ORIGINAL RESEARCH

# Effects of extract of *Urtica dioica* L. (stinging nettle) on the immune response of rats with severe malnutrition

Sara Beatriz Herrera[1,2], Leonor Rodriguez[2], María del Carmen García[3], José Luis Flores[2], Rodolfo Velasco[2]

[1]Posgrado en Biología Experimental, Departamento de Ciencias de la Salud, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, Ciudad de México, Mexico

[2]Departamento de Ciencias de la Salud, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, Ciudad de México, Mexico

[3]Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

## ABSTRACT

**Background:** Severe malnutrition is the main cause of immunodeficiency in children under 5 years in areas of economic depression in developing countries, and it is associated with generalized atrophy of lymphoid tissues. *Urtica dioica* L. is a medicinal herb with multiple pharmacological functions; it acts as modulator of the immune response, stimulating the proliferation of T lymphocytes *in vitro*.

**Objective:** To determine the ability of *U. dioica* L. to modulate the immune response of malnourished rats in the first 21 days of life.

**Methods:** Third-degree malnutrition during lactation was induced by food competition, and treatment with *U. dioica* L. was tested. Day-old Wistar rats were randomly assigned to three groups: (a) the well-nourished group, (b) the untreated malnourished group, and (c) the malnourished group treated with 0.2 g/ml *U. dioica* L. on days 14, 16, 18, and 20. On day 21, animals were euthanized, and the thymus, spleen, bone marrow, and blood were obtained. Lymphocyte subpopulations were determined in the thymus and leukocyte subpopulations in blood. The total number of nucleated bone marrow cells was determined, and the proliferative capability of spleen lymphocytes from malnourished rats treated with *U. dioica* L. was compared to that of other groups.

**Results:** *Urtica dioica* L. decreased CD4$^-$ CD8$^-$ lymphocytes and increased CD4$^+$ CD8$^+$ lymphocytes in the thymus, while in the blood, it increased CD4$^+$ cells, monocytes, and total T lymphocytes. The proliferation of spleen cells was stimulated in the malnourished rats, and a decrease in total nucleated bone marrow cells was observed. An increase in the red blood cell count, hemoglobin, and white blood cell count was observed in malnourished rats treated with *Urtica dioica* L. ($p \leq 0.05$).

**Conclusion:** *Urtica dioica* L. had immunomodulatory effects since it promoted the differentiation of CD4$^-$ CD8$^-$ T lymphocytes toward CD4$^+$ CD8$^+$ T lymphocytes and counteracted anemia and leucopenia associated with severe malnutrition.

## Introduction

Malnutrition is a public health problem that mainly affects developing countries as a consequence of poor economic and social conditions. Malnutrition results from an imbalance between food intake and nutritional requirements [1–3]. Severe malnutrition or protein energy malnutrition (PEM) is an extreme condition and is the main cause of immunodeficiency in children under 5 years [4]. In neonates and preschool children, PEM has been shown to impair the immune response and is associated with generalized atrophy of lymphoid tissues [5,6], causing a chain of immunological alterations characterized by leucopenia, a decrease in the ratio of T CD4$^+$/CD8$^+$ lymphocytes and an increase in T

Sara Beatriz Herrera, Leonor Rodriguez, María del Carmen García, José Luis Flores, Rodolfo Velasco

CD4$^-$/CD8$^-$ lymphocytes in circulating blood [7], anemia, and thrombocytopenia.

Different substances, such as flavonoids, poly-saccharides, lactones, and glycosides, are present in several plants and can affect humoral or cellular factors of the immune response [8,9]. Plants such as *Panax ginseng*, *Tinospora cordifolia*, and *Asparagus racemosus* have been shown to stimulate the immune system [10] and, therefore, have opened doors for the development of new plant-based immunostimulatory drugs. Stinging nettle, *Urtica dioica* L., has been proposed to have such immuno-stimulatory effects.

*Urtica dioica* L. (Urticaceae) is an herbaceous plant of economic importance due to its medicinal, food, nutritional complement, and textile proper-ties. This plant is widely distributed in America, Europe, and Asia [11,12]. *Urtica* grows in central and Southwestern Mexico, where it is known by many common names, including "Ortiga," "Chichicastle," and "Mala mujer" [13,14]. It is an annual or peren-nial plant that reaches up to 1 m in height and pres-ents stinging trichomes on the stem and leaves.

*Urtica dioica* L. has been recommended as an adjuvant treatment for rheumatic conditions, a nutri-tional tonic, and anti-anemic treatment, and has been suggested for the treatment of headache, eczema, skin care, edema, lower urinary tract infections, and allergies [15–17]. *Urtica dioica* L. has multiple phar-macological functions, including anti-inflammatory, analgesic, antioxidant, antiplatelet, antihyperglyce-mic, antihyperlipidemic, antiviral, anticancer, and antiandrogenic activities, among others [18].

*In vitro* studies have shown that the plant pos-sesses β-sitosterol and scopoletin, molecules that could be of clinical use in the management of benign prostatic hyperplasia because both inhibit 5α-reductase, which is associated with this condi-tion [19]. As mentioned previously, another import-ant activity of *U. dioica* L. is its role as a modulator of the immune response because it has been reported that *U. dioica* L. increases chemotaxis and the meta-bolic integrity of neutrophils from healthy humans *in vitro* [20]. Quercetin and two glycosidic flavo-noids from a methanolic extract of the aerial parts of this plant stimulated chemotaxis of neutrophils [21]. In addition, *U. dioica* L. has been shown to stimulate the proliferation of mouse spleen T lym-phocytes *in vitro* and result in a moderate increase in CD4$^+$ T lymphocytes [22]. An aqueous decoc-tion of the aerial structures of the plant stimulated the proliferation of mouse spleen lymphocytes *in vitro*, thereby confirming that the plant extract

can stimulate proliferation of cells of the immune system [23].

*Urtica dioica* L. has a wide range of compounds, including glycopyranosides, glycoproteins, proteins, flavonol glycosides, carotenoids, and biologically active compounds, such as caffeic acid, quinic acid, essential oils, formic and acetic acid, histamine, tan-nins, mucilage, and vitamins (A, B1, B2, C, K1, folic acid, and pantothenic acid), which can contribute to improved health in childhood malnutrition [24,25].

Considering that severe malnutrition causes immunodeficiency in children under 5 years of age, mainly in developing countries such as Mexico, which is rich in plants for medicinal use, among them *U. dioica* L.; the present study aimed to determine the effects of *U. dioica* L. on the proportion of T and B lymphocytes and the proliferative capacity of thymus, spleen, bone marrow, and blood cells using an exper-imental model of rats with third-degree malnutrition.

## Materials and Methods

### Chemicals and reagent tests

The following chemical and reagent tests were obtained: xylazine [Procin, México; Chemical Abstracts Service (CAS) 23076-35-9]; sodium chloride (NaCl; Meyer, México; CAS 7647-14-5); phosphate-buffered saline (PBS-DULBECCO; Microlab, Mexico; Mecanismo de Desarrollo Limpio MFCD00131855); monoclonal antibodies: anti-CD3, anti-CD4, anti-CD8, and anti-CD45 (Biolegend, San Diego, CA); fluorochromes [fluorescein isothiocy-anate (FITC), phycoerythrin (PE), peridinin chlo-rophyll protein (PerCP) or allophycocyanin (APC)] (BD Biosciences, San Jose, CA); Cell Counting Kit-8 (CCK-8, Dojindo Corp., Japan); trypan blue (CAS 72-57-1); Concanavalin A (ConA; CAS 11028-71-0); bovine serum albumin (Cohn Fraction V, pH 7.0; CAS 9048-46-8); and paraformaldehyde (CAS 30525-89-4, Sigma Chemicals Co., St. Louis, MO).

Stinging nettle (*U. dioica* L.) leaf extract (Enzymatic Therapy, LLC, part of Nature's Way Brands) was obtained. Stinging nettle (600 mg, serving size 2 capsules) was standardized to contain 1% silicic acid. This product contains natural ingredients. Entreprise de Taille Intermédiaire is a The Food and Drug Administration-registered drug establishment.

### Determination of the total polyphenol content of the extract of U. dioica L.

The total concentration of phenols was deter-mined by spectrophotometry using Folin–Ciocalteu reagent as the oxidant agent [26].

### Calibration curve

A standard solution of gallic acid (0.1 mg/ml) was used, from which volumes were taken from 0 to 100 µl in 20 µl intervals, and the volume of each solution was diluted up to 500 µl with distilled water. Determination of phenols in the powder of a capsule of *U. dioica* L. was performed. First, 5 mg of extract was dissolved in 1 ml of distilled water and diluted 1:10 with distilled water, and 40 µl of this solution was taken and then diluted to 500 µl with distilled water. Finally, to each of the standards and previously prepared plant sample, 250 µl of Folin–Ciocalteu 1 N reagent was added and agitated in a vortex for 5 minutes. Subsequently, 1,250 µl of 20% $Na_2CO_3$ was added and allowed to stand for 2 hours. The absorbance was measured at 760 nm. The results are expressed as a percentage of gallic acid equivalent per milligram dry matter (% GAE/mg dry matter).

### Experimental animals

Wistar rats were obtained from the animal facilities of the "División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Iztapalapa (UAM-I)" in Mexico City, Mexico. The animals were kept under controlled temperature (22°C–25°C) and 45% relative humidity with a 12–12 hour light–dark period (light 07:00–19:00 hour) with free access to food (Purina Mills International 5001, Richmond, VA) and water. Experiments were performed according to the guidelines of the UAM-I and the Official Mexican Guidelines (NOM-062-ZOO-1999). Nursing mother rats of approximately 350 g at 4 months of age were used.

### Induction of malnutrition and treatment U. dioica *L.*

Malnutrition during lactation was induced by food competition [27]. Rats (1 day of birth) from different litters were randomly assigned to one of two groups: (1) the well-nourished group (WN), eight pups with a nursing mother (control group), and (2) the malnourished group (MN), 16 pups with a nursing mother.

The pups were weighed every 2 or 3 days to estimate the body weight of each litter from the first day until weaning (day 21) to determine the degree of malnutrition compared to age-matched controls (WN). On day 14, rats with a weight deficit greater than 40% (rats with third degree or severe malnutrition) were used. Then, the MN group was distributed into two subgroups: (a) MN rats that received saline physiological solution and (b) MN rats treated with 0.2 g/ml *U. dioica* L. (MNUd), which was intragastrically administered through a cannula on days 14, 16, 18, and 20. On day 21, rats were anesthetized with xylazine, and blood was obtained by cardiac puncture with a heparinized syringe. Thymus, spleen and bone marrow were obtained. The weight (in grams) of the thymus and spleen of each rat was recorded and compared with the WN group. For the comparison, one-way analysis of variance (one-way ANOVA) was employed with the Tukey test as necessary to establish the statistical significance, for which 95% was taken as an index of reliability with a $p < 0.05$.

### Determination of lymphocyte subpopulations in thymus

The thymus was extracted, cells were obtained by homogenizing the organ and filtering through 85-mm mesh, and the disrupted cells were suspended in $Ca^{++}$ and $Mg^{++}$-free phosphate saline (PBS, pH 7.0). Cell viability was determined with 0.2% trypan blue and adjusted to $1 \times 10^7$ cells/ml, which were placed in 5 ml tubes (Falcon®). Cells were stained with 2 µl of monoclonal antibodies, anti-CD4 and anti-CD8 conjugated to APC and PE fluorochromes, for 30 minutes. Then, 2 ml of 0.5% PBS-albumin was added and centrifuged for 5 minutes at 1,500 rpm. Subsequently, 500 µl of fixative solution (1% paraformaldehyde) was added. Ten thousand cells were analyzed on a flow cytometer using the CELLQuest® program. The results are expressed as the percentage of positive cells for the corresponding marker. In each sample, CD4$^+$ CD8$^+$ (double positive, for both fluorescence markers), CD4$^-$ CD8$^-$ (double negative, for both fluorescence markers), CD4$^+$ CD8$^-$ (single positive, only for CD4 fluorescence), and CD8$^+$ CD4$^-$ (single positive, only for CD8 fluorescence) cells were identified.

### Determination of leukocyte subpopulations in blood

A total of 100 µl of blood from each rat was loaded into 5 ml tubes (Falcon®), and 2 µl of the following monoclonal antibodies conjugated to different fluorochromes were used: (1) CD3$^+$ FITC, CD8$^-$ PE, and CD4$^+$ APC for CD4 T lymphocytes, (2) CD3$^+$ FITC, CD8$^+$ PE, and CD4$^-$ APC for CD8 T lymphocytes, (3) CD3$^-$ FITC, CD8$^-$ PE, and CD4$^+$ APC for monocytes, (4) CD45-RA FITC and CD45$^+$ PerCP for B lymphocytes, and (5) CD3$^+$ FITC and CD45$^+$ PerCP for total T lymphocytes. After 30 minutes, 2 ml of lysis solution (FACS Lysing Solution, Becton Dickinson Immunocytometry Systems, San Jose,

CA) was added and incubated for 10 minutes at room temperature. Subsequently, each sample was centrifuged for 5 minutes at 500 g at room temperature, the supernatant was removed, and the cell button was washed with 0.5% PBS-albumin. Then, 500 μl of the paraformaldehyde fixative solution (1% in PBS) was added, and the cells were suspended prior to determination. At least 10,000 cells were analyzed by flow cytometry. The analysis was performed with CELLQuest®. The results were analyzed in dot graphs and are expressed as the percentage of positive cells for each marker. For each sample, four cell types were identified: CD4$^+$ CD8$^-$ cells (single positive, only for CD4 fluorescence), CD8$^+$ CD4$^-$ cells (single positive, only for CD8 fluorescence), monocytes, B lymphocytes and T lymphocytes. Granulocytes were not determined.

### Flow cytometry

Determination of lymphocyte subpopulations in the thymus and determination of leukocyte subpopulations in blood was performed with a FACSCalibur flow cytometer (Becton Dickinson, Immunocytometry System (BDIS), CA) with an argon laser (488 nm).

### Automated blood cell analysis

The total concentration of white blood cells (WBCs), red blood cells (RBCs), erythroid parameters, platelet count, and the lymphocyte/neutrophil relationship were determined using a KX-21N automatic analyzer (Sysmex, Japan).

### Total count of nucleated bone marrow cells

The right femur of each rat was dissected, their muscles were removed and 1 ml of physiological saline was injected through the femoral canal. The cells were collected in a plastic tube and homogenized by pipetting. The cell suspension was diluted 1:20 with Turk's solution, and total nucleated cells were counted in a Neubauer chamber under a light microscope.

### Determination of proliferation of spleen lymphocytes from malnourished rats treated with U. dioica L.

Under sterile conditions, the spleen of rats was isolated, dispersed mechanically on a metal 30 μm mesh, and washed with saline solutions. The cell suspension with 95% viability was added (trypan blue 0.2%) and adjusted to $2 \times 10^6$/ml in 96-well plates containing 100 μl aliquots in the presence of 10 mg/ml ConA and incubated at 37°C in an atmosphere of 5% $CO_2$ and humidity of 90% for 68 hours. As a control group, cells were cultured with RPMI-1640 medium without ConA. Each culture was performed in triplicate five times. Subsequently, 10 μl of CCK-8 (CCK-8, Dojindo Corp., Japan) was added to each well, and the plate was incubated for 4 hours at 37°C in a 5% $CO_2$ and 90% humidity atmosphere. The absorbance at 450 nm was read with an enzyme-linked immuno sorbent assay plate reader (BioTek, USA).

### Statistical analysis

The statistical program NCSS 2010 (Version: 07.1.20) was used. Values are expressed as the mean ± standard error (M ± SE). For the comparison between the means of the treated groups *versus* the control groups, we employed one-way ANOVA with Tukey's test and the Duncan test as necessary to establish statistical significance, using 95% as an index of reliability with a $p < 0.05$.

## Results

### Preliminary determination of total polyphenols in the extract of U. dioica L.

The concentration was calculated from a standard curve ($y = 0.0674X + 0.0082$; $R^2 = 0.9975$) made with gallic acid. The content of total polyphenols in *U. dioica* L. capsules was 2.43% GAE/mg dry matter.

### Body weight of well-nourished and experimentally malnourished rats left untreated and treated with U. dioica L. during the lactation period

Through the model of malnutrition, only 30%–40% of rats per litter reached the third degree of malnutrition; the cumulative data are shown in Table 1.

The average body weight of newborn rats (before the treatment) was 7.6 ± 0.1 g. At day 20, the average body weight of the WN group was 43.4 ± 0.6 g *versus* 26.1 ± 0.3 g in the MN group and 26.0 ± 0.7 g in the MNUd group. MN rats had a weight ten times lower than the WN rats, indicating that the MN and MNUd groups were still malnourished. In addition, no statistically significant difference was observed between the weights of the MNUd rats and the untreated MN rats (Table 1).

### Weights of the thymus and spleen

The average weight of the thymus in the WN rats was 0.145 ± 0.017 g, whereas the average weight

**Table 1.** Body weight observed during lactation in well-nourished rats and malnourished rats.

| Group | Day | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 4 | 7 | 9 | 12 | 14 | 16 | 18 | 20 |
| WN | 7.6 ± 0.1 | 11.0 ± 0.4 | 14.8 ± 0.5 | 20.1 ± 0.5 | 24.8 ± 0.5 | 29.6 ± 0.5 | 33.8 ± 0.5 | 38.1 ± 0.6 | 43.4 ± 0.6 |
| MN | 7.6 ± 0.1 | 9.2 ± 0.1 | 11.5 ± 0.1 | 14.8 ± 0.1 | 16.9 ± 0.2* | 20.3 ± 0.2* | 22.1 ± 0.2* | 24.2 ± 0.3* | 26.1 ± 0.3* |
| MNUd | 7.6 ± 0.1 | 9.2 ± 0.1 | 11.5 ± 0.1 | 14.8 ± 0.1 | 16.9 ± 0.2* | 19.6 ± 0.4* | 21.8 ± 0.4* | 23.9 ± 0.6* | 26.0 ± 0.7* |

WN = well-nourished rats ($n = 68$), MN = malnourished rats ($n = 161$), and MNUd ($n = 37$) = Malnourished rats treated with *Urtica dioica* L. Data are expressed as the mean ± SE. *$p < 0.05$, significant differences with respect to the WN group.

in the MN rats was 0.050 ± 0.007 g, and the average weight in the MNUd rats was 0.069 ± 0.008 g, indicating that no significant difference was observed among the malnourished groups. However, the WN group had a significantly higher value than the malnourished groups. In addition, no significant difference was observed in the total body weights of both malnourished groups.

The mean spleen weights for the WN, MN, and MNUd groups were 0.227 ± 0.017 g, 0.067 ± 0.007 g, and 0.090 ± 0.010 g, respectively. The spleen weight of the MN group was 70% less than the spleen weight of the WN rats, and the mean spleen weight of the MNUd rats was 60%, indicating that the spleen weight of this group gained 10% (Fig. 1).

### Thymocyte subpopulations

Rats from the MN group had a higher average percentage of CD4⁻ CD8⁻ thymocytes than rats from the other groups (3.04 and 1.8 times higher than the WN and MNUd group values, respectively), and the differences were statistically significant at $p < 0.05$. In contrast, the MN group presented a lower average percentage of CD4⁺ CD8⁺ than the other groups. No significant differences were observed between the WN and MNUd groups (Fig. 2).

### Distribution of circulating white blood cells

The mean percentages of CD4⁺ and CD8⁺ T lymphocytes and monocytes were lower in the MN group than in the WN group, except for B lymphocytes. The proportion of leukocytes in MNUd rats was similar to that in WN rats, except for T cells, which were decreased. There was a significant difference between the WN and MN groups; thus, malnutrition decreases the proportion of leukocytes. Although the MNUd group showed a higher proportion of CD4⁺ cells, CD8⁺ T lymphocytes, monocytes, and B cells than the MN group, only the CD4⁺ and monocyte counts showed statistically significant differences between the groups (Fig. 3).
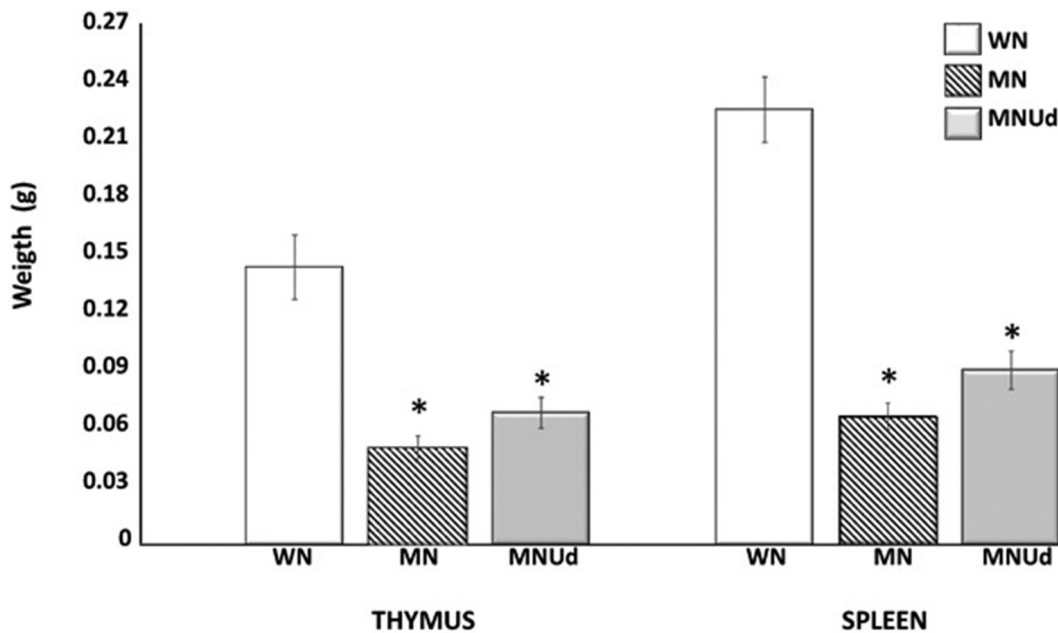


**Figure 1.** Weight of the thymus and spleen of malnourished rats. WN—Well-nourished rats ($n = 10$); MN—Malnourished rats ($n = 11$); and MNUd—Malnourished rats treated with *Urtica dioica* L. ($n = 15$). Data are expressed as the mean ± SE. *$p < 0.05$, significant differences with respect to the WN group.
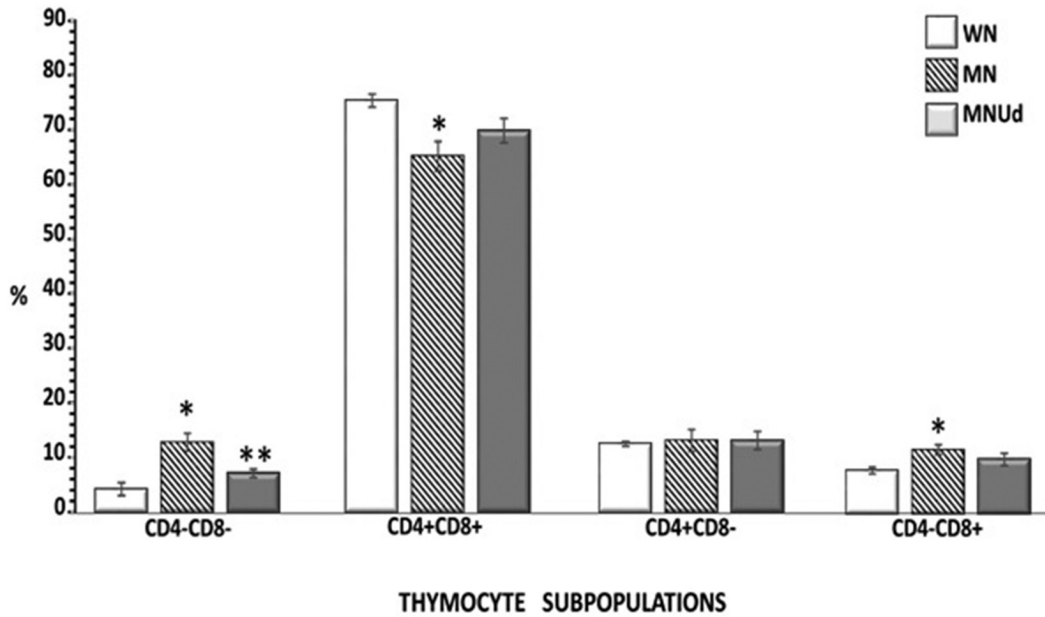
Sara Beatriz Herrera, Leonor Rodriguez, María del Carmen García, José Luis Flores, Rodolfo Velasco

**Figure 2.** Average percentage of thymocyte subpopulations of malnourished rats treated with *Urtica dioica* L. WN—Well-nourished rats ($n = 5$); MN—Malnourished rats ($n = 5$); and MNUd—Malnourished rats treated with *Urtica dioica* L. ($n = 5$). Data are expressed as the mean ± SE. *$p < 0.05$, significant differences with respect to the WN group. **$p < 0.05$, significant differences with respect to the MN group.
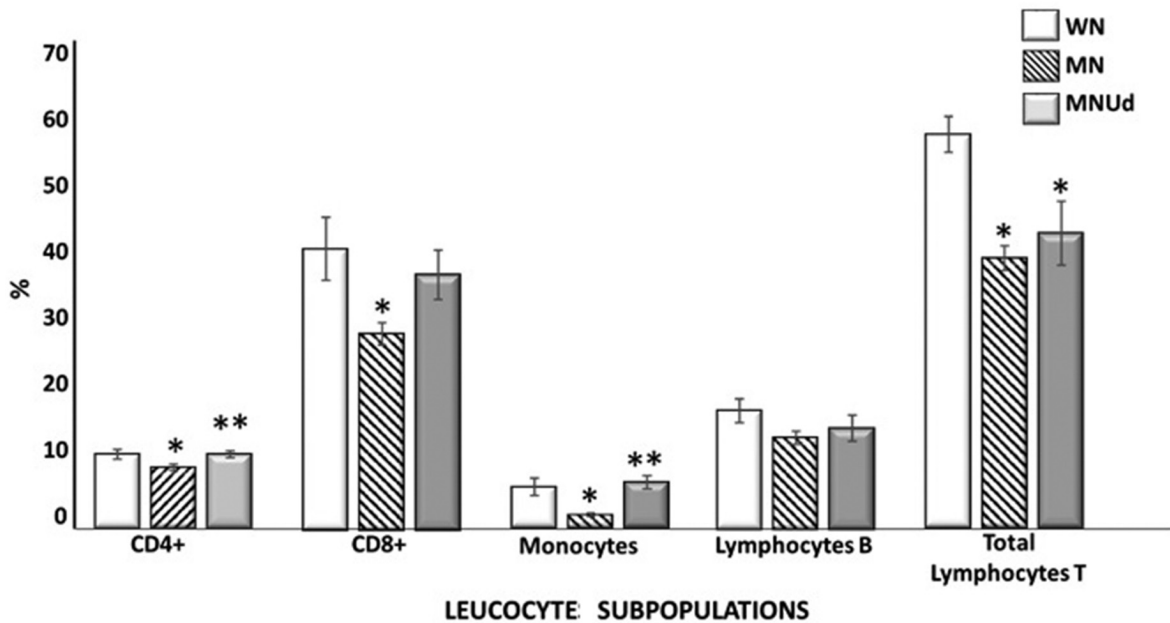


**Figure 3.** Average percentage of circulating WBCs in rats treated with *Urtica dioica* L. WN—Well-nourished rats ($n = 5$); MN—Malnourished rats ($n = 10$); and MNUd—Malnourished rats treated with *Urtica dioica* L. ($n = 10$). Data are expressed as the mean ± SE. *$p < 0.05$, significant differences with respect to the WN group. **$p < 0.05$, significant differences with respect to the MN group.

### *Hemocytometry*

The concentration of WBCs in the MN group decreased by 29% and 25% compared to that of the WN and MNUd groups, respectively. RBC concentrations and erythroid parameters, including hemoglobin, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), of the MNUd group tended to be higher than those of the MN group. However, no

**Table 2.** Hemocytometry of malnourished rats treated with *Urtica dioica* L.

| Group (*n*) | WBC × 10³/μl | RBC × 10⁶/μl | Hb (g/dl) | HCT (%) | MCV (fL) | MCH (pg) | MCHC (g/dl) | PLT × 10³/μL |
|---|---|---|---|---|---|---|---|---|
| WN (10) | 4.2 ± 0.6 | 5.3 ± 0.2 | 9.3 ± 0.4 | 31.3 ± 1.6 | 57.5 ± 1.6 | 17.0 ± 0.4 | 29.7 ± 0.7 | 408.8 ± 72.3 |
| MN (9) | 3.0 ± 0.2* | 4.1 ± 0.4* | 8.4 ± 0.6 | 27.0 ± 2.0 | 58.7 ± 2.0 | 19.0 ± 0.7 | 31.3 ± 0.4 | 341.0 ± 46.9 |
| MNUd (11) | 4.0 ± 0.4 | 4.7 ± 0.3 | 9.3 ± 0.6 | 29.3 ± 1.7 | 61.9 ± 0.9 | 20.2 ± 0.6* | 32.6 ± 0.7* | 290.4 ± 55.1 |

Hb = hemoglobin; PLT = platelet.
WN = well-nourished rats (*n* = 10), MN = malnourished rats (*n* = 9), and MNUd = malnourished rats treated with *Urtica dioica* L. (*n* = 11).
Data are expressed as the mean ± SE.
*$p < 0.05$, significant differences with respect to the WN group.

statistically significant differences between the WN and MNUd groups were observed, except for MCH and MCHC, which were higher in the MNUd group ($p \leq 0.5$) (Table 2).

The ratio of lymphocytes/neutrophils in the MN and MNUd groups showed a reduction of lymphocytes with respect to that of the WN group. However, this reduction was not statistically significant. Neutrophils increased in the MN and MNUd groups compared with the WN group (Fig. 4).

### Nucleated bone marrow cells

No statistically significant differences were observed among the WN, MN, and MNUd groups (Fig. 5).

### Spleen cell cultures

In the WN group exposed to ConA, there was a 19.1% increase in the absorbance, indicating increased cell proliferation with respect to the same culture without ConA. For the MN group, in the presence of ConA, the increase in absorbance was 9.2%. The MNUd group plus ConA showed an increase of 41.1% compared with the same group without ConA (Fig. 6).

### Discussion

In previous studies, researchers looking for *U. dioica* L. in the field collected plants with similar morphology that turned out to be *U. chamaedryoides* and *U. dioica* var. Angustifolia, which showed an ability to restore hematopoiesis in pregnant mice, preventing the development of malformations in fetuses [28,29]. In later collections, the specimens were identified as *U. urens*. In addition, aqueous extract of the leaf of *U. dioica* L. (capsules) commercialized as a nutritional supplement was purchased, and a comparative study was conducted between an aqueous extract of *U. urens* and the capsules of *U. dioica* L. to assess the ability of both to stimulate the
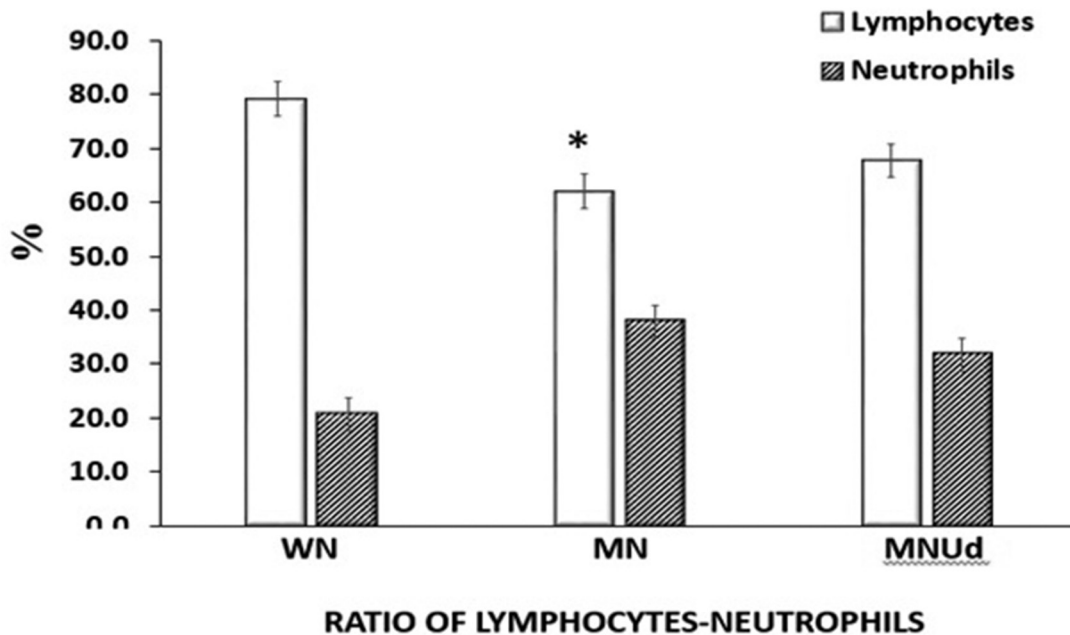


**Figure 4.** Lymphocyte-neutrophil count ratio of malnourished rats. WN—Well-nourished rats (*n* = 10); MN—Malnourished rats (*n* = 9); and MNUd—Malnourished rats treated with *Urtica dioica* L. (*n* = 11). Data are expressed as the mean ± SE. *$p < 0.5$, significant differences with respect to the WN group.

Sara Beatriz Herrera, Leonor Rodriguez, María del Carmen García, José Luis Flores, Rodolfo Velasco
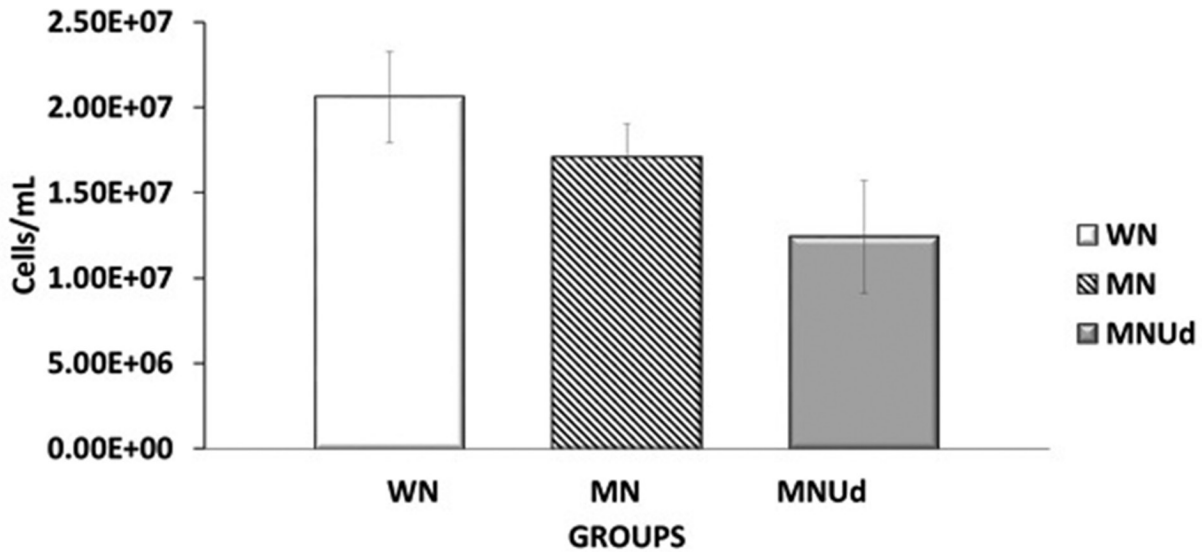
**Figure 5.** Total nucleated cells of bone marrow from malnourished rats. WN—Well-nourished rats ($n$ = 10); MN—Malnourished rats ($n$ = 10); and MNUd—Malnourished rats treated with *Urtica dioica* L. ($n$ = 10). Data are expressed as the mean ± SE. *$p$ < 0.5, significant differences with respect to the WN group.
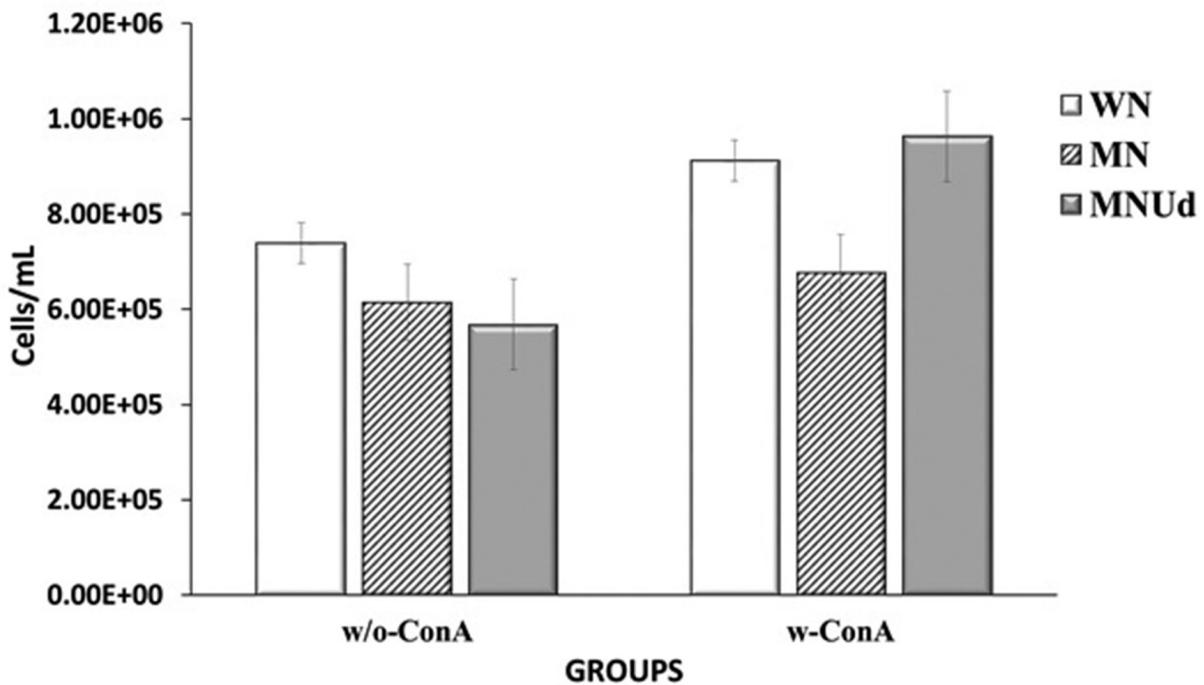


**Figure 6.** Proliferation of spleen cells from malnourished rats. The cultures were made in the absence (w/o-ConA) and presence of Concanavalin A (w-ConA). WN—Well-nourished rats ($n$ = 15); MN—Malnourished rats ($n$ = 8); and MNUd—Malnourished rats treated with *Urtica dioica* L. ($n$ = 10). Data are expressed as the mean ± SE. *$p$ < 0.05, significant differences with respect to the WN group.

proliferation of rat spleen lymphocytes *in vitro*. The highest and constant activity was obtained with the *U. dioica* L. capsules, and for this reason, after establishing a dose response curve (results not included), the present work was performed with *U. dioica* L. capsules.

The concentration of phenolic compounds in capsules of stinging nettle (*U. dioica* L.) leaf extract was 2.43% of dry matter by weight; this is an important finding with respect to the different biological properties of these metabolites and their possible application in various areas, such as food/feed,

cosmetics, phytomedicine, and textiles. However, it is important to note that phenolic compounds were determined only as reference due to their normal presence in the plant.

Our results showed that severe malnutrition was induced in Wistar rats by food competition, and the MN and MNUd groups presented a 40% body weight deficit, in addition to other signs, such as poor hair, brittle bones, and poor motor activity similar to those observed in malnourished children [30]. At the end of the experiment, the MN and MNUd groups presented reduced thymus and spleen weights and severe atrophy of the thymus [31], indicating that the *U. dioica* L. promoted cell proliferation, although it was not sufficient to increase the levels to the WN group levels.

Severe protein malnutrition causes leucopenia, a decrease in the proportion of $CD4^+$ and $CD8^+$ T lymphocytes, and an increase in the number of $CD4^-$ $CD8^-$ cells, plus immature T cells in the circulation [32,33], as we observed in the MN group. In the MNUd group, there was a restoration of hematopoietic cell levels, indicating that our extract may promote cell differentiation and maturation to almost normal levels. The data showed that treatment with *U. dioica* L. significantly increased the percentage of $CD4^+$ cells, monocytes, and T lymphocytes, resulting in cell counts in the MNUd group that did not significantly differ from those of the WN group. These results are in agreement with those reported by other authors regarding the capacity of *U. dioica* agglutinin (UDA) to stimulate the proliferation of T lymphocytes, although it does so in a delayed manner with respect to ConA [34]. The UDA behaves like a superantigen that activates the Vβ segments of the T lymphocyte receptor (TCR) by binding to molecules of the major histocompatibility complex type I and II [35]. Notably, the aforementioned agglutinin has been isolated from the rhizome of the plant.

Our study was carried out with a crude extract of the leaf that does not allow us to assume that this is the same mechanism of stimulation as that for the proliferation of T cells [36].

In blood cytometry analyses, the concentration of WBCs in the MN group was lower by 29% and 25% compared with that of the WN and MNUd groups, respectively. However, there were no significant differences between the WN and MNUd groups. Differences in circulating blood parameters between the MN and MNUd groups can be attributed to the plant extract because there were 7 days from the first administration of *U. dioica* L. to the sacrifice of the animals, a period that matches the term of 5–7 days average for the production of blood cells. That is, treatment with the plant extract in the MNUd group promoted the recovery of hematopoiesis, while in the untreated MN group, this recovery was not observed even with adequate time [37]. The total nucleated bone marrow cells were lower in the MNUd group than in the MN group, probably because the plant promotes the rapid release of cells from the bone marrow to the blood.

In Mexican traditional medicine, *U. dioica* L. is used to treat anemia due to its high iron content, which may contribute to the increase in erythrocytes and specifically to the mean corpuscular hemoglobin concentration. A comparable finding was observed when a decoction of *U. dioica* L. var. angustifolia prevented malformations in the fetuses of anemic mice, although the mother remained anemic [29]. Nettle plants are known to be very good sources of energy, proteins, fiber, and a range of bioactive compounds that have health benefits [38]. In Nepal, *U. dioica* L. is both a staple food and supplementary food, which is often the top cash crop of local communities, contributing to food security in the Nepal region. In the case of Mexico, although it is a developing country rich in flora and fauna, severe PEM has a high incidence in suburban and rural areas [39]. The effects of *U. dioica* L. on rats with immunodeficiency due to severe malnutrition observed in this work can be attributed to the plant acting as a phytomedicine reinforced by its role as a nutraceutical.

The immunostimulatory role of *U. dioica* L. has been previously reported and identified as a property of the root. In our work, the activity was detected in the leaf. Notably, although it is already known that *U. dioica* L. stimulates the proliferation of T lymphocytes in healthy individuals, our results reinforce this knowledge but in a pathological condition, malnutrition, which is frequent in developing countries, where malnutrition is observed in a high percentage of the child population.

## Conclusions

The aqueous extract of the leaves of *U. dioica* L. stimulates the immune response because it promotes the differentiation of T lymphocytes toward $CD4^+$ and $CD8^+$ cells, in addition to countering anemia and leucopenia associated with severe malnutrition. The effect of the nettle plant extract on lymphocyte activation and its production of cytokines is still being investigated.

Sara Beatriz Herrera, Leonor Rodriguez, María del Carmen García, José Luis Flores, Rodolfo Velasco

Considering the pharmacological functions of *Urtica dioica* L. and its role as an anti-inflammatory, analgesic, antiandrogenic, antihyperglycemic, anti-hyperlipidemic, antiviral, anticancer, and antianemic plant and that the treatment of diseases with patented medicines is becoming increasingly expensive, we note that it is important to highlight the action of *U. dioica* L. to counteract severe malnutrition, especially when a high percentage of the world's population lives in poverty and extreme poverty.

This plant could be used as a nutritional supplement or a nutraceutical even in those populations without sufficient resources, as their diet lacks the nutrients provided by *U. dioica* L.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Acknowledgments

## References

[1] UNICEF. La desnutrición infantil. Causas, consecuencias y estrategias para su prevención y tratamiento. Wendy Wisbaum. UNICEF, España, 2011.

[2] Black R, Victora C, Walker S, Bhutta Z, Christian P, De Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 2013; 382:427–51.

[3] Bourke C, Berkley J, Prendergast A. Immune dysfunction as a cause and consequence of malnutrition. Trends Immunol 2016; 37(6):386–98.

[4] Chandra RK. Nutrition and the immune system from birth to old age. Eur J Clin Nutr 2002; 56 Suppl 3:S73–6.

[5] Borchers AT, Hackman RM, Keen CL, Stern JS, Gershwin ME. Complementary medicine: a review of immunomodulatory effects of Chinese herbal medicines. Am J Clin Nutr 1997; 66:1303–12.

[6] Savino W. The thymus gland is a target in malnutrition. Eur J Clin Nutr 200; 56 Suppl 3:S46–9.

[7] Schaible U, Kaufmann S. Malnutrition and infection: complex mechanisms and global impacts. PLoS Med 2007; 4(5):e115.

[8] Tan B, Vanitha J. Immunomodulatory and antimicrobial effects of some traditional chinese medicinal herbs: a review. Current Med Chem 2004; 11:1423–30.

[9] Chin Y, Balunas M, Chai H, Kinghorn A. Drug discovery from natural sources. AAPS J 20068; (2):239–53.

[10] Bafna AR, Mishra SH. Immunomodulatory activity of methanol extract of roots of *Cissampelos pareira* Linn. Ars Pharm 2005; 46:25362.

[11] Kavalali GM. An introduction to *Urtica* (botanical aspects). In: Kavalali (ed.). *Urtica*. Therapeutic and nutritional aspects of Stinging Nettle. Medicinal and aromatic plants–Industrial Profiles. Taylor and Francis Co., London, UK, pp 1–11, 2003.

[12] Rafajlovska V, Kavrakovski Z, Simonovska J, Srbinoska M. Determination of protein and mineral contents in Stinging Nettle. QOL 2013; 4(1–2):26–30.

[13] Espinosa FJ, Sarukhán J. Manual de malezas del Valle de México. Claves, descripciones e ilustraciones. Universidad Nacional Autónoma de México, Fondo de Cultura Económica, México, 1997.

[14] Villaseñor RJ, Espinosa G. Catálogo de malezas de México. Universidad Nacional Autónoma de México, Consejo Nacional Consultivo Fitosanitario, Fondo de Cultura Económica, México, 1998.

[15] Akgül G, Yılmaz N, Celep A, Celep F, Çakılcıoğlu U. Ethnobotanical purposes of plants sold by herbalists and folk bazaars in the center of Cappadocica (Nevşehir, Turkey). Indian J Tradit Knowle 2016; 15(1):103–8.

[16] Polat R, Çakılcıoğlu U, Ulusan MS, Paksoy MY. Survey of wild food plants for human consumption in Elazığ (Turkey). Indian J Tradit Knowle 2015; 1(1):69–75.

[17] Mükemre M, Behçet L, Çakılcıoğlu U. Survey of wild food plants for human consumption in villages of Çatak (Van-Turkey). Indian J Tradit Knowle 2016; 15(2):181–91.

[18] Mamta S, Preeti k. *Urtica dioica* (Stinging nettle): a review of its chemical, pharmacological, toxicological and ethnomedical properties. Int J Pharm 2014; (4):270–7.

[19] Nahata A, Dixit VK. Ameliorative effects of Stinging Nettle (*Urtica dioica*) on testosterone induced prostatic hyperplasia in rats. Andrologia 2011; 44 Suppl 1:396–409.

[20] Basaran AA, Ceritoglu I, Undeger U, Basaran N. Immunododulatory activities of some Turkish medicinal plants. Phytother Res 1997; 11:609–11.

[21] Akbay P, Basaran AA, Undeger U, Basaran N. *In vitro* immunomodulatory activity of flavonoid glycosides from *Urtica dioica* L. Phytother Res 2003; 17:34–7.

[22] Harput US, Saracoglu I, Ogihara Y. Stimulation of lymphocyte proliferation and inhibition of nitric oxide production by aqueous *Urtica dioica* extract. Phytother Res 2005; 19(4):346–8.

[23] Tapia AR, Ramírez CA, Gónzalez GA, Contreras A, Velasco LR. Hematopoyetic activity of *Urtica dioica in vitro*. Memories of the 54 Annual meeting of the Western Pharmacology Society, Mexico City, Mexico, p 162, 2011.

[24] Güil GJL, Rebolloso FM, Isasa ME. Fatty acids and carotenoids from Stinging Nettle (*Urtica dioica* L.). J J Food Comp Anal 2003; 16(2):111–9.

[25] Degirmenci NS, Akal ZU, Alpsoy L. Cytotoxic and apoptotic effects of caffeic acid and caffeoyl malic acid on brain glioblastoma (U87-MG). Bull Pharm Res 2016; 6(1):7–13.

[26] García J, De la Rosa L, Herrera B, González A, López J, González G, et al. Cuantificación de polifenoles y capacidad antioxidante en duraznos comercializados en ciudad Juárez, México. Tecnociencia Chihuahua 2011; 5(2):67–75.

[27] Ortiz R, Cortés E, Pérez L, González C, Betancourt M. Assessment of an experimental method to induce malnutrition by food competition during lactation. Med Sci Res 1996; 24:843–6.

[28] Velasco LR, Fregoso PM, Flores SJL, Carrillo SJ, Herrera SSB, Vega AE, et al. Ability of *Urtica chamaedroydes* pursh to restore hematopoiesis in anemic pregnant mice. J Intercult Ethnopharmacol 2013; 2(1):37–42.

[29] Herrera SS, Flores SJ, Rodríguez CL, Aguilar C, Velasco LR. Administración de *Urtica dioica* var. Angustifolia Wedd como preventivo de malformaciones en fetos de ratones anémicas preñadas. Avances de las Mujeres en las Ciencias las Humanidades y todas las Disciplinas. Libro Científico Vol. II de Ciencias Biológicas y de la Salud y Ciencias Sociales y Humanidades, Mexico City, Mexico, 2015.

[30] Ortiz R, Cortés L, Gómez J, González C, Cortés E. Análisis con citometría de flujo de los diferentes tipos de timocitos en ratas desnutridas experimentalmente durante la lactancia. Ciencia en la frontera: revista de ciencia y tecnología de la UACJ. Biodiversidad 2000; 1(2):77–84.

[31] de la Rosa G. Análisis del número de células y peso de diferentes órganos de ratas desnutridas experimentalmente durante la lactancia. Tesis de pregrado, Universidad Autónoma Metropolitana-Iztapalapa, México, 2002.

[32] Schaible U, Kaufmann S. Malnutrition and infection: complex mechanisms and global impacts. PLoS Med 2007; 4(5):e115.

[33] Rytter M, Kolte L, Briend A, Friis H, Christensen V. The immune system in children with malnutrition—a systematic review. PLoS One 2014; 9(8). e105017; doi:10.1371/journal.pone.0105017

[34] Le Moal MA, Colle JH, Galelli A, Truffa-Bachi P. Mouse T-lymphocyte activation by *Urtica dioica* agglutinin. II.—Original pattern of cell activation and cytokine production induced by UDA. Res Immunol 1992; 143(7):701–9.

[35] Rovira P, Buckle M, Abastado JP, Peumans WJ, Truffa-Bachi P. Major histocompatibility class I molecules present *Urtica dioica* agglutinin, a superantigen of vegetal origin, to T lymphocytes. Eur J Immunol 1999; 29(5):1571–80.

[36] Ganeshpurkar A, Saluja AK. The pharmacological potential of rutin. Saudi Pharm J 2017; 25(2):149–64.

[37] Greer JP, Foerrster J, Rodgers G, Paraskevas F, Glader B, Arber DA, et al. Wintrobe's clinical hematology. 12th edition, Lippincott, Williams and Wilkins, Baltimore, MD, 2009.

[38] Adhikari BM, Bajracharya A, Shrestha AK. Comparison of nutritional properties of Stinging nettle (*Urtica dioica*) flour with wheat and barley flours. Food Sci Nutr 2015; 4:119–24.[39]. Monárrez J, Martínez H. Prevalence of malnutrition in Tarahumara children under 5 years of age in the municipality of Guachochi, Chihuahua. Salud Pública Mex 2000; 42:8–16.