Research article
Curr. Pers. MAPs, (2023); 6(2): 84-89

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

ENVIRONMENTAL POLLUTION DEGREE CHANGES THE BIOLOGICAL ACTIVITY OF NETTLE PLANT EXTRACTS IN CHICKENS

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https://doi.org/10.38093/cupmap.1337889
Received: 09/08/2023
Accepted: 06/09/2023

Abstract

The positive effects of plants, including stinging nettle (*Urtica dioica L.*) were reported by numerous researchers (1) as well as changes in the chemical composition of the plant due to heavy metal pollution (2, 3). This study aimed to compare the changes in body wight, total leukocyte numbers (TL) and delayed type hypersensitivity (DTH) in 28 days old chickens (n=12/group) injected subcutaneously twice (days 0 and 7), with 0.5 ml of alcoholic nettle extracts harvested from both unpolluted (group III) and polluted areas (group IV), against untreated (group I) and 70^0 alcohol treated (group II) controls. Blood was sampled three times, on days 0, 7 and 14, while the wattle test to a homologous lymphocyte suspension was performed on day 14 of the experiment.

There were no significant differences in weight gain/period between groups III (0.979 kg) and IV (0.967kg), against each other and controls (0.991 kg-group II and 1.029 kg - group I, respectively). As opposed to the slight changes of the TL in control groups, there was a constant increase in group III (from 15,400±3,421/mm^3 to 17,125±4,231/mm^3) and a decrease in group IV (17,611.1±2,401/mm^3 to 17,166.7±2,522/mm^3).

The wattle test results after 48 h were similar in groups I, II and IV, the differences ranging between 0.23 and 0.28 mm, while the unpolluted nettle extract diminished the DTH to a difference of 0.07 mm.

The polluted nettle extract induced the lowest weight gain and TL numbers, and increased the wattle reactivity when compared to the unpolluted one.

Keywords: *Urtica dioica L.,* chickens, weight, leukocytes, adaptive immunity, wattle test

1. Introduction

Urtica dioica (stinging nettle) is a plant well known for the medicinal effects of all its parts: roots, leaves and *herba*. Researches indicated that *U. dioica* L. could be considered as a natural alternative source for various fields of human activity (Yildirim et al., 2013). Characterization of the biological properties (antioxidant and antiradical) of phenolic compounds in nettle extracts is important for their possible applications in industrial areas, such as food/feed, cosmetics and also phytomedicine (Pinelli et al., 2008; Borsuk et al., 2011). Some studies propose that the therapeutic benefit of *U. dioica* extract could be partly attributed to its potential inhibition

of oxidative processes (Husein et al., 2014). Numerous therapeutic trials indicated that the use of various extracts from the plant provided beneficial effects in benign prostatic hyperplasia (Chrubasik, 2007), urinary tract problems, arthritis, neuralgia, cardiovascular disease, mild menorrhagia, gout, allergic rhinitis and others (Samakar et al., 2022).

In vitro assays indicate that *U. dioica* extract represented a significant source of natural alternatives to antimicrobial therapy, thus avoiding excessive antibiotic use (Simule, 2011). The potential employment as antimicrobial agent of this extract in pharmaceutical and food industry was also supported (Modarresi-Chahardehi et al., 2012). Thus, *Urtica dioica* leaves gave the best inhibitory activity against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (Stanciuc et al., 2011). Crude extracts obtained by Soxhtlet extractor with ethyl acetate and hexane exhibited stronger antimicrobial effects against the Gram-positive bacteria than the Gram-negative bacteria (Modarresi-Chahardehi et al., 2012).

Immunological effects of *U. dioica* extracts have also been well documented. Dietary incorporation of *U. dioica* at 5% in *L. victorianus* (the ningu, a ray-finned fish in the family *Cyprinidae*) led to significantly higher relative percentage (95%) survival against *Aeromonas hydrophila* (Ngugi et al., 2015). Similarly, *Urtica dioica* agglutinin (UDA), which is a lectin found in stinging nettle, was capable of activating T-lymphocyte in a way not observed with any other known plant lectin (Galelli and Truffa-Bachi, 1993; Musette et al., 1996; Krystofova et al., 2010).

UDA appeared to prevent formation of a systemic lupus erythematosus-like condition in mice and has *in vitro* antiviral activity (Balzarini et al., 1992; Musette et al., 1996). UDA also antagonizes the epidermal growth factor receptor, a beneficial effect which could interfere with the pathogenesis of BPH (Wagnet et al., 1995). The stimulatory effect of *U. dioica* alcoholic extract on the phagocytic function in young, equine infectious anemia virus recently infected horses, was followed by an inhibitory effect which could be explained by the possible rapid carbon particle ingestion and an increase in cell membrane fragility (Bolfã et al., 2011; Borsuk et al., 2011).

The changes in the stinging nettle chemical composition due to heavy metal pollution were reported by numerous researchers (Notten et al., 2005; Krystofova et al., 2010). Based on studies regarding the extraction efficiency of metals in aqueous preparations, the elements in herb infusions were classified into highly-extractable (>55%)(K), moderately-extractable (20-55%)(Mg, Na, P, B, Zn and Cu) and poorly-extractable (<20%)(Al, Fe, Mn, Ba, Ca and Sr) (Pytlakowska et al., 2012).

Models were developed to estimate de heavy metal concentration in plants, *Urtica dioica* included, based on soil quality. The most important soil property that influenced *U. dioica* metal concentrations was the clay content, while pH affected Cu and Zn concentrations (Notten et al., 2005; Boshoff et al., 2014). Comparing in terms of heavy metal accumulation *T. officinale, U. dioica*, *R. pseudoacacia* and *M. recutita*, it was concluded that the first were better metal accumulators, while *M. recutita* was a metal avoider (Gjorgieva et al., 2011).

Other studies indicated that medicinal plants often represented subjected to heavy metal contamination exceeding permissible levels for some species and thus, collecting medicinal plants from contaminated area should be discouraged/banned [22]. Heavy metal ions in contaminated soils may easily enter the human food chain through crop plants, depending on geochemical characteristics of the soil and the ability of plants to selectively

accumulate some of these elements [23]. Prior to daily consumption of significant amounts of edible/medicinal plants, better compositional analyses should be performed (Mithril and Dragsted, 2012). This study aimed to investigate the biological activity of alcoholic extracts of nettle plants collected from diffrent areas known to be polluted (heavy autoroute traffic) and unpolluted (mountainy area) on chickens' growth rate and immunity.

2. Material and Methods

2.1. Plant material

Two alcoholic extracts of stinging nettle (*Urtica dioica* L.) were obtained by percolation, the plant material representing 9.5 g of the drug in each 100mL of tincture, by the Faculty of Pharmacy in Cluj-Napoca, according to the provisions of the General Pharmacopoeia. The stinging nettle plants were harvested from *a*) an unpolluted mountain region (SE Carpathian arch, Sibiu region) and *b*) from the edges of the city of Cluj-Napoca, considered to be an area polluted at a medium level, where exceedings of the maximum permissible concentration (MPC) of copper and lead, established by Order no. 756/1997 for the approval of the Requirements for environmental pollution assessment of the Romanian Government were recorded (Sarma et al., 2011).

2.2. Biological material

The experiment was carried out on 28 days old chickens, divided in four groups (n=12/group), injected subcutaneously twice (days 0 and 7), with 0.5 ml of alcoholic nettle extracts harvested from either unpolluted (group III) and polluted areas (group IV), against untreated, environmental and handling control (group I) and 70^0 alcohol treated, solvent control (group II). Blood was sampled three times, on days 0, 7 and 14, when the birds were weighed and leukocytes were counted (Türk method) (Davis et al., 2008), while the wattle test to a homologous (chicken) lymphocyte suspension was performed on day 14 of the experiment.

2.3. Lymphocyte suspension

Blood was sampled from conventionally farmed hens on heparin (50IU/ml, 5 ml)to prevent clotting, then centrifuged in density gradient on Ficoll® Paque Plus at 1500 rpm (Sigma Aldrich, 4 ml) and the buffy coat was separated. The cells were then washed in RPMI 1640, supplemented with 5% FCS and antibiotics (penicillin 1000IU/mL and streptomycine 1000μg/mL) by centrifugation at 1500 rpm (EBA 270, Hettich, Germany) for 10 min, three times and diluted to a final concentration of 4 x 10^6/ml in the supplemented RPMI 1640.

2.4. Delayed type hypersensitivity test

Wattle measurements using callipers were performed in each group, then the birds were injected with 0.1 ml of a chicken lymphocyte suspension in one of the wattles and RPMI 1640 in the other one.Both wattles were measured in each bird 48 h later.

Average values and standard deviation were calculated for all parameters, while the statistical significance of the differences between the polluted and unpolluted stinging nettle extracts were estimated by Student-t test.

3. Results and Discussion

Urtica dioica is a wide-spread herbaceous perrenial flowering plant of Order *Rosales*, Family *Urticaceae*, Genus *Urtica*. Numerous analyses of nettle plnt have revealed the presence of more than fifty different chemical constituents, showing anti-oxidative, anti-microbial, anti-ulcer and anesthetic capacity. The main immunologically active components were present in the roots of the plant, but the leaves also contained substantial amounts of poliphenols, known for their immunoactive effect (Samakar et al., 2022).

In the present study, no significant differences in weight gain/period between groups III (0.979 kg) and IV (0.967kg), against each other and controls (0.991 kg-group II and 1.029 kg - group I, respectively) were found (Fig 1).

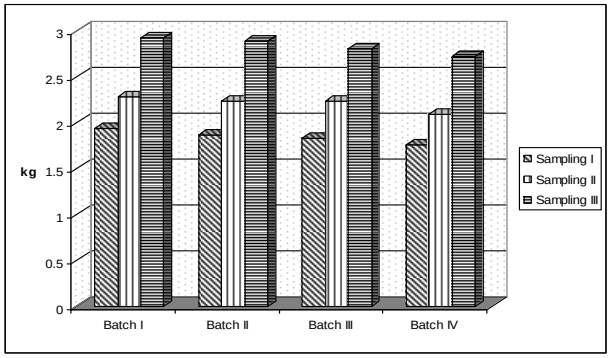

Figure 1: Dynamics of the body weight of chickens in the experimental groups (x ± s)(kg)

Although the birds in group IV had the least increase in weights, the treatment with the extract of stinging nettle collected from the polluted area did not significantly decrease the weight gain per the experimental period. As opposed to the slight changes of the TL in control groups (Fig 2), the parameter constantly increased in group III (from 15,400 ± 3,421/mm^3 to 17,125± 4,231/mm^3) and decreased in group IV (17,611.1 ± 2,401/mm3 to 17,166.7 ± 2,522/mm3) at 7 days.

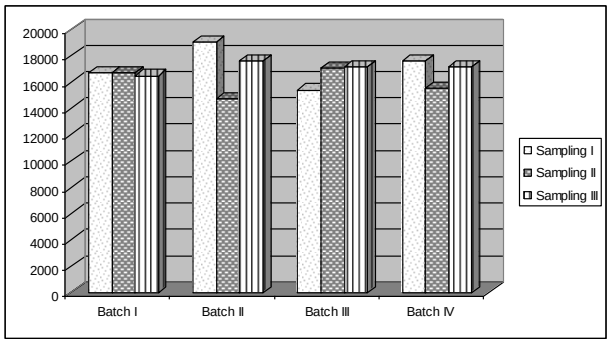

Figure 2: Total leukocyte numbers' development during the experimental period (x ± s)

The only statistically significant ($p < 0.05$) decrease was observed in the TL of group II, on day 7. Thus, the medium pollution level with metals on the edges of Cluj did not statistically significantly influence the body weight or the numbers of leukocytes.

Subsequently, the consumption of nettle plant from either unpolluted or polluted areas would not change the weights of chickens raised in either of the locations and would presumably preserve, with minor changes the immune response to microbial aggressors.

The wattle test results (Fig 3) after 48 h were similar in groups I, II and IV, the differences ranging between 0.23 and 0.28 mm, while the unpolluted nettle extract diminished the DTH to a difference of 0.07 mm.

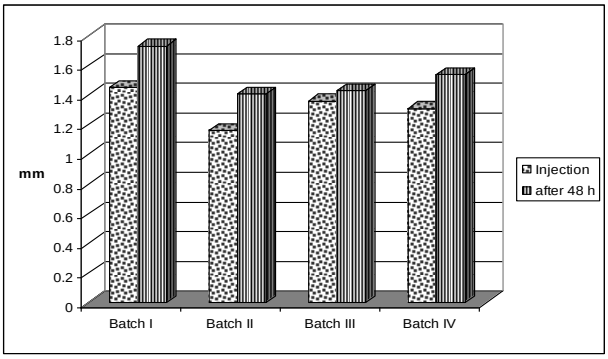

Figure 3: Wattle test responses subsequent to *in vivo* unpolluted and polluted nettle plant extract treatments in chickens (x ± s) (mm)

Pollution influenced to some extent the quality of the alcoholic nettle extracts and therefore their biological effects, , the functionality of leukocytes being changed by the pollution degree. Thus, the leukocytes from chickens treated with the nettle extract collected from the polluted area were more prone to respond to the agrression, exceding a normal response, while the non-polluted nettle extract preseved the DTH within a physiological range. Therefore such evaluations could be usefull for detecting an excessive aggression level and the response to it when the chickens are exposed to different levels of metal pollution. Further studies could elaborate a correlation between the heavy metal type and concentration and DTH, providing an evaluation grid.

4. Conclusion

In spite of the fact that, based on literature, *U. dioica* seems to be a heavy metal accumulator, the results of this experiment did not indicate an influence of the officially reported medium pollution level in the soil of Cluj-Napoca surroundings or the lack of pollution in the mountains, at the site of the nettle plant collection, sufficient to induce statistically significant changes in the food conversion rate, numbers of leukocytes or their adaptive function in the graft rejection test. Further studies could explicit, if present, a dose-effect correlation.

Acknowledgements

The authors wish to thank for laboratory assistance to biotechnologist eng. Elena Marian and for technical support to senior technician Traian Jucan.

Author Contribution

All authors declare equal contribution to the study design and experimental work, interpretation of the resultsand editing the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest during the accomplishment of this research. None of the authors has any financial and/or personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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