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GRAPTOPHYLLUM PICTUM RESTORES IN THREE DAYS HAEMATOLOGIC PARAMETERS AFTER PHENYLHYDRAZINE-INDUCED ANEMIA OF INFLAMMATION IN WISTAR RATS

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ABSTRACT: *Graptophyllum pictum* (GP) is used to treat anemiain some traditional communities. To understand this practice, we had prepared GP tea leaves for the management of phenylhydrazine-induced anemia in rats. An experimental protocol was built with six groups of six rats each. Rats in normal group were fed with standard food and water *ad libitum*. The rest of rats received an intraperitoneal injection of 40 mg/kg of phenylhydrazine for two days to induce anemia. The GP tea leaves (GPTL) had an extraction percentage of 40.8% and contained: ron (32.89 mg/100 g dry material (DM)), carotenoids (649.01 mg/100g DM), phytates (0.22 mg/100g DM) and oxalates (3.21 mg/100g DM For the *in-vivo* experiment, intraperitoneal injection of phenylhydrazine leads to decreases: hemoglobin, hematocrit, red blood cells, MCH, MCHC; increases in white blood cells and MCV threshold in anemic rats was observed. After 3 days feeding period of rats with 210 mg / kg of (GPTL) or 0.3mg / kg of Ranferon®, a restoration of all the parameters mentioned was observed. (GPTL) restored of hematological parameters in the case of anemia compared to Ranferon®, a pharmaceutical drug. Thus, GPTL exhibits therapeutic anti-anemia effects and could be used as a natural alternative to treat anemia of inflammation.

INTRODUCTION: Worldwide, anemia affects 1.62 billion persons. It represents a global public health problem. South-East Asia and Africa are among the most affected regions of the world [1]. The prevalence in Cameroun represents 40% of pregnant women and 60% of children under the age of five [2]. In general, anemia is characterized by a deficiency of red blood cells or hemoglobin in the blood, which disrupts oxygen transport [3].

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For the anemia of inflammation, also known as the anemia of chronic disorders is a type of anemia that develops in the context of chronic inflammatory conditions. Concerning Phenylhydrazine (Phz), it reacts with hemoglobin and leads to the formation of various oxidative products. The next step is red blood cell damage and lysis. However, Phz-induced anemia by destroying red blood cell and increasing the release of cellular contents can trigger inflammatory responses [4].

To treat anemia in some communities, including the Cameroon or Papua Communities, *Graptophyllum pictum* (GP) is used. This plant, also known as the caricature plant, is a medicinal shrub belonging to the Acanthaceae family. It is probably native to New Guinea and has since

spread widely to India, Mexico, United States of Amerca, Ghana, Cameroun, Bolivia, and Asia. Currently, it is commonly found in some regions of Indonesia and is known locally as Daun Ungu or Daun Wungu. GP is traditionally used as it is believed to possess anti-anemic properties. This medicinal plant is also known as batik leaf. It has been incorporated into drinks to treat anemia. However, infusion of *Graptophyllum pictum* leaves seems to have potential to be explored [5]. The present work is designed to check if the traditional tea of GP has an *in-vivo* effect through phenylhydrazine-induced anemia in Wistar rats.

MATERIALS AND METHODS:

Preparation of *Graptophyllum pictum* Aqueous Extracts: *Graptophyllum pictum* leaves were collected from the quarter Ngoa Ekelé in the 3th sub-division of Yaoundé. The plant had been identified at the Cameroon National Herbarium in Yaoundé. Plant was washed and dried at laboratory temperature. After grounding aqueous extraction was made in triplicate. The formula "yield = 100 x (Mass of extract) / (Mass of leaves powder)" was used to calculate extraction yield.

Graptophyllum pictum leaf powder (100 g) was infused in 2 L of hot distilled water (100 °C) for 10 minutes. The resulting filtrate was dried in an oven at 50 °C and stored in clean, dry bottles. For maceration, powder of *Graptophyllum pictum* leaves (100g) was put in 2L of distilled water. The mixture was left to macerate for 24 hours and filtered. The filtrate obtained was dried and stored for further experiments. While for decoction, one hundred grams (100 g) of powder was mixed in 2 L of distilled water. The mixture was brought to a boil at 100 °C for 30 min. After cooling, we had obtain dry extract.

Determination of Anti-nutritional Factors in Aqueous Extracts: Total tannins were determinated by spectrophotometric method as described by Bainbridge *et al.* [6]. Briefly, 0.5 mL of the extract was mixed with 3 mL of the butanol-HCl (95:5), and 0.1 mL of the ferric reagent. The mixture was shaken and incubated in a water bath at 100°C for 1 hour. The absorbance was read at 550 nm for the tannin content determination. The oxalate content was determined by the method described by Aina *et al* [7]. In short, after extracting 1 g of lyophilized herbal tea from H_2SO_4 (3 mol/L), the oxalate content was calculated by taking 1 ml of $KMnO_4$ as equivalent to 2.2 mg of oxalate. The results were expressed in mg/100 g of extract.

The phytic acid content was determined by the Olayeye *et al*. method [8]. One gram of the powder was mixed with 2% HCl. The resulting filtrate, 25 mL, was mixed with 5 mL of 0.3% ammonium thiocyanate solution and 53.5 mL of distilled water to achieve the desired acidity. The resulting solution was titrated with standard iron (III) chloride solution (0.00195 g iron per mL) until a persistent brownish-yellow color was observed for 5 minutes. The phytate content was then calculated.

Determination of the Nutritional Potential in Aqueous Extracts: Vitamin C was determined by the colorimetric method as previously described [9]. Five ml of herbal tea were mixed with 1 mL of 90% acetic acid. The mixture was titrated with DCPIP solution (50 µmol) until a pale pink color was obtained. The volume was recorded (Vt). This titration was repeated with 5 mL of distilled water for the blank (Vb) and 5 mL of ascorbic acid solution (0.1 mg/mL) for the standard (Vst). The vitamin C content of the samples was then calculated with the formular:

$$\text{Vitamin C (mg / mL)} = \text{Vt-Vb / Vst-Vb} \times \text{Dilution factor}$$

Total carotenoids were determined as previously described [10]. Briefly, optical density was read at 450 nm. The level of total carotenoids was obtained with the formula:

$$\text{Total carotenoids (µg/g)} = A_{450} \times V \times 10^4 / A1\%/1CM \times M$$

A_{450} = absorbance at 450 nm; V = volume in mL; A1cm / (1%) = absorption coefficient of total carotenoids in petroleum ether.

Iron was assessed by colorimetric enzymatic ferene method using a commercial kit (SG Mitalia, Roma Italia). The procedures were carried out as indicated in the manufacturer's instructions [11]. The ash contents were determined by simple incineration at 550 °C according to A.O.A.C. protocol's [12].

Determination of Secondary Metabolites; Anti-Oxidant and Antiradical Activities in Aqueous Extracts: Total polyphenols were determined as

described by Singleton and Rossi [13]. Gallic acid was used as a standard. The polyphenols content were expressed in mg gallic acid equivalent/g of dry matter (mg EAG/g DM).

Total flavonoid determination was made as described by Zhishen *et al*.[14]. Catechin was used as the standard; the flavonoid content was deduced from the calibration line and expressed in mg catechin equivalent/100 mL. The total antioxidant capacity (TAS) of the extracts was determined based on the reduction of molybdenum, in the form of molybdate ion $M0O_4^{2-}$, to molybdenum $M0O^{2+}$ as previously described [15].

Briefly, a volume of 200 µL of each extract at different concentrations was mixed with 2 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. After cooling, the absorbance of the solutions was read at 695 nm, against the blank which contained 200 µL of the reagent solution and 0.2 mL of methanol, under the same conditions. The results obtained were expressed in mg gallic acid equivalent per gram of dry matter of the extract (mg EAG/g DM).

Ferric-reducing antioxidant power (FRAP) of extracts was determined by its action to reduce ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}). The Fe^{2+} ions formed interact with 2,4,6-tris(2pyridyl)-s-triazine (TPTZ) to form a complex that absorbs at 593 nm [16]. Briefly**,** to 0.1 mL of extract, 3 mL of freshly prepared FRAP reagent were added. After 5 min of incubation, the absorbance of the reaction medium was read at 593 nm. Gallic acid was used as a reference and the tests were carried out in triplicate. The antioxidant potential was determined from the regression line of the calibration curve. The results were expressed in mg of Fe (II)/100 g of plant material.

Animal Experiment: In this study, 36 healthy Wistar rats weighing between 150 and 180 g were used and randomly divided into six groups of six individuals each. Rats were kept under 12 h light/12 h dark in normal standard environmental condition (temperature (25–28 °C), humidity (35–60%)). They were allowed free access to water and feed. Experimental procedures and animal handling were approved by the Yaoundé I University, Research Ethical Clearance Committee (Ethical clearance reference N°: BTC-JIRB2024-117).

Induction of Anaemia: Before induction of anemia, baseline haematological parameters in rats were determined. Phenylhydrazine (PHZ) was administered intraperitoneally at the dose of 40 mg/kg for two consecutive days [17] to induce anaemia to the rats of group 2 to group 6. We had considered induction of anemia when RBC level as well as haemoglobin concentration of the blood reduced to 30 % or less.

After induction of anemia, the groups of 6 rats were fed daily from the 3rd day for 3 consecutive days as follows:

- Group 1: receiving standard food + Water.
- Group 2 (negative control): receiving the standard food + water.
- Group 3 (positive control): receiving standard feed + water + Ranferon (a pharmaceutical drug) 0.3 mg/kg for 3 days (dose required for treatment).
- Group 4: receiving standard feed + water + 7 mg/kg of our extract for 3 days (for treatment).
- Group 5: receiving standard feed + water + 70 mg/kg of our extract for 3 days (for treatment).
- Group 6: receiving standard feed + water + 210 mg/kg of our extract for 3 days (for treatment according to traditional indication.

Blood Analysis: Exactly 3 days post treatment with Graptophyllum pictum traditional tea, rats were fasted overnight, anaesthetized and sacrificed. Blood samples were collected by cardiac puncture and dispensed into EDTA (Ethylenediaminetetraacetic acid) container for haematological tests. Red blood cell (RBC) indices, Haemoglobin (Hb) level, hematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and white blood cell (WBC) were estimated following the methods described previously [18, 19].

Statistical Analysis of the Results: Statistical analyses were performed using SPSS version 20.0

for Windows. XLSTAT software was used to perform principal component analysis (PCA) for classifying herbal teas based on the correlations of the different variables studied. ANOVA combined with a Tukey post-hoc test was used to analyze the results of the bioactive component analyses. For hematological parameter assays, data was analyzed with GraphPad prism version 8.0.2 and results expressed as mean ± standard deviation. Multiple comparisons were done following ordinary one-way ANOVA and then Tukey post hoc test to determine significant differences between groups. p value<0.05 was considered statistically significant.

RESULTS AND DISCUSSION:

Aqueous Extractions of *Graptophyllum pictum* Leaves: Beside the traditional tea leaves of *Graptophyllum pictum* preparation, we had implemented two other types of preparations including maceration and decoction in order to retain the best method for the management of phenylhydrazine-induced anemia in Wistar rats. The average yields obtained are presented below in **Table 1.** A significant difference ($p<0.05$) was noted between the yields of the three extracts. It follows that the highest extraction yield was obtained with the infusion.

TABLE 1: EXTRACTION YIELDS OF AQUEOUS EXTRACTIONS OF *GRAPTOPHYLLUM PICTUM*

Samples	Yield (%)
Maceration	25.75 ± 0.02^{c}
Decoction	27.73 ± 0.02^{b}
Infusion	40.8 ± 0.2^{a}

The assigned values of different letters are significantly different ($p < 0.05$).

Determination of Macronutrients Potential of *Graptophyllum pictum* Extracts: The following **Table 2** presents the water, ash and iron contents. It appears that decoction, maceration and infusion had comparable contents of water, ash and iron.

Therefore, maceration and decoction aqueous extracts could meet the recommended nutritional intake of minerals.

Also, the contents of iron, with significant difference at $P<0.05$, varies from 37.37mg/100g DM for decoction, 35.28 mg/100 g DM for maceration at 32.89 mg/100g DM for infusion. Iron is a very important mineral due to its many biological roles.

It is an essential trace element for the synthesis of hemoglobin and myoglobin [20]. It is involved in the transport and storage of oxygen in the body; it is also a part of the composition of enzymes that intervene in metabolic reactions and also plays a role in the process of cell division and normal cognitive function [21].

Recommended iron doses vary from 7 mg/day to 35 mg/day depending on age, sex and physiological status [22]. Iron is important in the diet of pregnant and breastfeeding women as well as children [23].

With these iron levels, many women would benefit from consuming these herbal teas during pregnancy and even after childbirth. Heme iron is easily assimilated by the body, while plant-based iron requires the presence of Vitamin C.

TABLE 2: CONTENTS IN MACRONUTRIENTS OF THE AQUEOUS EXTRACTS (MG/100 G DM)

Samples Content	Maceration	Decoction	Infusion
Water	91.46 ± 1.43^{a}	94.36 ± 0.50^{b}	92.25 ± 0.34^{a}
Ashes	13.69 ± 0.26^{c}	11.84 ± 0.09^{b}	9.84 ± 0.08^{a}
Iron	35.28 ± 0.21^{c}	37.37 ± 0.06^{b}	32.89 ± 0.10^{a}

The assigned values of different letters are significantly different ($p < 0.05$).

Determination of *Graptophyllum pictum* Extracts Bio-Actives Components: The following **Table 3** represents the contents of bioactive compounds. Vitamin C contents ranged from 645.96 mg/100 g DM (decoction) to 1151.22 mg/100 g DM (infusion). The Consumption of our herbal teas could cover 100% of the recommended daily vitamin C requirements for all age groups of the population [24]. Indeed, vitamin C is an extremely important nutrient for the body where it plays multiple roles [25]. It allows the maintenance of the proper functioning of the immune system and especially the fact that it promotes the absorption of minerals contained in food, particularly non-heme iron [26]. Its presence in these extracts would contribute to the improvement of the bioavailability of iron. However, as far as the carotenoid contents are concerned in the extracts, **Table 3** shows: 4.32; 5.79 and 6.49 µg/100g DM for maceration, decoction and infusion respectively.

TABLE 3: CONTENTS IN BIO-ACTIVES COMPONENTS OF THE AQUEOUS EXTRACTS (MG/100 G DM)

Samples	Maceration	Decoction	Infusion
Vitamin C	716.31±1.77	645.96±0.50	1151.22±2.38
Carotenoïds	4.32±1.57	5.79±2.76	6.49±8.76

The assigned values of different letters are significantly different ($p < 0.05$).

Determination of Antinutrients Potential of *Graptophyllum pictum* Extracts: Table 4 below shows the tannin, oxalate and phytate contents. It appears that tannin contents in this study ranged from 2.22±0.07 mg/100g DM for the infusion, 3.00±0.04 mg/100g DM for the maceration to 3.18±0.02 for the decoction. Those contents are higher in the decoction and maceration extracts compared to the infusion. The safe value is 150 to 200 mg/100g as reported previously [27]. Tannins reduce the absorption of nutrients such as iron, vitamin B12 [28]. Subsequently, the oxalate contents varied from 2.73mg/100g DM for the decoction, 2.75mg/100g DM for the maceration to 3.21mg/100g DM for the infusion. The toxic dose in humans is 200 to 500mg / 100g. The safety limits of oxalate content in humans vary from 3 to 5 mg/Kg as reported previously [29]. The oxalate value for those extracts ranges from 2.73 to 3.21 mg, which is within the safe limit and below the toxicity threshold and therefore will not cause any harm.

Finally, the tea leaves of *Graptophyllum pictum (L.)* Nee Griff phytate contents were: 0.10±0.00 mg/100g DM for maceration, 0.18± 0.01 mg/100g DM for decoction and 0.22 mg/100g DM for infusion. The daily intake of these compounds by people with a vegetarian diet should be in the range of 2000 to 2600 mg/100g /day [30], for those with a mixed diet, the phytate content should be between 150 – 1400g/day. The contents obtained in these herbal teas are less than 1400 mg, so they are not likely to cause harm if consumed. It is known that phytates reduce the bioavailability of Fe [31, 32]. On the other hand, Hurrel *et al* [33]., showed that a consumption of 4 to 9 mg of phytates reduces iron absorption 4 to 5 times.

TABLE 4: CONTENTS OF *GRAPTOPHYLLUM PICTUM* EXTRACTS (MG/100G DM)

Samples	Maceration	Decoction	Infusion
Tanins	3.00 ± 0.04^{b}	3.18 ± 0.02^{c}	2.22 ± 0.07^{a}
Oxalates	2.75 ± 0.11^{a}	2.73 ± 0.09^{a}	3.21 ± 0.09^{b}
Phytates	0.10 ± 0.00^{a}	0.18 ± 0.01^{b}	0.22 ± 0.00^{c}

The assigned values of different letters are significantly different ($p < 0.05$).

Antioxidants Capacity of *Graptophyllum pictum* Extracts: Flavonoids and total polyphenols are known for their antioxidant capacities. These phytochemical compounds and antioxidant tests carried out with the extracts are recorded and presented in **Table 5** below. The phenolic compound contents ranged from 2365.54 ± 4.22 mgEAG/gDM, 2520.44 ±7.71 mgEAG/ gDM to 2675.20± 3.67 mgEAG/ gDM for maceration, decoction, and infusion respectively with significant differences ($P<0.05$). Infusion have the highest content followed by decoction and finally maceration. Phenolic compounds are natural antioxidants with protective properties for the heart [34]. Flavonoid contents are 1897.60; 2520.80 and 2499.07 mg E. catechin/100 g DM for maceration, decoction and infusion respectively. These values show that the different preparation methods have an effect on the amount of flavonoids, known to have antioxidant properties. According to several authors, antioxidant activity can be affected by many factors. The structure of phenolic compounds, in particular the degree and position of hydroxyl groups on the aromatic ring of the molecule, glycosylation and the presence of other proton-donating groups [35]. The three aqueous extracts have comparable FRAP activity. This test involves the ability to reduce Fe^{3+} to Fe^{2+}. Fe^{2+} is a powerful pro-oxidant that can react with hydrogen peroxide (H_2O_2) to produce hydroxyl radicals (HO) [36]. The total antioxidant capacity (TAC) ranged from 1002.52±1.65 mg EAG/g for maceration, 666.67±1.67 mg EAG/g extract for decoction to 613.69±1.25 mg EAG/g for infusion. These results show with a significant difference ($p<0.05$), that maceration has the highest antioxidant power (1002.52±1.65 mg EAG/g extract), followed by decoction (666.67±1.67 mg EAG/g extract) and finally infusion (613.69±1.25 mg EAG/g extract). All extracts have shown high

antioxidant activities **Table 5.** However, infusion extract have highest: yield of extraction; contents of bio-active molecules (Vitamin C and carotenoid), polyphenols and flavonoids. They were also highlighted in the study of Djuikwo and collaborators [5]. Bearing all this in mind, we have selected the infusion extract for anti-anemic study.

TABLE 5: LEVELS OF FLAVONOIDS, TOTAL POLYPHENOLS AND ANTI-OXIDANT ACTIVITIES (FRAP, TAC)

Samples	Maceration	Decoction	Infusion
Phenolic compound (mg EAG /100g DM)	2365.56 ± 4.22[b]	2520.44 ± 7.71[a]	2675.20± 3.67[c]
Flavonoids (mg E. catéchine /100g DM)	1897.60 ± 3.67[a]	2320.80 ± 2,76[b]	2499.07 ± 7.97[c]
FRAP (mg Fe II /100g DM)	811.51 ± 1.14[c]	746.66 ± 3.67[b]	728.48 ± 0.69[a]
TAC (mg EAG/g extract)	1002.52 ± 1.65[c]	666.67 ± 1.65[b]	613.69 ±1.25[a]

The assigned values of different letters are significantly different ($p < 0.05$); EAG = Gallique Acid Equivalent.

Hematological Parameters: Induction of anemia by phenylhydrazine in rats resulted in a significant decrease in the number of red blood cells, hemoglobin and HTC. However, there was an increase in white blood cells (WBC) and MCV compared to control rats (non induced) as shown in the **Table 6**. Phenylhydrazine is an antipyretic drug which has the property of causing hemolytic anemia in both humans and rats. The infusion administrated at concentrations of 210mg/Kg (traditional dose) showed a significant increase at the 5% threshold of red blood cells, hemoglobin and HTC; a decrease in white blood cells, MCV as shown in **Table 6**. The increase in white blood cells as well as MCVis a defense reaction of rats to foreign substances, which alter their normal physiological processes. Phenylhydrazine causes oxidative damage to red blood cells by increasing the formation of reactive oxygen species [37]. The infusion with 210 mg/Kg made from the leaves of *Graptophyllum pictum* (L.) Nee. Greff. Leds to an improvement in the values of all the parameters that were affected. All previous results indicate that these extracts contain bioactive agents that are potent antioxidants that prevent or repair damage to cells caused by free radicals or highly reactive oxygen species [38]. The result of this study suggests that these studied herbal tea showed positive hematological activities in rats and could be used in the management of anemia of inflammation.

TABLE 6: INCIDENCE OF *GRAPTOPHYLLUM PICTUM* INFUSION IN ANEMIC RATS

***In-vivo* experimentation results**							
Parameters	**References**	**Values**					
		Non induced	**Induce and non treated**	**Induce and treated with Ranferon**	**Induce and treated with GP extract**		
					7 mg/kg	**70 mg/kg**	**210 mg/kg**
WBC (109/L)	4.0-10.0	7.95±1.46	10.32±0.99	8.11±1.57	11.1±0.13[e]	8.75 ±,01[b]	7.1 ±0.4
RBC (10 ^ 6) / mu * l)	7.00-8.70	7.52±1.06	3.88±0.41	7.51±0.69	4.17±0.20[e]	4±0.78[b]	8.26±0.81
Hb (g / d * l)	10.0-16.0	13.12±2.1	8.52±1.36	14.48±1.08	6.36±0.3	7.18±0.13	15.33 ±2.12
HTC (%)	35-54	39.21±4.41	24.8±2.14	42.95±4.65	24.17±0.32[e]	34.1±0.10[c]	44.56±4.93
MCV (FI)	80-100	94.5±2.01	119.63±2.12	96.74±4.03	25.43±0.10[c]		9.28±5.77

Values with different superscript letters in the same row are significantly different ($p<0.05$). Red blood cell (RBC) indices, Hemoglobin (Hb) level, Hematocrit (HCT), mean corpuscular volume (MCV) and white blood cell (WBC).

After induction of anemia by phenylhydrazyne, we observed an increase in the white blood cell count (leukocytosis) and mean corpuscular volume (macrocytosis). On the one hand, with leukocytosis the body's immune system often releases white blood cells to fight off pathogens or repair damaged tissue. This could be a sign of inflammation or infection and the increase of white blood cells may be considered as a reliable biomarker of inflammation [39]. On the other hand, macrocytosis could derive from Inflammation through various mechanisms, including nutrient deficiencies (like vitamin B12 or folate) or through bone marrow problems (direct effects on red blood cell production) [40]. After administration of *Graptophylum pictum* leaves infusion, we observed a normalization of all hematological parameters observed (RBC indices, Hb level, HCT, MCV WBC), including white blood cell count and mean corpuscular volume. We could suggest that

Graptophylum pictum infusion according to the indications of the traditional Cameroonian pharmacopoeia is a preparation with anti-anemic effects.

CONCLUSION: The traditional herbal tea made from *Graptophyllum pictum* (L.) Nee Griff. leaves in the form of an infusion has a good yield of extraction (40%). It is rich of potential macronutrients as nutritional intake of minerals. Its level of vitamin C cover completely the recommended daily requirements. The extract have a good anti-oxidant activity and has also shown anti-anemic activity after Phz-induced anemia. Infusion in traditional dose (210 mg/Kg) restores hematological parameters monitored within three days after induction of anemia with phenylhydrazine. This recovering is in a manner comparable to the administration of Ranferon©(a pharmaceutical drug). Those preclinic results may be scientific support to the traditional used of *Graptophyllum pictum* as anti-anemic. Moreover, a clinical survey validation is important to promote the consumption of the infusion made from the leaves of *Graptophyllum pictum* (L.) Nee Griff. to improve iron, anti-oxidant or anti-inflammatory and vitamin C status.

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CONFLICT OF INTEREST: Ferdinand Kouoh Elombo has no conflict of interest. Chancella Eléonore Yando has no conflict of interest. Aikore Abdul Hamed has no conflict of interest. Ruth Viviane Djuikwo has no conflict of interest. Elie Fokou has no conflict of interest. Paul Moundipa Fewou has no conflict of interest.

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