Original Article

Differential Influence of Seabuckthorn on the *In vivo* and *In vitro* Cell-Mediated Immune Responses in Chickens with Gumboro Disease

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ABSTRACT

Objective/Purpose: The study intended to clarify the restoring effects of an alcoholic seabuckthorn extract on the cell-mediated immunity in chickens with Gumboro disease. **Material and Methods:** Graft rejection was performed *in vivo* on healthy, Gumboro virus infected and convalescent chickens, by injecting 0.1 ml of a sheep lymphocyte suspension ($5x10^6$ cells/ml) mixed 1:1 with either saline or an alcoholic seabuckthorn extract. Wattle thickness was measured before and 24, 48 and 72 hours post injection and rejection indices were calculated. An *in vitro* blast transformation test was carried out on heparinized blood samples from the three groups. Blast transformation indices were calculated after 48 h at 37°C, for PHA M and seabuckthorn treated cultures, by glucose concentration measurements. The statistical significance of the differences was interpreted by Student's t-test. **Results & Discussion:** The wattle thickness decreased from 24 onwards in all categories, while seabuckthorn increased the wattle response the most in healthy (40 ± 0.43mm) and the least (19.03 ± 0.11) in diseased chickens ($p<0.01$-0.001), which also showed the strongest inhibitory activity during blast transformation (-1.5 ±2.7%). **Conclusion:** The *in vivo* cell-mediated immune response seemed more fit than the *in vitro* test to evaluate the immunological role of alcoholic seabuckthorn extracts.

Keywords: Chicken, Gumboro Disease, Immunity, Hippophae.

INTRODUCTION

The Sea buckthorn (*Hippophae rhamnoides L.*) is a deciduous shrub from the Order: *Rosales*, Family: *Elaeagnaceae*, Genus: *Hippophae L.*, well known worldwide for its nutritious and theraputical qualities. The berries contains more than 190 bio-active components of a broad diversity, including sugars, sugar alcohols, fatty acids, vitamins (C, E, and K), phenolic compounds, carotenoids, fibre, amino acids, minerals, plant sterols.[16] The majority (90%, 1.5 mil ha) of its natural habitat is found in China, Mongolia, Russia, Northern Europe and Canada.[9] Total berries contains a high level of carotenoids (9.4-34.5 mg/100g) and vitamin C (360-2500 mg/100g).[3] Similarly, the biological effects are multiple, such as antioxidant, antiedematous, *antiinflamatory, antibacterian,* analgesic, anti-arteriosclerosis, cardioprotector, anticancer, antiaging, immune enhancer, gastrointestinal treatment, radiation effect relief, astringent, tonic.[1-4-11-13-14]

The Gumboro disease (infectious bursitis) is a viral, highly pathogenic and economically impacting disease of poultry. The main consequences of the disease development are caused by the immune suppression subsequent to viral activity, since it could be self-healing, rather than the morbidity and mortality.[17]

The study intended to clarify the effects of an alcoholic seabuckthorn extract on the *in vivo* and *in vitro* cell-mediated immunity in

DOI: 10.5530/ijper.51.3s.38

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chickens in different developmental stages of Gumboro disease and monitor its immune restoring potential.

MATERIALS AND METHODS

The study was carried out on three groups of 18 to 24 days old chickens: (I) healthy (n = 16), (II) naturally infected with clinical signs (n = 18) and (III) convalescent (n = 15). Blood samples were collected on heparin (50 IU/ml) from each group, by the wing vein puncture for the *in vitro* test. The alcoholic sea-buckthorn extract (Plantextract, Romania) used was a commercially available one, prepared according to the provisions of the German Pharmacopoeia.

The *graft rejection* test was performed *in vivo* by injecting 0.1 ml of a sheep lymphocyte suspension ($5x10^6$ cells/ml) mixed 1:1 with either saline or the alcoholic sea-buckthorn extract. Wattle thickness was measured before and 24, 48 and 72 hours post injection and rejection indices were calculated as a percentage of the measurements.

Leukocyte blast transformation test [12]. An *in vitro* blast transformation test was carried out on heparinized blood using the sea-buckthorn extract in whole blood cultures. For that, 0.25 ml of each blood sample was diluted with four times the amount of RPMI 1640 supplemented with 5% FCS and antibiotics, at pH 7.4 (Sigma-Aldrich, USA). The mixture was distributed in 96-sterile-well plate (200 µl per well) in duplicate. Three *in vitro* experimental variants were tested for each individual bird, namely (1) untreated control culture, (2) phytohaemagglutinin-M (PHA)(1µ per well), (3) sea-buckthron treated culture (5 µl/well). The cultures were incubated for 48 h at 37.5°C in a 5% CO_2 atmosphere, glucose residue was quantified by the orto-toluidine test. The stimulation/inhibition indices (S/I) were calculated. The statistical significance of the differences between the groups was interpreted by Student's t-test.

RESULTS & DISCUSSION

The Gumboro disease virus infects the bursa of Fabricius, one of the major primary immune organs in chickens, populated by B cells, thus impeding on its development and hindering their functioning during the immune response. Not only the B cells, but also the phagocytes, antigen presenting cells and T lymphocytes suffer, thus both the innate and the adaptive immunity are severely suppressed.[17] The anatomical and functional drawbacks at the immune system level open the floor for development of bacteria, most of them epiphytic, which leads to aggravation of the disease, further increasing the morbidity, mortality and condemnation of carcases.[17]

Due to increased antibiotic resistance present in farmed chicken at present leading to inefficacy of traditional therapies, vegetal extracts were intensively studied for their antioxidant, antibacterial and antiviral effects.[2-5,6,7] Furthermore, immune enhancing activities were tested in healthy and diseased patients.[8-10-15] The wattle test (Figure 1, 2, 3) indicated that the sea-buckthorn extract significantly increased the local response against xenogenic lymphocytes in infected and in convalescent chickens ($p<0.05$, 4.5 x and 4.7 x, respectively). Nevertheless, it did not improve the *in vitro* response in any of the groups (Table 1), possibly due to a dose dependant effect, which was not tested in this experiment.

CONCLUSION

Sea buckthorn extract induces an emphasized reaction at the injection site but also an increased flexibility of

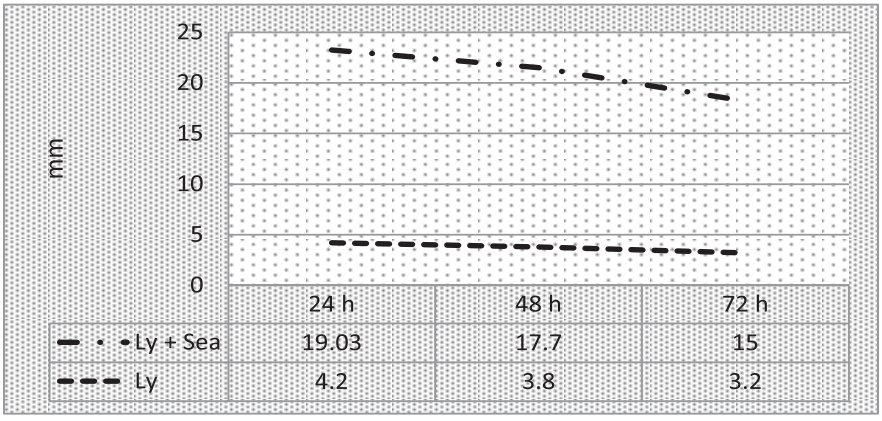

Figure 1: Wattle graft rejection indices in infected chickens

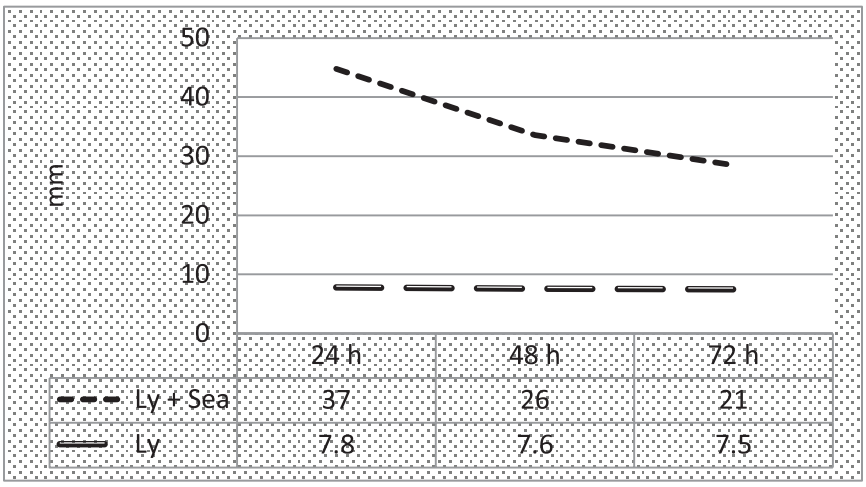

Figure 2: Wattle graft rejection indices in convalescent chickens

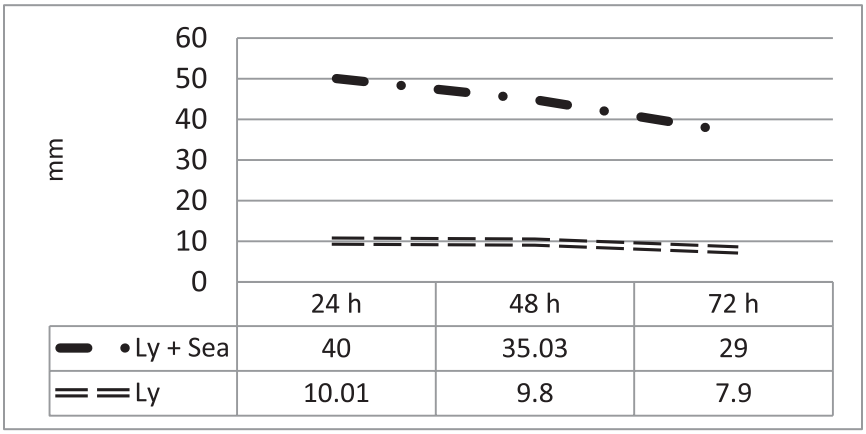

Figure 3: Wattle graft rejection indices in healthy chickens

Table 1: Comparative values of blast transformation indices in Gumboro infected, convalescent and healthy chicken groups (%)

Group	Variant	x	s	s^2
Healthy chickens (I)	Control	0.46	0.16	0.02
	PHA	-0.12	0.14	0.02
	Seabuckthorn	-0.5	0.3	0.07
Diseased chickens (II)	Control	0.6	0.25	0.06
	PHA	-0.46	0.87	0.76
	Seabuckthorn	-1.5	2.7	7.3
Convalesc. Chickens (III)	Control	0.43	0.165	0.0275
	PHA	0.16	0.09	0.009
	Seabuckthorn	-0.33	0.4	0.15

the cell-mediated immune response**.** The *in vivo* cell-mediated immune response response, probably targeting *in situ* a broader cell-population, seemed more fit than the *in vitro* test to evaluate the immunological role of a seabuckthorn extract. Dose-dependent protocols should be defined in further studies.

ACKNOWLEDGEMENT

This research was supported by grant PNII PNCD 61/2012 of MEN Romania.

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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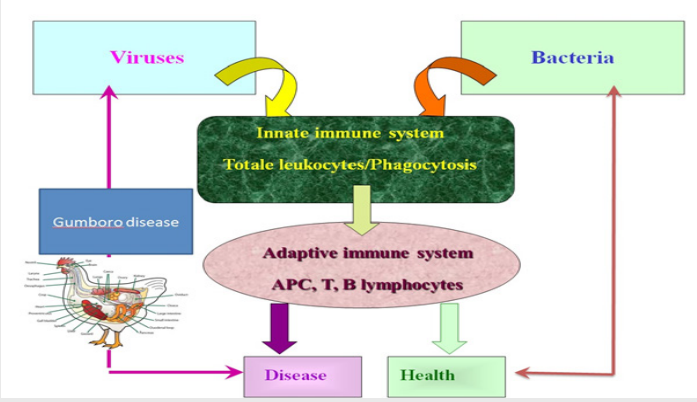
PICTORIAL ABSTRACT



SUMMARY

- This study investigated the *in vivo* and *in vitro* effects of an alcoholic sea-buckthorn extract on the cell-mediated immunity of chickens 18 to 24 days old chickens with and without Gumboro
- The leukocyte blast transformation and graft rejection tests were performed to estimate the *in vitro* and *in vivo* adaptive cell mediated responses, respectively.
- The sea-buckthorn extract significantly increased the local response against xenogeneic lymphocytes in infected and in convalescent chickens ($p < 0.05$, 4.5 times, and 4.7 times, respectively) but did not improve the *in vitro* response in any of the groups.
- The *in vivo* cell-mediated immune response, probably targeting in situ a broader cell-population, seemed more fit than the *in vitro* test to evaluate the immunological role of a sea-buckthorn extract.

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Cite this article: Sandru CD, Niculae M, Pall E, Brudasca F, Vasiu A, Cerbu C, Giupana R, Spinu M. Differential Influence of Seabuckthorn on the *In vivo* and *In vitro* Cell-Mediated Immune Responses in Chickens with Gumboro Disease. Indian J of Pharmaceutical Education and Research. 2017;51(3)Suppl:S318-22.