Original Article

Prolonged *in vivo* Stinging Nettle Treatment Impacts on Functional Capacity of Leukocytes in Immunologically Mature Chickens

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ABSTRACT

Objective: The experiment aimed at establishing the effects of an alcoholic *Urtica dioica* extract on the *in vitro* blastogenic response in antigen stimulated, immunologically mature chickens. **Materials and Methods:** Three equal groups (n = 17) of 47 days old, Rock x Cornish chickens were subjected to oral administration of: a) 0.5 ml/chicken/day of an alcoholic stinging nettle extract (I), b) 0.5 ml/chicken/day alcohol (II, solvent control), and c) 0.5 ml chicken/day water (III-environment control) for seven days. All birds were injected with 0.5 ml/bird of a 5% SRBC suspension (days 0 and 7). Leukocyte numbers (Burker Turk method) and their blastogenic capacity (blast transformation test) were monitored on days 0, 7 and 14. The specific response was evaluated against a SRBC lysate. Student's t-test was used to evaluate the statistical significance of the differences. **Results and Discussion:** The leukocyte numbers increased to from 18,083.33 ±4,879.81/mm^3 to 42,833.33±7,547.99/mm^3 by day 14. *In vitro* responses to *C. officinalis* and *E. angustifolia* decreased while SRBC response increased in the nettle treated group. **Conclusion:** The results did not validate the implemented protocol for the alcoholic stinging nettle treatment. Other administration routes, schemes or dosages should be tested to improve the functional capacity of the leukocytes.

Key words: Chicken, Immunologically mature, Leukocytes, Blast transformation, Nettle extract.

INTRODUCTION

Medicinal plants possess various biological activities and have been used for medicinal purposes.[1,2] Phytochemical and biological investigation have demonstrate their antimicrobial, antifungal, antitumor, antioxidant**,** antimicrobial and immunomodulatory activity.[3,4] Immunomodulation using medicinal plants may provide novel immunomodulatory agents to supplement the present conventional therapy for a variety of diseases.[1,5] Stinging nettle (*Urtica dioica*, *Urticaceae* family) is a wild-growing, widespread perennial plant with marked immunomodulatory, antimicrobial, antitumoral, anti-inflammatory, antioxidant, antiviral, activities.[6,7] The chemical composition of stinging nettle shows the presence of vitamins (A, B, B_{12}) acetylcholine, histamine, serotonine, minerals, chlorophyll, salicylic acid, lecithin, carotenoids, flavonoids, sterols and formic acid.[8,9] Based on the complex biological activity and chemical composition the present experiment aimed at establishing the effects of an alcoholic *Urtica dioica* extract on the *in vitro* blastogenic response in antigen stimulated, immunologically mature chickens.

MATERIAL AND METHODS

The crossbred Rock x Cornish chickens (47 days old) were randomly allocated to three treatment groups (n=17). All the groups were

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subjected to relatively constant environmental conditions (temperature, relative humidity, ventilation) and similar management practice. A commercially available alcoholic nettle plant extract (Plantextract, Romania) was used. The birds were subjected to oral administration of: a) 0.5 ml/chicken/day of an alcoholic stinging nettle extract(I),b) 0.5ml/chicken/dayalcohol(II,solventcontrol), and c) 0.5 ml chicken/day water (III-environment control) for seven days. All birds were injected with 0.5 ml/bird of a 5% SRBC suspension (days 0 and 7) and blood sample were collected.

The *total number of leukocytes* was evaluated using the classical Bürker-Türk method.

In vitro cell-mediated reactivity was assessed by blast transformation test[10] on days 0, 7 and 14.

The *in vitro* response was measured against phytohemagglutinin-M (PHA-M, Sigma-Aldrich) and commercial alcoholic extracts for human use of *Calendula officinalis* and *Echinaceea purpureea* (Plantextract, Romania) produced according to the German Pharmacopeia. One ml of each blood sample was diluted with four times the amount of RPMI 1640 (Sigma-Aldrich, USA) supplemented with 5% FCS and penicillin+streptomycine, at pH 7.4 and placed in 96-sterile-well plate (200 µl per well). The *in vitro* experimental variants were namely (1) untreated control culture, (2) phytohaemagglutinin-M (PHA)(1µ per well), (3) 70° alcohol and (4–5) alcoholic vegetal extracts of *Calendula officinalis*, and *Echinacea purpurea*. The specific response was evaluated against a SRBC lysate. Subsequent to an incubation at 37°C in a 5% CO_2 atmosphere for 48 h, glucose consumption was evaluated by an ortotoluidine colorimetric method with a spectophotometrical reading at 610 nm wavelength (SUMAL PE2, Karl Zeiss, Jena, Germany), using the reagent as a blank.[11,12] The stimulation/inhibition index (S/I) was calculated as: S/I %=[(IG–GR)/IG]´100, where S/I = blast transformation index, where IG= the initial glucose concentration in the culture medium and GR=glucose residue in the sample after incubation.[13]

Statistical analyses

Student's t-test was used to evaluate the statistical signi ficance of the differences. A value of $p < 0.05$ was considered statistically significant. All data were expressed as the mean ± SD.

RESULTS AND DISCUSSION

Antibiotics were used as dietary supplements for a long period of time in chickens. The spread of antibiotic resistance at high levels led to a ban on further use of antibiotics as growth promoters, therefore alternative solutions were subject to numerous researches. Plants provide a handy and highly bioavailable source for dietary supplements in farmed animals improving the weight gain and health due to their antibacterial and immune stimulating effects.[2,14] There is evidence to suggest that herbs, spices and various plant extracts have appetizing and digestion-stimulating properties and antimicrobial effects which stimulate the growth of beneficial bacteria and minimize pathogenic bacterial activity in the gastrointestinal tract of chickens. Immunomodulation using different medicinal plants can offer an alternative to conventional treatment for a variety of infections.[1]

There are many studies on the effect of *U. dioica*. The results of Franciškovi ć *et al.* (2017)[8] using root and herbal extract of *U. dioica* shows that root extracts can decrease the thromboxane production in human platelets, while the herb extracts of *U. dioica* have inhibitory effect toward 12-LOX (lipoxygenase-type enzyme) pathway, and increase the chemokine release from intestinal epithelial cells.

We have previously reported that subcutaneously injected *U. dioica* alcoholic extract in chicken, showed significant immune stimulating response.[14] In the present study we examined the possible stimulating potential of *U. dioica* alcoholic extract after oral administration in crossbred Rock x Cornish chickens. As it is shown in Figure 1 the total numbers of leukocytes significantly ($p<0.001$) increased during the experimental period in all groups. Although non-significant when compared to the other groups, the increase was the highest in the nettle extract treated group. The nettle extract treatment *in vivo* did not improve the *in vitro* response of the group

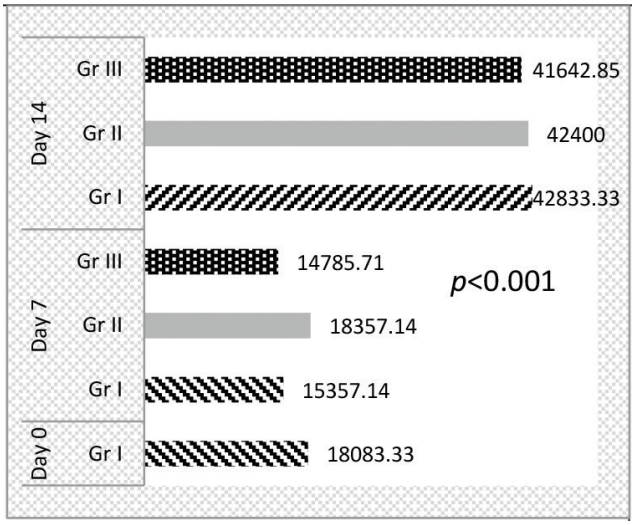
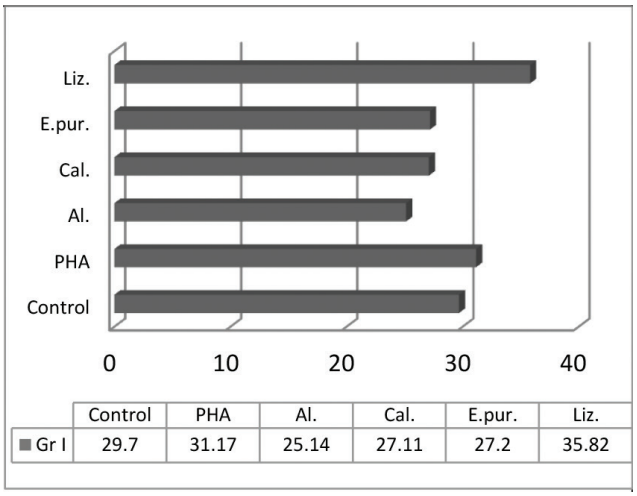
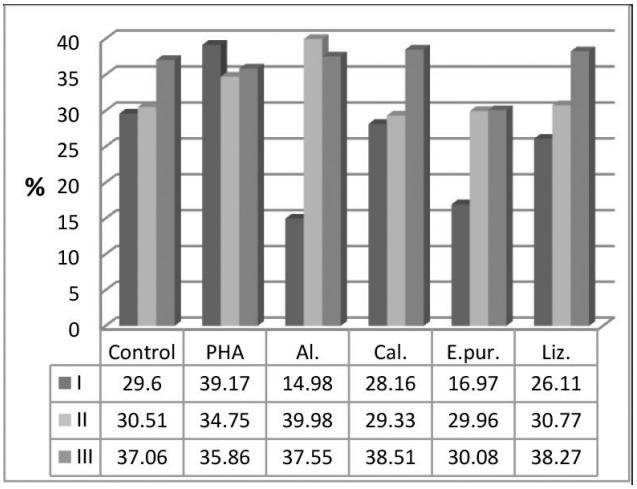


Figure 1: Changes in total leukocyte numbers during the experiment.



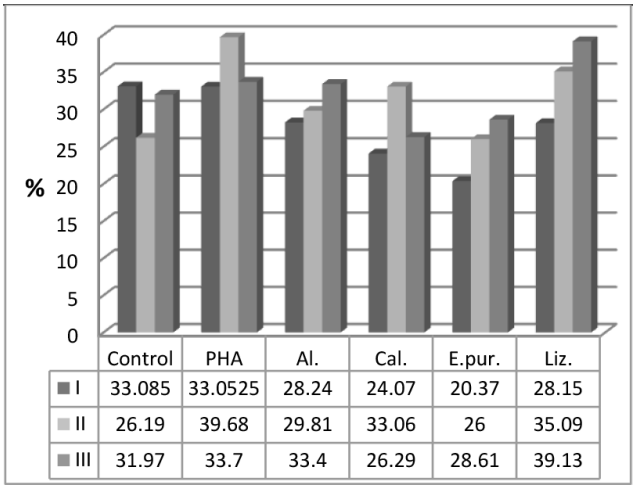
	Control	PHA	Al.	Cal.	E.pur.	Liz.
Gr I	29.7	31.17	25.14	27.11	27.2	35.82

Figure 2: SI% values prior to the treatment-day 0.



	Control	PHA	Al.	Cal.	E.pur.	Liz.
I	29.6	39.17	14.98	28.16	16.97	26.11
II	30.51	34.75	39.98	29.33	29.96	30.77
III	37.06	35.86	37.55	38.51	30.08	38.27

Figure 3: Values of SI% for the experimental groups after the nettle treatment after 7 days.



	Control	PHA	Al.	Cal.	E.pur.	Liz.
I	33.085	33.0525	28.24	24.07	20.37	28.15
II	26.19	39.68	29.81	33.06	26	35.09
III	31.97	33.7	33.4	26.29	28.61	39.13

Figure 4: Values of SI% for the experimental groups at the end of the experiment – day 14.

to the *Calendula* and *Echinacea* alcoholic extracts (Figure 2-3). The effect of nettle administration towards the *in vitro Calendula* response was short-lasting, the decrease being obvious by day 14 (Figure 4). There was an increase in the response of group III control towards the antigen *in vitro*, but this response was not supported statistically.

CONCLUSION

The results did not validate the implemented protocol for the alcoholic stinging nettle treatment in significantly stimulating the *in vitro* to antigen. Other administration routes, schemes or dosages should be tested, to also improve the functional capacity of the leukocytes not only their numbers.

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CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

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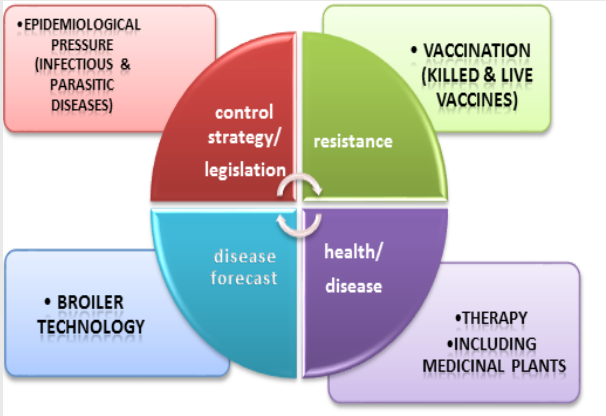
PICTORIAL ABSTRACT



SUMMARY

- The research aimed at establishing the effects of an alcoholic *Urtica dioica* extract on the *in vitro* blastogenic response in antigen (SRBC) stimulated, immunologically mature chickens
- Total leukocyte numbers were counted (Bürker-Türk method) and their *in vitro* blastogenic activity was evaluated by a glucose consumption test.
- The *in vitro* response to the SRBC antigen and other plant extracts was not augmented by the *in vivo* stinging nettle treatment.
- Other administration routes, schemes or dosages should be tested, to also improve the functional capacity of the leukocytes not only their numbers.

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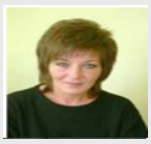

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