Ability of *Urtica chamaedroydes* pursh to restore hematopoiesis in anemic pregnant mice

**Rodolfo Velasco Lezama**¹, **Martha Fregoso Padilla**², **José Luis Flores Sáenz**¹, **Jorge Santana Carrillo**¹, **Sara Beatriz Herrera Solís**³, **Elisa Vega Avila**¹, **Eduardo Barrera Escorcia**²

¹Departamento de Ciencias de la Salud. Universidad Autónoma Metropolitana-Iztapalapa. Av. San Rafael Atlixco 186. Col. Vicentina. Iztapalapa, México, D. F. 09340.

²Laboratorio de Biorregulación, Facultad de Estudios Superiores-Iztacala. Universidad Nacional Autónoma de México. Avenida de los Barrios s/n, Los Reyes, Tlahuapan. Estado de México. 54090.

³Herbario Metropolitano “Ramón Riba” Universidad Autónoma Metropolitana-Iztapalapa. Av. San Rafael Atlixco 186. Col. Vicentina. Iztapalapa, México, D. F. 09340.

Received: September 01, 2012

Accepted: December 05, 2012

Published Online: December 24, 2012

DOI: 10.5455/jice.20121205083808

**Corresponding Author:** Rodolfo Velasco Lezama, Universidad Autónoma Metropolitana-Iztapalapa. Av. San Rafael Atlixco 186. Col. Vicentina. Iztapalapa, México, D. F. 09340. rodolfo.velasco2003@yahoo.com.mx

**Keywords:** Iron-deficiency anemia, *Urtica chamaedroydes*, Hematopoiesis.

### Summary

Aim/Background: Iron-deficiency anemia (IDA) is associated during pregnancy with increased mortality and morbidity maternal-infantile rate, malformations and fetal or neonatal death. To avoid the death or malformations of fetuses the Indian communities use *Urtica chamaedroydes*. The purpose of this study was to investigate the ability of the decoction of *U*. *chamaedroydes* (chichicastle) to restore the hematopoiesis in pregnant mice and to avoid malformations in fetuses.

Methods: Forty female mice CD1, 8–12 weeks were distributed in groups A, B and C with ten animals each. Anemia was induced in groups A, B and C by courts in tail twice a week during 15 days. Hematological determinations were performed on days 0, 15 and 34. On day 15 the pregnancy was induced in all anemic mice and the healthy control group (D). During the gestation, groups A and D did not receive treatment. Group B was treated with a decoction of *Urtica chamaedroydes* (4 g/L) as drinking water and Group C with two sc administrations of 200 mg/kg of FeSO₄. On day 34 all groups were sacrificed.

Results: Groups treated with the decoction of *U. chamaedroydes* or FeSO₄ recovered the normal level of erythrocytes and platelets, none statistically significant differences were found between the concentration of these elements among days 0 and 34 within the same group. Neither the decoction nor FeSO₄ allowed recover the normal level of leukocytes. From the total fetuses, malformations were observed in 80 20, 5, 10 % of Groups A, B, D and C, respectively.

Conclusion: These results could support the traditional medicinal use of this plant in the treatment of the anemia and to prevent malformations.

© 2012 GESDAV

### INTRODUCTION

Iron deficiency is the most common nutritional disorder that affects at least one third of the world’s population. It is a frequent cause of anemia during pregnancy, provoking a high risk of maternal-fetal mortality [1]. Generally 52% of pregnant women present anemia and more than 90% of them reside in developing countries [2].

In Mexico, some researchers [3] reported that anemia has higher incidence in pregnant women that inhabit indigenous communities (24.02%) that those not indigenous (14.67%), and pointed out that the prevalence of anemia during the first trimester of the pregnancy goes from 3.5 to 7.4% and reaches 15.6 to 55% in the third trimester. The importance of nutrition before and during pregnancy determines the risk late in
the intra-uterine growth, which is bigger when the women's state is unfavorable before the pregnancy [4]. Some studies have shown that when the women present Iron deficiency anemia (IDA) during the first and second trimesters of gestation, there is an increment in the rate of childbirth post-term, low weight when being born, fetal deaths, delayed development motor and neural, in extreme cases, increase in the risk of maternal-fetal mortality [5].

Investigations concerning the iron-deficiency anemia in the embryonic period, particularly the formation of organs produces defects in the generation of cerebral cells, and several morphological malformations. During the gestation, anemia can also alters the cell proliferation by changes in the pattern of synthesis of diverse neurotransmitters, fatty acids, cholesterol and myelin, as well provokes a decrease in the synthesis of DNA since the ribonucleotide reductase requires iron as cofactor [6]. To avoid such effects in modern medicine, IDA is treated by oral or injected administration of FeSO₄. In Mexico, anemia in indigenous women is associated with malnutrition as a result of low resources and at the cost of the treatment. To counteract this suffering and the effects in pregnant women and over the gestated product, in indigenous communities far from hospitals and doctors, people use as alternative medicinal plants [7], among them the Urtica dioica, Urtica chamaedroydes and Urtica urens.

For the present study Urtica chamaedroydes Pursh was selected, because is empirically used in pregnant women with iron-deficiency anemia to counteract this syndrome and to avoid malformations in the fetus [8].

Urtica chamaedroydes Pursh. (Urticaceae), reaches 30 to 80 cm tall, branched and covered with stinging hairs. It has oval, or elongated leaves also covered with stinging hairs. The plant is widely distributed from the Center to the North of Mexico. The decoction is given in cases of anemia, asthma, and for purifying the blood. The plant is commonly known as white grass, chichicastle and ortiguilla [9].

In a preliminary study, our group reported as abstract, hematopoietic activity of Urtica chamaedroydes in anemic pregnant rats and the ability to reduce the number of malformations in their gestated products [10]. Although, this plants is used as antianemic, especially by indigenous groups in the country, no experimental information over its pharmacological action was found. And we aimed to determine the ability of the decoction of Urtica chamaedroydes Pursh to restore the hematopoiesis in anaemic pregnant mice and to avoid malformations in the fetuses.

MATERIAL AND METHODS

Plant material

Aerial parts of the plant were collected by one of the authors (Rodolfo Velasco) in Alto Lucero town, Veracruz in January 2009, and authenticated at the Herbario Metropolitano of the Universidad Autónoma Metropolitana (UAM) Iztapalapa, where a voucher specimen of the plant (70446) is stored.

Preparation of the decoction

The aerial parts were dried at room temperature, protected from dust and sunlight and grounded in a hand mill (Victoria, Colima). 500 g of the ground material were boiled 15 min using 3 liters of distilled water. The decoction was filtered and evaporated to dryness under reduced pressure, at 35°C in a Savant Speed Vac plus SC210A concentrator (Farmingdale, USA), then placed in vials and frozen at -20°C until its administration to mice.

Phytochemical screening

A preliminary phytochemical study of the decoction was performed by coloring and precipitation assays as reported previously [11]. Total phenolic compounds were measured by Folin-Ciocalteau Method [12], Flavonoids were determined as described by Wolf, et al. [13].

Induction of iron-deficiency anemia

Forty female CD₁ mice between 8-12 weeks old were obtained from the animal facilities of the Facultad de Estudios Superiores Iztacala of the Universidad Nacional Autónoma de México. Animals were maintained with alternating 12 h periods of light and darkness and allowed free access to food and water according to the statutes of the CICUAL (Comité Institucional para el Uso y Cuidado de los Animales de Laboratorio) of the official Mexican norm for the production and maintenance of laboratory animals. NOM-062-200-1999 [14]. Mice were weighed and distributed in the following groups with ten mice each.

A. Induction of iron deficiency anemia without post-treatment (IDAw/o)
B. Induction of iron deficiency anemia post-treated with the aqueous Urtica chamaedroydes (IDAUch)
C. Induction of iron deficiency anemia post-treated with ferrous sulfate (IDAFe)
D. Healthy control (HC)

Iron-deficiency anemia was induced by cuts in the tail of mice on days 0, 4, 7, 11, 15. To evaluate the hematological conditions, animals were bleeding on days 0 and 15, the blood was collected in plastic tubes.
containing dry heparin sodium (Pisa, Mex.). Hematological determinations were performed with an analyzer Sysmex KX-21N (Sysmex Corp. Japan). Also blood films were prepared and stained by the Wright’s method (Hycel, Mex.). Once confirmed anemia, the pregnant was induced in all groups included the control group. During the gestation, group A (IDAw/o) anemic mice did not received any treatment, for group B (IDAUch) the decoction of aerial parts of U. chamaedroydes (4 g/L) was given as drinking water, group C (IDAFe) received two sc applications of 200 mg/kg of FeSO₄ (Nycomed, Mex.) and group D (HC) without treatment. On day 19 of gestation all mice were sacrificed in a CO₂ camera and the fetuses examined visually and under a stereoscopic microscope to detect the number and type of malformations. Malformations in fetuses from anemic mice from groups B and C, were compared against the healthy control group (group D), and those anemic mice without treatment (group A). Results are expressed as mean ± standard desviation. The comparison among Groups was made using variance analysis (ANOVA) and the LSD Fischer test. A p value less than or equal to 0.05 (p<0.05) was considered statistically significant.

RESULTS

From 500 g of ground material 49 g of dry decoction were obtained, a yield of 9.8%.

Phytochemical screening

Phenolic compounds and flavonoids were detected in the decoction, but no saponins or alkaloids were found. Total phenolic compounds and flavonoids in the dry decoction were 3.49% and 1.5%, respectively compared with their standards of gallic acid and catechin, respectively.

Induction of iron-deficiency anemia.

On day 15 mice from groups A, B and C presented signs of iron-deficiency anemia, manifested as lethargy, generalized pallor, 10% of them spent slept most of the time. On this time no significant differences of corporeal weight were found these groups, however such weight was 35% lower than mice from control group (D).

Erythrocytes

Mice from groups, A (IDAw/o), B (IDAUch) and C (IDAFe) after induction of anemia (day 15) showed 54% average lower concentration of erythrocytes compared their own lecture on day 0, and the healthy control group at the same time. However, only group IDA/w/o kept low concentration of erythrocytes until the end of experiment (day 34). Table 1.

Table 1. Concentration of erythrocytes and platelets in healthy mice and mice with iron deficiency anemia.

| Group  | Day | Erythrocytes X10¹²/l | Platelets X10¹⁵/l |
|--------|-----|---------------------|------------------|
| HC     | 0   | 1.06±0.10           | 1.35±0.24        |
|        | 15  | 1.12±0.10           | 1.40±0.18        |
|        | 34  | 0.98±0.20           | 1.18±0.16        |
| IDA    | 0   | 1.10±0.12           | 1.43±0.20        |
|        | 15  | *0.58±0.08          | 0.89±0.22        |
|        | 34  | *0.50±0.06          | *0.98±0.14       |
| IDAUch | 0   | 1.20±0.08           | 1.52±0.15        |
|        | 15  | *0.63±0.03          | *1.10±0.19       |
|        | 34  | 0.92±0.10           | *1.57±0.12       |
| IDAFe  | 0   | 1.15±0.10           | 129±0.11         |
|        | 15  | *0.68±0.10          | *0.90±0.10       |
|        | 34  | 0.99±0.05           | 119±0.15         |

n=10, Mean ± Standard deviation, *p<0.05
HC= Healthy Control, IDA= Iron-deficiency anemia with out posttreatment, IDA= Iron-deficiency anemia postreated with Urtica chamaedroydes,
IDAFe= Iron-deficiency anemia postreated with Ferrous sulphate.

In group D (HC) the concentration of erythrocyte on day 34 was 18 % lower than their own lecture on day 0, change that is not statistically significant. Anemic mice treated with the decoction of Urtica chamaedroydes (IDAUch) or ferrous sulfate (IDAFe) at the end of the experiment recovered 76 % and 86%, of concentration of erythrocytes, respectively compared to their own concentration on day 0. In both cases the differences are statistically significant p<0.05, which means that treatment of pregnant anemic mice either with the decoction or FeSO₄ do not recover the normal level of erythrocytes at 34 day. Table 1. Examination of the stained blood films on day 15 for groups IDA/w/o, IDAUch and IDAFe revealed morphological alterations in erythrocytes as; poikilocytosis; elliptocytosis and anysocytosis, besides hypochromic microcytes (small erythrocytes), morphology that it is usually in IDA [15].

Platelets

In control group (HC) the concentration of platelets at the end of the experiment was 87%, regarding day 0,
however the difference is not statistically significant. Although, platelets and leukocytes are usually in normal levels in patients with IDA, in these experiments, mice from group IDAw/o presented 31% of reduction of platelets, while in groups treated with the decoction of *U. chamaedroydes* or FeSO₄, the final concentration (day 34) was 103% and 92%, respectively compared to their own lectures on day 0. Both groups recovered the normal level of platelets, however in group treated with the decoction (IDAUCH), such increase was significantly higher respect control group (HC) and group treated with FeSO₄, p <0.001. Table 1.

**Leukocytes**

In groups IDAFe, IDAUCH and IDAw/o, the concentration of leukocytes diminished an average of 31% on day 15 of the experiment. Groups treated with the decoction of *U. chamaedroydes* or with FeSO₄ at the end of the experiment did not recover the normal levels of leukocytes regards day 0 (Figure 1).

**Effect of Urtica chamaedroydes on malformations in fetuses**

The results shown that 80% of fetuses from group of anemic mice without treatment (IDAw/o) presented malformations, meanwhile those treated with the decoction had 20%, fetuses from group treated with FeSO₄ 10%, and control group 5%. Figure 2. Being malformations of nasal graves and of palate the most observed.

**DISCUSSION**

On day 34, erythrocytes from groups HC, IDAUCH and IDAFe had almost normal size and color (normocytic-normochromic), which means normal level of hemoglobin. Also erythrocyte indices were normal in groups IDAUCH and IDAFe. The almost normal counts of erythrocytes in groups treated with the decoction of *U. chamaedroydes* or FeSO₄ requires a previous synthesis of erythropoietin (EPO), and considering that this hormone shares 30% of similarity with thrombopoietin [16], hormone responsible for the production of platelets [17]. It is possible that the increase of platelets concentration seen could be due to an indirect stimulating effect of EPO on thrombopoiesis, triggered by the decoction or FeSO₄ as well, as a stimulating effect of EPO on the precursor cells of platelets (megakaryocyte), through a common cell, since both cell lines share the hematopoietic precursor cells BFU-EMeg [18].

The low concentration of leukocytes in these groups respect control group are statistically significant p <0.001. Then the conclusion is that the decoction of the plant does not present leukopoietic activity, even some authors have reported that other member from the generous *Urtica* (*dioica*) stimulates in vitro the proliferation of lymphocytes [19]. Also a mitogenic activity on T lymphocytes is related with polysaccharides from the roots and leaves of *Urtica dioica* [20]. Besides, an immunomodulatory activity of this plant has been attributed to the flavonol glycosides isolated the leaves of *U. dioica* [21].
activities are primarily dependent of the hematopoietic activity of the plant. However, such activities have not been described until now for *U. chamaedroydes*.

Generally, anemic mice without treatment had a lower number of fetuses but they were bigger and heavier than those from other groups. Fetuses from the healthy mice were smaller than those from other groups. Decoction of *U. chamaedroydes* contributes to restore the erythropoiesis and thrombopoiesis, also reduces the number of malformations in the gestated products versus fetuses from pregnant anemic mice without treatment.

Remains to know, if the thrombopoietic effect seen with the decoction is direct through the stimulation on the line megakaryocyte-platelet, or indirect as result of its erythropoietic activity. Also it is necessary to determine the toxicity of this plant, since genotoxic, embryotoxic, mutagenic and abortive effects has been reported for *U. dioica* [22].

The decoction of *U. chamaedroydes* contributes to restore the erythropoiesis and to reduce the number of malformations in products of gestation. These results could explain and support the traditional medicinal use of this plant in the treatment of the anemia and to prevent malformations.

ACKNOWLEDGMENTS

The authors gratefully thank Dr. Margaret Lee from Universidad Autónoma Metropolitana, for style revision.

REFERENCES

1. Andrews NC. Iron deficiency and related disorders. Eds. : Greer JP, Foerster J, Rodgers GM, Paraskevas F, Glader B, Arber DA. Means RT Jr. In: Wintrobe’s Clinical Hematology. Vol. I. 12th Edition. Philadelphia; Wolters Kluwer/Lippincott. Williams & Wilkins; 2009. p. 810-834.

2. WHO/CDS. World prevalence of anemia 1993-2005; global database on anemia. 2008.

3. Martínez SH, González CT, Flores MR, Rivera DJ, Lezama MA, Sepúlveda AJ. Anemia en la mujer en edad reproductiva. Salud. Pub. Méx. 1995; 37(12): 108-19.

4. Bobadilla J, Langer A. La mortalidad infantil en México, un fenómeno de transición. Rev. Mex. Sociol. 1990; 1: 111-31.

5. Collard KJ. Iron homeostasis in the neonate. Pediatrics. 2009; 23(4):1208-16.

6. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J. Nutr. 2001; 131(2S-2):649S-666S; discussion 666S-668S.

7. Flora Medicinal Nahua de San Miguel Tzinacapan Cuetzalan, Puebla. Eds. : Instituto Nacional Indigenista In: Flora Medicinal Indígena de México. Vol II. 1994. 985.

8. Argueta VA, Cano AL, Rodarte ME. Atlas de las Plantas de la Medicina Tradicional Mexicana. Vol. I. 1994. Instituto Nacional Indigenista, México. p. 412-15.

9. Cano AL. Flora medicinal de Veracruz. Universidad Veracruzana, Veracruz. 1997. México.

10. Velasco LR, Yesca MMA, Gallegos CA, Arratia CLM, Zamora GR, Tapia AR, Vega AE. Empleo de *Urtica (chamaedroydes)* en el tratamiento de ratas con anemia ferropénica y su efecto sobre los productos de gestación. Bioquimia. 2009; 34: 76.

11. Alarcón AF, Vega AE, Almanza PJ, Velasco LR, Vázquez CL, Román RR. Hypoglycemic effect of *Plantago major* seeds in healthy and alloxan-diabetic mice. Proc. West. Pharmacol. Soc. 2006. 48; 150-53.

12. Shohael AMD, Ali MB, Yu KW, Hahn EJ, Lee HL, Paek KY. Process Biochem. 2006; (41): 1179-85.

13. Wolfe K, Wu X, Liu RH. J. Antioxidant activity of apple peels. Agric. Food Chem. 2003; 51: 609-14.

14. Norma Oficial Mexicana NOM-062-ZOO-1999. Diario Oficial de la Federación, 6 de diciembre de 1999.

15. Hoffbrand V, Pettit JE. *Clinical Haematology*. 3rd edition. 2000. Mosby, London.

16. Suzuki N, Ohneda O, Takahashi S, et al. Erythroid-specific expression of the erythropoietin receptor rescued its null mutant mice from lethality. Blood. 2002; 100: 2279-88.

17. Lok S, Foster DC. The structure, biology, and potential therapeutic applications of recombinant thrombopoietin. Stem Cells. 1994; 12: 586-598.

18. Kaushansky K. and Drachman JG. The molecular and cellular biology of thrombopoietin: The gen primary regulator of platelet production. Oncogene. 2002; (21): 3359-67.

19. Lima GPB, Cabral AGS, Furtado FF, Barros LI,
Macedo RO. Urtica dioica: uma revisão dos estudos das suas propriedades farmacológicas. Rev. Bras. Farm. 2008; 89: (3) 199-06.

20. Wagner H, Willer F, Kreher B. Biologically active compounds from the aqueous extract of Urtica dioica. Planta Med. 1989; 55(5): 452-54.

21. Akbay P, Basaran A, Undeger U, Basaran N. In vitro immunomodulatory activity of flavonoid glycosides from Urtica dioica L. Phytother. Res. 2003; 17(1): 34-7.

22. Basaran AA, Yu TW, Plewa MJ, Anderson D. An investigation of some Turkish herbal medicines in Salmonella typhimurium and in the comet assay in human lymphocytes. Teratogen Carcinogen Mutagen. 1996; 16(2): 125-38.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.