IJP (2021), Vol. 8, Issue 4 **(Research Article)**

Received on 24 March 2021; received in revised form, 25 April 2021; accepted 29 April 2021; published 30 April 2021

ANTI-ANAEMIC EFFECT OF AQUEOUS LEAVES EXTRACT OF *HIBISCUS ACETOSELLA* WELW. EX HIERN (MALVACEAE) ON TWO EXPERIMENTAL MODELS OF ANAEMIA INDUCED BY 2, 4-DINITROPHENYL-HYDRAZINE AND BLOOD LOSE IN WISTAR RAT

Mbock Armel Junior [1], Nguemfo Edwige Laure [* 2], Bogning Zangueu Calvin [1], Magne Fongang Annie Laure [1] and Dongmo Alain Bertrand [1]

Laboratory of Biology and Physiology [1], Faculty of Sciences, P.O. Box 24157, University of Douala, Cameroon.
Department of Biological Science [2], Faculty of Medicine and Pharmaceutical Sciences, P. O. Box 27021, University of Douala, Cameroon.

Keywords:

Bleeding anaemia, Haemolytic anaemia, *Hibiscus acetosella*

Correspondence to Author:
Nguemfo Edwige Laure

Department of Biological Science, Faculty of Medicine and Pharmaceutical Sciences, P.O Box 27021, University of Douala, Cameroon.

E-mail: enguemfo@yahoo.com

ABSTRACT: Anaemia globally affects 1.62 billion peoples in the world. To treat anaemia, *Hibiscus acetosella* is sometimes used traditionally. This study has been conducted to evaluate the anti-anaemic effects of the aqueous leaves extract of *Hibiscus acetosella*, on two experimental models of anaemia. Bleeding anaemia was induced by retro-orbital puncture of 2-2.5 mL of blood during two days, while haemolyticanaemia was induced by intra-peritoneal injection of 2. 4 dinitro-phenylhydrazine (40 mg.kg bw) during 7 days. After induction, plant extract (100; 200 or 400 mg.kg bw) was daily administrated by oral route for 14 days. During experience, haematological analysis was done at the beginning, at the end of induction and every week during treatment. During haemolytic anaemia; aqueous extract significantly increased haematocrit and red blood cell with the maximum of 45% and 32% respectively at the dose of 400 mg.kg.bw; haemoglobin significantly increased in rats with a maximum of 32% at the dose of 200 mg.kg bw. In bleeding anaemia model, *H. acetosella* significantly increased the haematocrit as well as red blood cell with a maximum increase of 14% and 31%, respectively as compared to the anaemic group. This result suggests that *H. acetosella* would have an important anti-anaemic effect.

INTRODUCTION: Anaemia is a pathological state in which the number of erythrocytes is insufficient to cover the physiological needs of the organism, characterize by a reduction of the normal quantity of haemoglobin in blood's circulation less than 13 g/dl or 12 g/dl respectively for men and women [1].

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.IJP.8(4).146-54

The article can be accessed online on
www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.8(4).146-54

According to the world health organization, it occurs at all stages of the life cycle, associated with many pathologies [2]. It has been reported that 50% of the cases of anaemias are due to the deficiency of iron (UNICEF/UNU/WHO) [3]. Among the other causes of anaemia, heavy blood loss as a result of menstruation or parasite infections can lower blood haemoglobin (HB) concentrations. The most serious anaemia's consequence on the health resides in the increase of the maternal and infantile mortality risk observed in the severe forms, but also an increase of the risks of postoperative mortality among the anaemic patients WHO [2].

Anaemia becomes global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development WHO [3]. According to WHO [3], anaemia globally affects 1.62 billion peoples, which corresponds to 24.8% of the population, the highest prevalence being in preschool-age children. They also reported that for preschool-age children, pregnant and non-pregnant women, the highest proportion of individuals affected are in Africa (WHO, 2008). In Cameroon, the prevalence of anaemia was of 68.3% in 2004 and 60.3% in 2011 for the children of 6 to 59 months, 44.9% in 2004 and 39.5% in 2011 for the women of 15 to 49 years [4, 5].

The treatment of the anaemias changes according to the type of anaemia. For the effective control of this disease, the contributing factors must be identified and addressed. Treatment is based either on an oral provision in iron, B12 or B9 vitamin, but also on a treatment with immuno-suppressants or corticosteroids, administration of erythropoietin, blood transfusions, or transplantation of bone marrow [6]. However, most of these treatments are enough expensive and therefore, less accessible to the populations in the under-developed and developing countries.

It became necessary to find new alternatives as to the ones from the medicinal plants that often are available. It is the case of *Hibiscus acetosella* Welw. Ex Hiern Malvaceae in the treatment of anaemia. *Hibiscus acetosella (H.a)* is a popular vegetable that grows in Africa. In folk medicine, different parts of this plant are uses to treat: fever, anaemia, headache, rheumatism, inflammations, conjunctivitis, haemorrhoids, tumours, ringworms, sores, abscesses, as a diuretic, sedative, anti-scorbutic, intestinal antiseptic, to stimulate lactation in breastfeeding women and as a blood purifying tonic [7].

In angola infusions made from leaves are used as a post-fever tonic and as a medicine to treat anaemia. In Nigeria the leaves are used for the treatment of dysentery, to control menstrual disorders and after-birth problems. In DRC, it is a vegetable prescribed by health care workers to diabetics. In East Africa, children with an aching body are washed in cold water to which some mashed *Hibiscus acetosella* leaves have been added [7]. In Cameroon, *Hibiscus acetosella* is one of the plants used to cure anaemia. Previous phytochemical work on *Hibiscus acetosella* revealed the presence of caffeic acid, gallic acid, gallocatechin, coumaric acid, 3, 4-dihydroxy-benzoic acid gallic tannins, anthocyanin, mucilage, anthrax-quinone, saponins, terpenes, sterols, glycosylated flavonoids, phenolic acids, fibbers and sugar [8, 9, 10].

Pharmacological investigations of the hibiscus genus reported that species displayed biological activities, including antioxidant, antigenotoxic, anti-inflammatory, anti-microbial, anti-diabetic, hepato-protective and anticancer activities [11, 12, 13, 14, 15]. In continuation of the previous studies carried out on *Hibiscus species*, the present work describes the anti-anaemic potential of the aqueous leaves extract of *Hibiscus acetosella* on two experimental models of anaemia induced by haemolytic activity of the 2, 4 DNPH and by bleeding in rats.

Experimental Animals: Wistar rats of 2-2.5 months of age, weighing 200-220 g were selected for these experiments. The animals were raised in colony cages, in the animal house of the University of Douala, Cameroon, under standard conditions of light (12 h light/dark cycle) and temperature (25-27 °C). They received a standard commercial diet and tap water *ad libitum*. The experimental protocol was approved by the Institutional Ethics Committee of the University of Douala (N°2304 CEI-Udo /05/2020/T) in accordance with the guidelines for the care and use of Laboratory Animal.

MATERIALS AND METHODS:

Material:

Plant Material: The fresh leaves of *Hibiscus acetosella*, domesticate and grows in the botanical garden at the University of Douala (Littoral region – Cameroon) were collected. Identification was confirmed by Pr Richard PRISO, a botanist at the Department of biology and physiology of Plant organisms of the University of Douala.

Methods:

Extraction Procedure: The leaves of *H. acetosella* were dried at room temperature and crushed in powder. The powder (1000 g) was boiled for 45 min and filtered through whatman filter paper No. 3. The filtrate was oven-dried at 40-50 °C to obtain a residue.

Phytochemical and Phyto-Analytical Studies:

Phytochemical Screening: Basic qualitative phytochemical screening of acute leaves extract of *Hibiscus acetosella* was carried out by testing the presence or absence of the following plant constituents: alkaloids, saponins, flavonoids, phenol, steroids, triterpenes, coumarins, anthocyanins, and anthraquinones using the methods outlined by Trease and Evans [16].

Estimation of Iron: A total of 100 mg of extract was transferred in 10 ml of water and transferred into a 100 ml volumetric flask, 5 ml of 2.058 mol/ml potassium thiocynate, and 3 ml of 6 mol/ml nitric acid were added to develop the color. A volume was made to 100 cm 3 marks with deionized water. Absorbance readings were measured for each sample (extract and standard solutions) at 579 nm using a UV-visible spectrophotometer [17].

Oral Acute Toxicity Study: Oral acute toxicity study was carried out following OECD guidelines number 423. The principle was based on the limit test method at the dose of 2000 mg/kg; nine 09 female rats were distributed in three (03) groups corresponding to one control group and two tested groups. Extract-treated rats received plant preparation at the single dose of 2000 mg/kg by oral route while control group received the vehicle (distilled water). The signs of toxicity and number of deaths were recorded after 48 h of observation. Mortality, lethal dose 50 (LD_{50}), and therapeutic dose (TD) were estimated by the OECD method [18].

Anti-anaemic Effect Study:

Anaemias Induction and Administration of Extract: Two methods of anaemia's induction were used. Haemolytic anaemia was induced by intra-peritoneal injection of 2, 4 dinitrophenyl hydrazine (2, 4 - DNPH) from sigma – Aldrich (CAS No. 119-26-6) at 40 mg.kg bw to each rat for 7 days, while bleeding anaemia was done by daily bleeding of rats (2-2.5 ml of blood from retro-orbital puncture for 2 days.

Before induction's days, approximately one milliliter of venous blood was collected for haematological studies by nipping the tails of the rats. The haematological parameters as a red blood cell, haematocrit (Ht) and haemoglobin (HB) were determined using automatic counter. After induction, rats with haematocrit and haemoglobin ≥ 30% reduction were considered anaemic and used for this experiment [19]. Following anaemia induction rats of each method of induction were divided into five groups of five rats each as following: group I considered as a normal group received water; group II served as anaemia group received 2-4-dinitrophenylhydrazine Groups III to V that was the tests groups received 2,4 - dinitrophenyl-hydrazine and the aqueous extract. The extract chosen doses were 100, 200, and 400 mg/kg/bw. Distilled water or aqueous extract were administered once daily for 14 consecutive days *per os*. Blood was collected in heparin capillary tubes under tail veins. The estimation of various haematological parameters was evaluated by the automatic counter as described by [20].

Haematological Assessment: Blood venous was collected before and after induction of anaemia as well as two times during the administration of the extract. The volume of blood collected (0.25 to 0.45 ml) did not affect blood parameters. The red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (Ht), mean cell volume (MCV), mean cell haemoglobin (MCH), mean corpuscular haemoglobin, and concentration (MCHC) were determined by Haematological analyser (automatic counter Nihon Kohden MEK 6411K, Japan).

Statistical Analysis: The results were expressed as mean ± SEM. The data obtained were analysed using analysis of variance (ANOVA) on Graph pad 7.0 software. The significance of differences was evaluated by means of two-way ANOVA (analysis of variance) followed by Bonferroni post hoc test for multiple comparisons. P values lower than 0.05 were considered significant.

RESULTS AND DISCUSSION: *Hibiscus acetosella* is a popular vegetable in Cameroon. It is used as a medicinal plant to treat diseases such anaemia [21].

Extract Yield and Qualitative Phytochemical Screening of *Hibiscus Acetosella*: The yield of the extract was observed to be 117 g (W/W: 11, 7%). The qualitative phytochemical screening, as observed in **Table 1** showed the presence of Alkaloids, saponins, steroids, and coumarins, while flavonoids, phenols, terpenoids, anthocyanins and Anthraquinones were not detected in the sample.

The bioactive compounds detected, such as saponins and steroid, are respectively known for their anti-haemolytic activities (case of saponins) and their capacity to stimulate the bony marrow to stimulate the production of erythropoietin, or to incorporate iron-on red blood [22, 23, 24].

TABLE 1: QUALITATIVE PHYTOCHEMICAL SCREENING OF *HIBISCUS ACETOSELLA*

S. no.	Compounds tested		Founded/ Unfounded
1	Alkaloids		Founded
2	Saponins		Founded
3	Flavonoids		Unfounded
4	Phenols		Unfounded
5	Steroids		Founded
6	Terpenoids		Unfounded
7	Coumarins		Founded
8	Anthocyanins		Unfounded
9	Anthraquinones	Bounded quinones	Unfounded
		Unbounded quinones	Unfounded

Estimation of Iron: Iron content was estimated at 10.77 ± 2.43 mg/kg of extract in the human body, iron loss is normally at the range of 0.17 - 0.90 mg/day[25]. World health organization recommended iron intake for growth at the ranges of 0.23 – 0.60 mg/day according to age and sex [25].

Decrease levels of iron is one of the major indexes of anaemia. This condition is observed when iron demand by body is more than its availability in body [26]. This could also be due to in sufficient iron intake, impairment in the mechanism of iron absorption and transportation and/or chronic blood loss [27]. The present work demonstrated that extract containing iron and can be used to treat iron deficiency anaemia

Determination of the Lethal Dose 50 (LD_{50}) and the Therapeutic Doses of *Hibiscus Acetosella*: Acute toxicity study in rats show neither adverse effects nor mortality after single administration of extract at the dose of 2000 mg/kg.

The lethal dose 50 was considered to be higher than 2000 mg/kg. *H. acetosella* extract has been classified in the "category 5" or unclassified of chemicals, category characterizing by non-toxic substances. It's kwon that unclassified substances are safe for humans and animals [18]. Therapeutic dose (TD) determine by the OECD method (TD = LD_{50} /10) has been calculated at 200 mg/kg.

Effect of *Hibiscus acetosella* Extract on Both Form of Anaemia: Anaemia is a common clinical, haematological syndrome, which is characterized by a reduction of haemoglobin concentration and erythrocytes in blood and would reduce oxygen delivery to tissue [28]. Haemoglobin is a natural constituent of RBCs and biochemically adapted to carry oxygen in the lungs and deposit it at tissues for oxidative metabolism [28]. It has been characterized to also play a major role in physiological carbon dioxide removal and acid-base balance; an increased production of haemoglobin is an advantage to an organism [28].The present work also gives the effect of aqueous leaves extract of *Hibiscus acetosella* on some haematological parameters in 2, 4 - dinitrophenyl hydrazine and bleeding-induced anaemia in rats.

Effect of Treatment with Aqueous Leaves Extract of *H. a* on Haemolytic Anaemia:

Effect on Haemoglobin Level Red Blood Cell and Haematocrit: We observed that administration of 2,4-dinitrophenyl hydrazine significantly decreases in all groups, Ht (max in group II, $p < 0,01$), RBC (max in group IV, $p < 0,01$) and HB (max in group V, $p < 0,001$)when compared to normal group. All extract doses of plant tested significantly increased Ht, RBC, and HB previously decreased by administration of 2,4 - dinitrophenyl hydrazine in anaemic group. Ht level and RBC significantly increased ($p < 0,001$) with the maximum of 45% and 32% respectively at the dose of 400 mg.kg bw after 14 days of treatment as compared to the anaemic group **Fig 1A** & **B**. Plant extract also increased significantly ($p < 0,001$) the HB in rats with the maximum of 32% at the dose of 200 mg.kg bw (seventh day) when compared to the anaemic group **Fig 1C**.

Effect on Mean Cell Volume, Mean Cell Haemoglobin Concentration and Mean Cell Haemoglobin Levels: Administration of 2,4 - dinitrophenyl hydrazine significantly increased the mean cell volume (max in group V, $p < 0,01$) and decreased mean cell haemoglobin level (max in group III, $p < 0.001$). We also observed decrease of mean cell haemoglobin concentrations compares to the normal group. After 7th day of treatment with aqueous H.a, MCV decreased when MCHC and MCH increased non-significantly in all treated groups compared with anaemic group **Fig. 2A** & **C**.

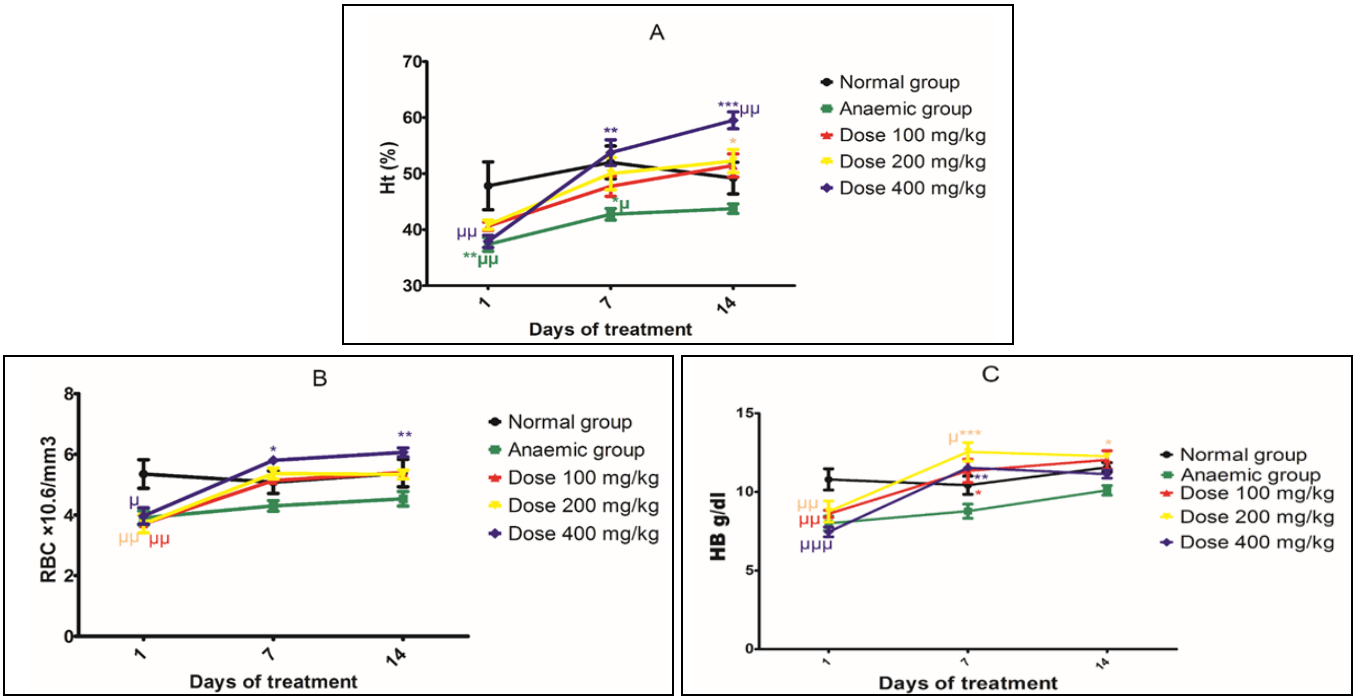

FIG. 1: EFFECT OF AQUEOUS LEAVES EXTRACT OF *H.a* ON HAEMATOCRIT (A), RED BLOOD CELL (B) AND HAEMOGLOBIN LEVEL (C) IN HAEMOLYTIC ANAEMIA HB = Haemoglobin; RBC = Red blood cell; Ht = Haematocrit. *P < 0.05;**P < 0.01; ***P < 0.001, significantly different versus anaemic group µP< 0.05; µµP <0.01 and µµµP< 0.001, significantly different versus normal group.

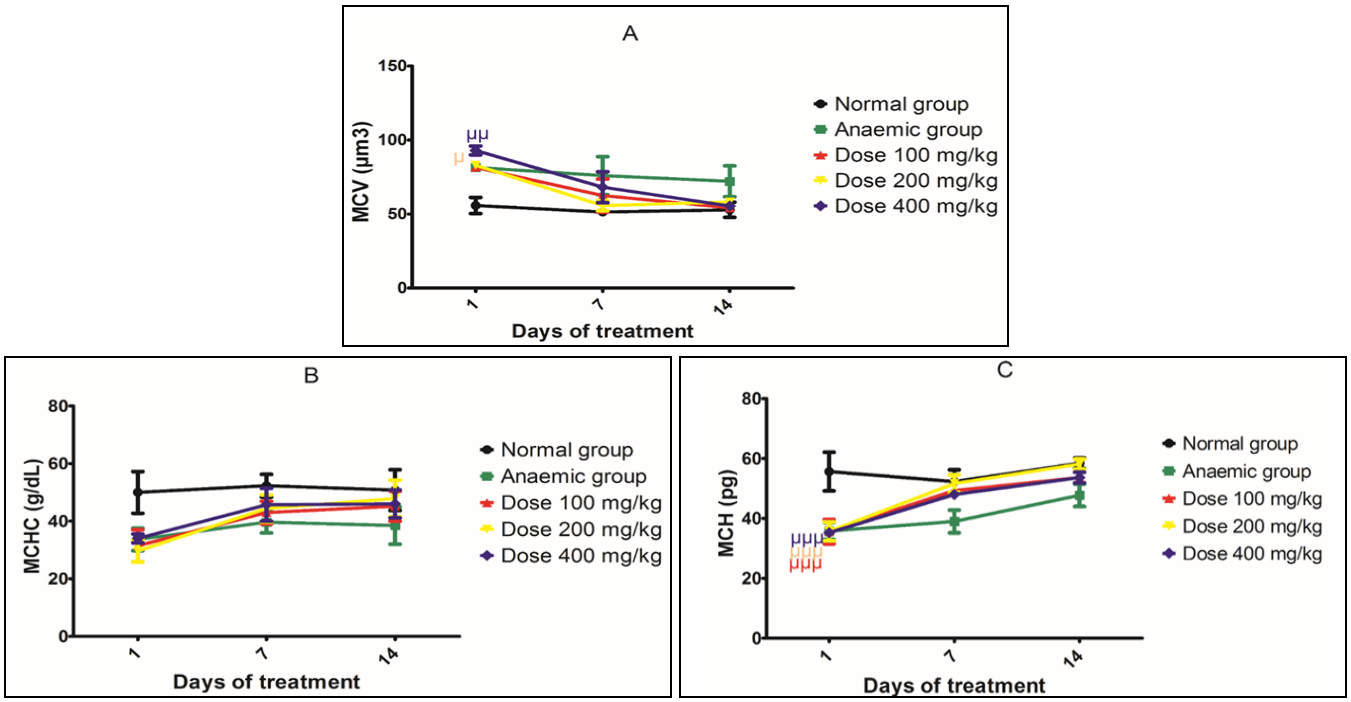

FIG. 2: EFFECT OF AQUEOUS LEAVES EXTRACT OF *H.a* ON MCV (A), MCHC (B), MCH (C) IN HAEMOLYTIC ANAEMI MCV = Mean cell volume; MCHC = Mean cell haemoglobin concentration; MCH = Mean cell haemoglobin level.µP< 0.05; µµP <0.01 and µµµP< 0.001, significantly different versus normal group.

Phenyl hydrazine and its derivative 2,4-DNPH are recognized for their capacity to cause haemolysis both in vitro and *in-vivo* by the formation of aryl and hydroxyl radicals, which has been demonstrated to be associated with its interaction with erythrocytes [20].

The detoxifying capacity of the red cell due to the accumulation of hydrogen peroxide may lead to the oxidation of essential cellular constituents, including membrane phospholipids, contributing to the eventual haemolysis of affected cells [29]. This leads to a lack of circulating erythrocytes and

haemoglobin. In this study, administration of 2,4-DNPH (40 mg.kg bw for 7 days) in rats induced a haemolytic anaemia characterized by decreased RBC, Hb and Ht.

Treatment of anaemia with extract of *H. a* resulting in increase of all those haematological parameters. Thus, the aqueous extract of H amay act, inhibiting the haemolysis induced by 2, 4-DNPH. It has been reported that rats intoxicated with phenyhydrazine showed an increase MCV due to the enlarged cell volumes of the reticulocytes transiently to the circulation [30]. It also confirmed with [31], that MCV was higher than expected because the calculated value includes the size of the red blood cells and immature erythrocytes.

In this study, administration of the 2, 4-dinitropheny hydrazine induced an increase of MCV, indicating the haemolytic anaemia in rat [32]. The treatment of the anaemic rat with H an induced the decrease of this haematological index showing thus, would influence the pathogenesis of the anaemia. Moreover, it has been reported that H. a possessed antioxidant activity [11].

Since the intoxication of 2,4-DNPH is related to the formation of aryl and hydroxyl radicals and the accumulation of hydrogen peroxide in the red cell, the aqueous extract of H. a could interfere with the oxidation of cellular constituents.

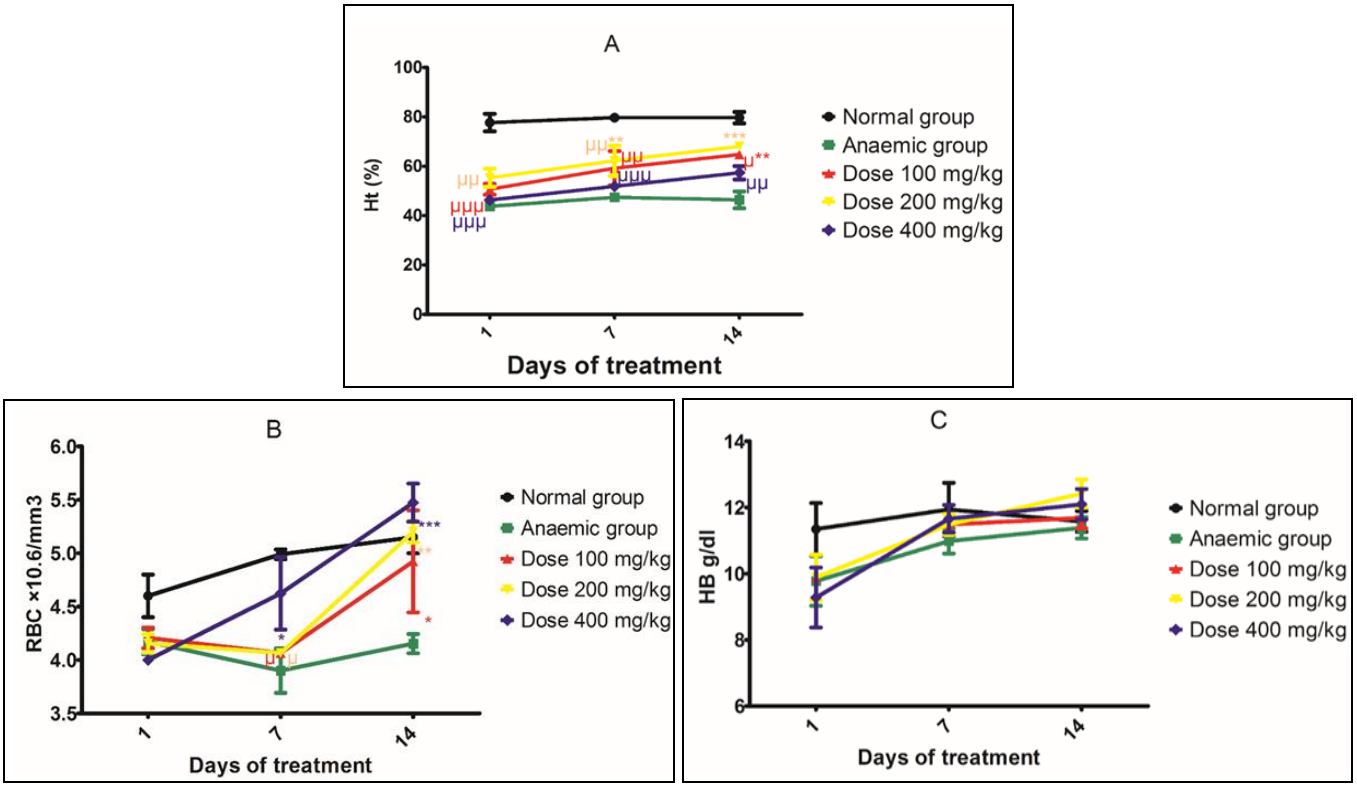

FIG. 3: EFFECT OF AQUEOUS LEAVES EXTRACT OF *H.a* ON HAEMATOCRIT (A), RED BLOOD CELL (B) AND HAEMOGLOBIN LEVEL (C) IN BLEEDING ANAEMIA HB = Haemoglobin; RBC = Red blood cell; Ht = Haematocrit. *P < 0.05;**P < 0.01; ***P < 0.001, significantly different versus anaemic group µP< 0.05; µµP <0.01 and µµµP< 0.001, significantly different versus normal group.

Effect of Treatment with Aqueous Leaves Extract of H. A on Bleeding Anaemia: Another anaemic condition was induced experimentally in the blood loss by a retro-orbital puncture in rats during 2 days. Bleeding anaemia was induced by the method of successive daily bleeding [33].

Effect on Haematocrit, Red Blood Cell and Haemoglobin Level: Bleeding decreased Hb, RBC and significantly Ht (max in group V, p < 0.001) as compared to the normal group **Fig. 3A** and **B**. During 14 days of treatment, *H. acetosella* increased haemoglobin, and significantly the haematocrit as well as red blood cell at all doses tested with a maximum increase of 14% at 200 mg.kg bw (p < 0.05) and 31% at 400 mg.kg bw (p < 0,001) respectively as compared to the anaemic group **Fig. 3**.

Effect on Mean Cell Volume, Mean Cell Haemoglobin Concentration and Mean Cell Haemoglobin Levels: In experimental bleeding anaemia, the MCV level increase significantly (max in group III, p < 0,001) without significant changes in the MCHC and MCH levels **Fig. 4**. Administration of extract on bleeding anaemia induced significant decreased of MCV after 14 days of treatment at a dose of 400 mg.kg bw (p <0.01) as compared to the anaemic group.

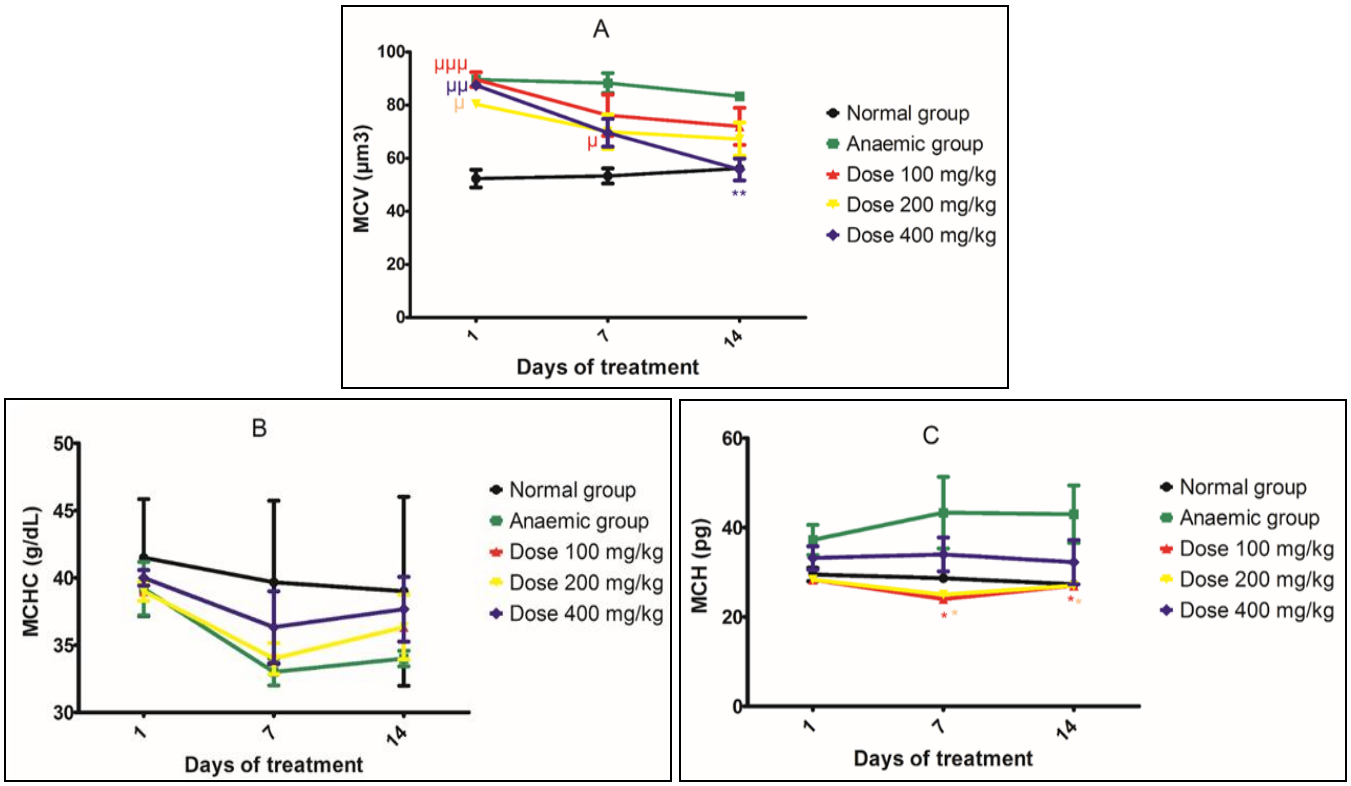

FIG. 4: EFFECT OF AQUEOUS LEAVES EXTRACT OF *H.a* ON MCV (A), MCHC (B) AND MCH (C) IN BLEEDING ANAEMIA MCV = Mean cell volume; MCHC = Mean cell haemoglobin concentration; MCH = Mean cell haemoglobin level. *P < 0.05;**P < 0.01, significantly different versus anaemic group µP< 0.05; µµP <0.01 and µµµP< 0.001, significantly different versus normal group.

In the previous model, changes of haematological parameters during the anaemia induced by phenyhydrazine are different [34]. In the course of treatment with phenylhydrazine, haematological values (haematocrit and haemoglobin level, number of erythrocytes) decrease after the third day, but they can return to the normal level when the percentage of reticulocytes become highest [33]. This state has been observed in our results when bled animals showed a reduction in the haemoglobin concentration, red blood cell number, and significantly, the haematocrit. Fourteen days of treatment of rats with aqueous extract increased the haematocrit, red blood cell, and haemoglobin level. Anaemia of blood loss can lead to the loss a large number of red blood cells. Loss of RBC decreases levels of haemoglobin and iron in the body [35]. It has been reported that the anaemia caused by blood loss similar to iron deficiency anaemia [36]. Thus, the presence of iron in the extract would be an asset in the treatment of bleeding anaemia. The increase in haematocrit is an indication of an increase in haemoglobin concentration that may result from increased RBC count [35]. Increased production of haemoglobin is an advantage to an organism according to its roles like a natural constituent of RBC, to carry oxygen in the lungs and deposit it at tissues for oxidative metabolism and to remove carbon dioxide [37]. The increase of the haematological indices exhibited by *H. acetosella* extract might be due to classes of metabolites (saponins, steroids) and minerals (iron), contents in the aqueous extract. It could also be due to other compounds as folic acid and ascorbic acid. Indeed, deficiency of folic acid in the body and the reduced level of ascorbic acid and/or its decreased catabolism is also a condition observed during anaemia [26]. These constituents may act as

haemopoietic factors that have directly influenced on the production of blood in the bone marrow. Like anaemia caused by blood loss similar to iron-deficiency anaemia, the leaves' aqueous extract of *Hibiscus acetosella* contain the iron that could help improve haematological parameters.

Further study is necessary to determine the folic acid and the ascorbic acid rate in the extract. These findings agree with folk medicine reporting that aqueous extract of *H. acetosella* leaves has anti-anaemic effect, what justifies the traditional use of this plant in the treatment of anaemias.

ACKNOWLEDGEMENT: We thank Professor Richard PRISO of the Department of Biology and Physiology of the Vegetal Organisms in the Faculty of Sciences of the University of Douala for the achievement of the *Hibiscus acetosella* identification. AB Dongmo acknowledges the Alexander von Humboldt Foundation for the grant apparatus.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Kanfer EJ and Nicol BA: Haemoglobin concentration and erythrocyte sedimentation rate in primary care patients. J Roy Soc Med 1997; 90: 116
2. WHO: WHO library catalogue-in-publication data Surgical care at the district hospital. Genève 2003.
3. WHO: Worldwide prevalence of anaemia in WHO global database on anaemia mclean eeglii and cogswell m eds bruno de benoist geneva switzerland eth zurich. Switzerland Atlanta Georgia 2008; 1993-05.
4. INS and ICFL: International health and demographic investigation to multiples informers of cameroon in Calverton. Maryland USA INS and ICF International 2012.
5. INS and ORC: Macro health and demographic investigation of cameroon in calverton. Maryland USA INS ETORC Macro 2004.
6. Movaffaghi Z and Hasanpoor M: Effect of therapeutic touch on blood hemoglobin and hematocrit level. J Holist Nurs 2006; 24: 41-8.
7. Denton OA, Grubben GJH, Messiaen CM, Schippers RR, Lemmens RHMJ and Oyen LPA: Plant resources of tropical africa prota foundation. Backhuys Publishers CTA Wageningen Netherlands 2004; 312-13.
8. Vilela TC, Leffa DD, Damiani AP, Damazio DDC, Manenti AV, Carvalho TJG, Ramlov F, Amaral PA and De Andrade VM: *Hibiscus acetosella* extract protects against alkylating agent-induced DNA damage in mice. An Acad Bras Ciênc 2018; 90(3).
9. Obouayeba AP, Meité S, Boyvin L and Yeo D: Cardio-protective and anti-inflammatory activities of a polyphenols enriched extract of *Hibiscus sabdariffa* petal extracts in wistar rats. J Pharmacognosy Phytochem 2015; 4(1): 57-63.
10. Mukunda L, Elumbu S, Kalenga K, Utshudienyema N, Ikolonga P, Mango M, Likwela L and Agasa B: Fiber and sugar contents in vegetables prescribed and consumed by diabetics in Kisangani. J Exp Biol Agr Sci 2015; 3(3).
11. Kapepula MP, Kabamba NP, Tshisekedi TP, Tsumbu CTF, Mouithys-Mickalad A, Mumba D, Tshala-Katumbay D, Serteyn D, Tits M, Angenot L, Dibungi KTP and Frédérich M: Comparison of metabolic profiles and bioactivities of the leaves of three edible Congolese *Hibiscus species*. Nat Prod Res 2017; 31(24): 2885-92.
12. Adetutu A, Odunola OA, Owoade OA, Adeleke AO and Amuda OS: Anticlastogenic effects of *Hibiscus sabdariffa* fruits against sodium arsenite-induced micronuclei formation in erythrocytes in mouse bone marrow. Phyt Other Res 2004; 18: 862-64.
13. Farombi EO and Fakoya A: Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of *Hibiscus sabdariffa* L. Mol Nutr Food Res, 2005; 49; 1120-28.
14. Olalye MT and Rocha JBT: Commonly used tropical medicinal plants exhibit distinct in vitro antioxidant activities against hepatotoxins in rat liver. Exp Toxicol Pathol 2007; 58: 433-38.
15. Rosa RM, Melecchi MIS, Da Costa HR, Abad FC, Simoni CR, Caramão EB, Henriques JAP, Saffi J and DE Paula Ramos ALL: Antioxidant and anti-mutagenic properties of *Hibiscus tiliaceus* L methanolic extract. J Agric Food Chem 2006; 54; 7324-30.
16. Trease GE and Evans WC: A textbook of pharmacognosy 13th ed wb saunders. London 1989; 176-80.
17. Vogel AI: Quantitative inorganic analysis 4th ed. London Longman 1983; 122-25.
18. OECD: Acute oral toxicity-acute toxic class method, OECD. guideline n ° 407 for testing of chemicals 2001.
19. Damilola A, Omoboyowa, Agha O, Aja Florence E, Kerian C and Ngobidi: Effects of methanol seed extract of *Aframomummelegueta* alligator pepper on wistar rats with 2, 4-dinitrophenyl hydrazine-induced hemolyti canemia. RABM 2017; (3): 11-17.
20. Ravi UT, Bhavin AV, Shrikant VJ, Paras KP and Dinesh RS: Effect of dehydrated water extract of fruits of opuntia ficus indica on experimentally-induced hemolyticanemia in rats. IJPRD 2011; 4(03): 185-91.
21. Denton OA, Grubben GJH, Messiaen CM, Schippers RR, Lemmens RHMJ and Oyen LPA: Plant resources of tropical africa prota foundation. Backhuys Publishers CTA Wageningen Netherlands 2004; 312-13.
22. Van-Burden TP and Robinson WC: Formation of complexes between protein and vidalia onions. J Agr Food Chem 1981; 50(19): 5338-42.
23. Sébahoun G, Costello R, Rossi D, Tostain J and Androgens: Haematopoïesis and immunity-androgens and haematopoiesis. AFU Prof Urol 2004; 14, 797-800
24. Molinari PF and Rosenkrantz H: Erythropoietic activity and androgenic implication of testosterone derivatives in orchiectomized rats. J Lab Clin Med 1971; 41, 149-152.
25. UNICEF, UNU and WHO Iron deficiency anaemia assessment, prevention and control. A Guide for Programme Managers 2001.
26. Anupam J, Aditya G, Ankita AD and Nazneen D: Protective effects of beetroot extract against phenyl hydrazine induced anaemia in rats. Phcog J 2014; 6: 5.
27. Clark SF: Iron deficiency anemia. NCP 2008; 23: 128-41.
28. Mohammed SOM, Mahmoud SS, Mohammed AMH and Abdelnaeem AA: Iron Deficiency and Iron Deficiency Anemia in Adolescent Girls in Rural Upper Egypt; Inter Blood Res Rev 2016; 5(4): 1-6.
29. Ferrali M, Cinzia S, Sugherini L, Pompella A Lodovici M, Caciotti B, Ciccoli and Comporti M: Release of free

redox-active iron in the liver and DNA oxidative damage following phenylhydrazine intoxication. Bio Chem Pharmacol 1997; 53: 1743-51

30. Dolznig H, Bartunek P, Nasmyth K, Müllner EW and Beug H: Terminal differentiation of normal chicken erythroid progenitors shortening of G1 correlates with loss of d-cyclin/cdk4 expression and altered cell size control. Cell Growth Differ 3995; 6(11): 1341-52.
31. Angermeier E, Domes K, Lukowski R, Schlossmann J, Rathkolb B, Hrabě M, Angelis and Franz H: Iron deficiency anemia in cyclic GMP kinase knockout mice. Haematologica 2016; 101: 49.
32. Föller M, Feil S, Ghoreschi K, Koka S, Gerling A, Thunemann M, Schuler B, Vogel J, Pichler B, Ravi S, Kasinathan P, Nicolay M, Huber F and Feil R: Anemia and splenomegaly in CGKI-deficient mice, proceedings of the. National Academy of Sciences of the United States of America 2008; 105(18): 67-75.
33. Marković SD, Marijana P, MiloševićNataša Z, Đorđević Branka I, Ognjanović A, Štajn Zorica S, Saičić MB and Spasić: Time course of hematological parameters in bleeding-induced anemia. A Biol S 2099; 61(2): 165-70.
34. Kostić G, Cvetković M and Kostić MM: Time course Time course of experimental phenylhydrazine-induced anemia in rats. Bilten Hematol Transf 1988; 16: 53-61.
35. Finch C: Regulator of iron balance in humans' blood. Blood 1994; 84: 1697-1702.
36. Saljooghi AS and Delavar-mendi F: The effect of mercury in iron metabolism in rats. J Clinic Toxicol 2013.
37. Musyoka TM, Dorothy NW, Wycliffe AM, Juma KK, Nzioka MD, Maina D, Waithaka SK, Ngugi MP, Orinda GO, Karau GM and Njagi ENM: *In vivo* anti-anaemic effect and safety of aqueous extracts of *Erythrina abyssinica* and *Zanthoxylum usambarensis* in mice models. J Hematol Thrombo Dis 2016.

How to cite this article:
Mbock AJ, Nguemfo EL, Bogning ZC, Magne FAL and Dongmo AB: Anti-anaemic effect of aqueous leaves extract of H*ibiscus acetosella* Welw. Ex Hiern (Malvaceae) on two experimentals models of anaemia induced by 2, 4-dinitrophenyl-hydrazine and blood lose in wistar rat. Int J Pharmacognosy 2021; 8(4): 146-54. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.8(4).146-54.