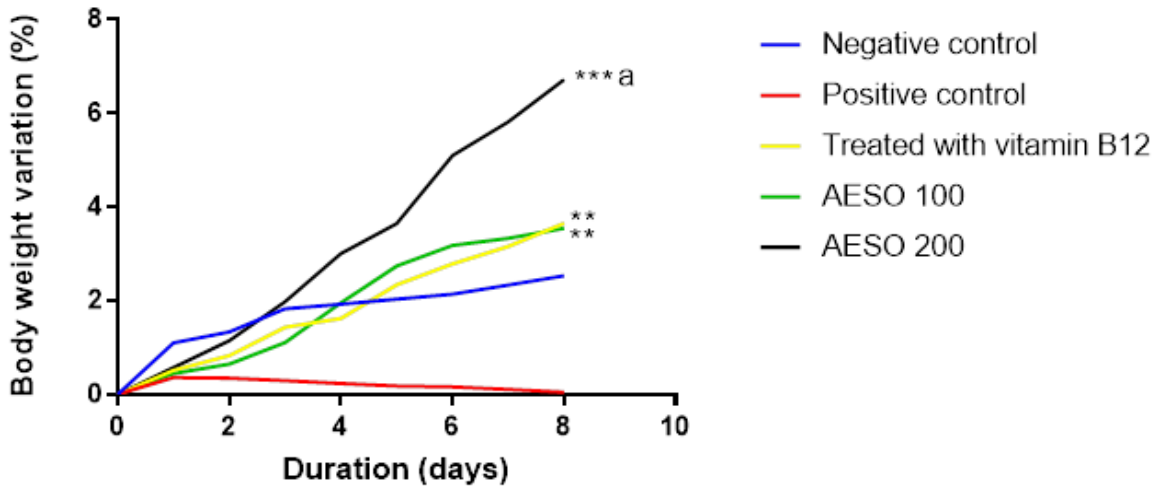


Figure 6: Effects of different treatments on the evolution of body weight in mouse

***** : significant difference compared to positive control ; **a**: significant difference compared to negative control

$AESO_{100}$: aqueous extract of *Spinacia oleracea* (100 mg/kg), **AESO $_{200}$** : aqueous extract of *Spinacia oleracea* (200 mg/kg), **B 12** : Vitamine B 12

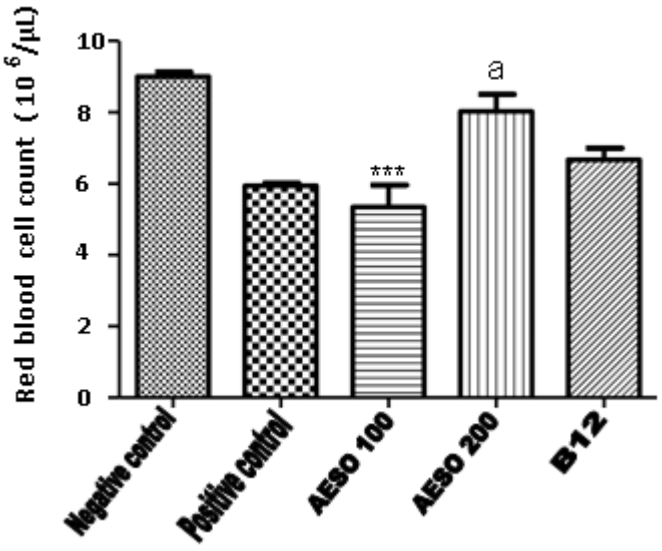


Figure 7: Effects of different treatments on red blood cell after induction of anemia by Phenylhydrazine hydrochloride

***** : significant difference compared to negative control ; **a,b,c**: significant difference compared to positive control

AESO$_{100}$: aqueous extract of *Spinacia oleracea* (100 mg/kg), **AESO $_{200}$** : aqueous extract of *Spinacia oleracea* (200 mg/kg), **B 12** : Vitamine B 12

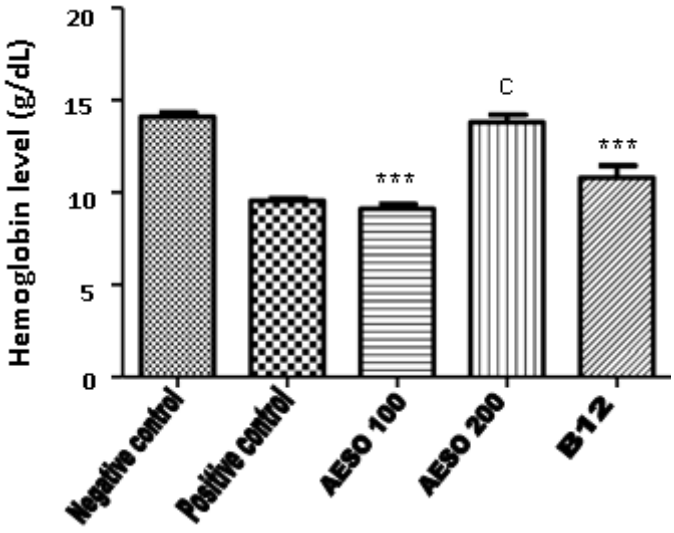


Figure 8 : Effects of different treatments on hemoglobin level after induction of anemia by Phenylhydrazine hydrochloride

***** : significant difference compared to negative control ; **a,b,c**: significant difference compared to positive control

AESO$_{100}$: aqueous extract of *Spinacia oleracea* (100 mg/kg), **AESO $_{200}$** : aqueous extract of *Spinacia oleracea* (200 mg/kg), **B 12** : Vitamine B 12

On hematocrit level, the results showed significant reductions ($p<0.05$) following administration of $AESO_{100}$ which is $28,66\pm4,44.10^3/\mu L$ and vitamin B12 which is $35,77\pm3,87.10^3/\mu L$ compared to negative control which is $47,55\pm0,85.10^3/\mu L$. No significant changes were observed in animals treated with vitamin B12 and treated animals with $AESO_{100}$ compared to positive control. Administration of $AESO_{200}$ on hematocrit level showed a significant increase, resulting in an increase of 30.68% compared to positive control. No significant change ($p>0.05$) was observed between the negative control and $AESO_{200}$ **(Figure 9).**

- ***Effects of l'EASO on white blood cells***

On white blood cells, the results showed no significant change ($p>0.05$) following the administration of $AESO_{200}$ which is $10,20\pm0,95.10^3/\mu L$ and vitamin B12 which is $8,50\pm0,79.10^3/\mu L$ compared to negative control which is $10,95\pm1,18.\ 10^3/\mu L$. Likewise, no significant changes were observed following the administration of $AESO_{200}$ and vitamin B12 compared to positive control. Administration of $AESO_{100}$, on white blood cells, showed a significant reduction ($p<0.05$), resulting in a reduction of 35.58% compared to positive control. Likewise, on white blood cells count, a significant reduction was observed between $AESO_{100}$ and negative control, resulting in a reduction of 33.88% **(Figure 10).**

- ***Effects of AESO on platelets***

On platelets, administration of various treatments ($AESO_{100}$, $AESO_{200}$ and vitamin B12) showed no significant change ($p>0.05$) compared to negative control. Compared to positive control, administration of different doses of the aqueous extract of *Spinacia oleracea* ($AESO_{100}$ et $AESO_{200}$ increased blood platelet count. These increases resulting to 81.99% and 85.03%, at the respective doses of 100 mg / kg and 200 mg / kg BW of the aqueous extract of *Spinacia oleracea* compared to positive control **(Figure 11)**.

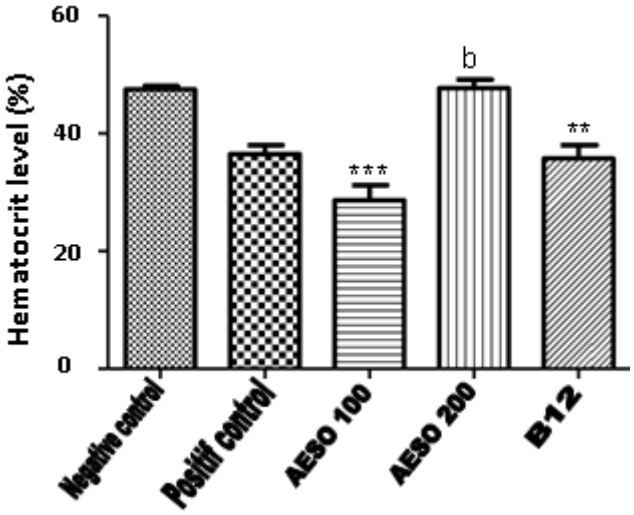


Figure 9 : Effects of different treatments on hematocrit level after induction of anemia by Phenylhydrazine hydrochloride

***** : significant difference compared to negative control ; **a,b,c**: significant difference compared to positive control

AESO$_{100}$: aqueous extract of *Spinacia oleracea* (100 mg/kg), **AESO $_{200}$** : aqueous extract of *Spinacia oleracea* (200 mg/kg), **B 12** : Vitamine B 12

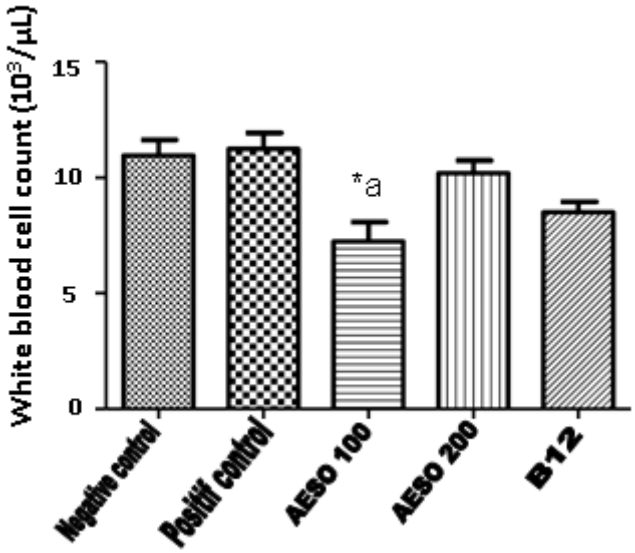


Figure 10 : Effects of different treatments on white blood cell after induction of anemia by Phenylhydrazine hydrochloride

***** : significant difference compared to negative control ; **a,b,c**: significant difference compared to positive control

AESO$_{100}$: aqueous extract of *Spinacia oleracea* (100 mg/kg), **AESO $_{200}$** : aqueous extract of *Spinacia oleracea* (200 mg/kg), **B 12** : Vitamine B 12

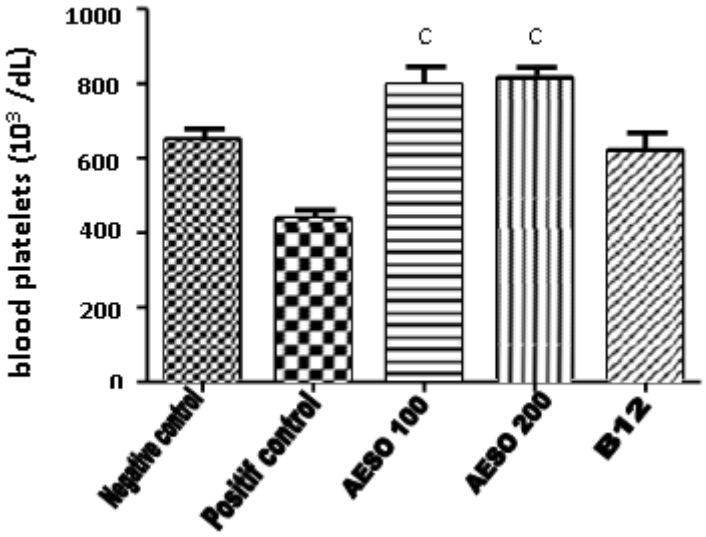


Figure 11 : Effects of different treatments on blood platelets after induction of anemia by Phenylhydrazine hydrochloride

***** : significant difference compared to negative control ; **a,b,c**: significant difference compared to positive control

AESO$_{100}$: aqueous extract of *Spinacia oleracea* (100 mg/kg), **AESO $_{200}$** : aqueous extract of *Spinacia oleracea* (200 mg/kg), **B 12** : Vitamine B 12

DISCUSSION

Phytochemical analysis of the aqueous extract of *Spinacia oleracea* revealed presence of sterols, polyphenols, flavonoids, alkaloids and saponosides. The results obtained are similar to those of **Luka *et al.***, in study of aqueous extract of *Spinacia oleracea* [7]. Phytochemical analysis revealed presence of saponosides and alkaloids, these secondary metabolites have anti-anemic potential [8].

The results of acute oral toxicity showed that the lethal dose of this plant is higher than the limit dose of 2000 mg/kg of BW. In addition, no change in behavior and toxic signs were observed during the 14 days of observation. These results are similar to those of **Gomathi *et al.*,** which observed no toxicity in study of antidiabetic activity of leaves of *Spinacia oleracea* [9]. These results are also similar to those of **Nagar *et al.*,** in the study of anti-inflammatory potential of *Spinacia oleracea* leaf extract[10]. According to the globally harmonized classification system of guideline OECD 423, *Spinacia oleracea* is in Category 5 or may not be classified.

The results relating to the induction of anemia by phenylhydrazine hydrochloride for 2 days showed that this injection significantly reduced erythrocyte parameters (red blood cells, hemoglobin, hematocrit) of treated animals compared to the control who received only distilled water. These results obtained are similar to those of **Ebuehi et Mbara,** who observed that administration of phenylhydrazine for 2 days in mice reduced the values of red blood cells, hemoglobins and hematocrit[11]. This anemia results from an early lysis of the erythrocytes which results in the change of the color of the blood (change from bright red to dark red). Induction of anemia for 2 days with phenylhydrazine showed a significant increase in white blood cells and a significant decrease in blood platelets compared to the control group. The modifications of these parameters could be explained by the presence of bioactive compounds in the aqueous extract of Spinacia oleracea which could justify these changes. In addition, the increase in white blood cells reveals the body's defense reaction to foreign elements or substances induced in the body.

The weights of animals administered at the dose of 200 mg / kg of BW significantly increased compared to controls during the 7 days of treatment. These effects are similar to those of **Narhari *et al.***, who observed an increase in body weight in rats treated with methanolic extract of *Terminalia citrina* leaves[12]. The increase in body weight could be attributed to the stimulation of appetite (orexigenic effect) due to administration of extract.

The results relating to the administration of the extract for 7 days after induction of anemia on erythrocyte parameters showed a significant increase in this parameter. The level of red blood cells, hemoglobin and hematocrit increased significantly, especially at the 200 mg/kg dose of the aqueous extract of *Spinacia oleracea* compared to the positive control. These effects could indicate the ability to stimulate the release of erythropoietin (humoral regulator) by kydney, which in turn stimulates the production of erythrocytes[13]. On white blood cells, no significant difference was observed in animals treated with 200 mg / kg BW of aqueous extract of *Spinacia oleracea* compared to controls. This could suggest that the administration of the aqueous extract *Spinacia oleracea* for 7 days after induction of anemia, could contribute to the improvement of the leukocyte parameters. The results for blood platelets showed a significant increase in this parameter in animals treated with different doses of the aqueous extract ($AESO_{100}$ and $AESO_{200}$) compared to positive control. These results are similar to those

of **Mohammad *et al.***, who observed an increase in blood platelets in animals treated with aqueous extract of *Allium eriophyllum* leaves compared to untreated control[14].

CONCLUSION

The present study showed that the administration of the aqueous extract of *Spinacia oleracea* in a single dose does not cause any signs of toxicity and mortality. Anemia caused by injection of phenylhydrazine hydrochloride was characterized by decreased levels of red blood cells, hemoglobin and hematocrit in mouse. Treatment of animals for 7 days after induction of anemia showed a significant increase in erythrocyte parameters (red blood cells, hemoglobins and hematocrit) mouse treated at a dose of 200 mg/kg BW. This study shows that leaf vegetable, as *Spinacia oleracea* (spinach) has anti-anemic potential. However, complementary studies are necessary in order to know more clinical information on this plant.

ACKNOWLEDGEMENT

We thank the Laboratory of Endocrinology and Biology of Reproduction (UFR Biosciences) of Félix Houphouët Boigny University

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