Industrial Crops and Products 88 (2016) 44–47

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Vegetal extracts influence *in vitro* on the cell-mediated immunity in carnivores depending on health status, target species and plant taxonomy

Marina Spînu*, Mihaela Niculae, Anamaria Ioana Paştiu, Carmen Dana Şandru, Emoke Pall, A. Vasiu

Department of Infectious Diseases, University of Agricultural Sciences and Veterinary Medicine, 3–5 Mănăştur Street, Cluj-Napoca 400372, Romania

ARTICLE INFO

Article history:
Received 30 June 2015
Received in revised form 24 February 2016
Accepted 27 February 2016
Available online 7 March 2016

Keywords:
Plant extracts
Leukocytes
Adaptive immunity
Foxes
Dogs
Mink

ABSTRACT

Impaired immune response in individuals subjected to various stress factors leads to disease or low protective responses following vaccination, therefore modern immunology is seeking for alternative immune stimulating compounds for which plants could represent a source. The aim of this study was to establish the influence of several ethanolic vegetal extracts on *in vitro* adaptive cell-mediated reactivity in wild (mink, n = 70, silver foxes, n = 22) and domestic (dogs, n = 32) carnivores and compare the responsiveness of these species under physiological (antigen-primed) and pathophysiological (aleutian disase of mink) conditions. Mink and foxes were primed with a 5% SRBC suspension (0.5 and 1 ml respectively), twice, 14 days apart and blood was sampled on days 0 and 14, while untreated dogs were sampled only once. Ethanolic extracts of *Calendula officinalis*, *Symphytum officinale*, *Arnica montana*, *Echinaceea angustifolia* and *Echinacea purpurea* were tested for immune activity in whole blood cultures by the use of the *in vitro* blast transformation test, where cell growth was monitored spectrophotometrically, by the glucose consumption test and stimulation/inhibition indices (S/I) were calculated.

E. angustifolia acted stimulating in Aleutian disease positive mink (S/I SRBC primed 76.75 ± 12.52%, non-primed 19.58 ± 18.07, $p < 0.001$) and foxes (S/I primed 20.28 ± 21.27%, non-primed 20.13 ± 13.10), while *S. officinale* was most stimulating (3.85 ± 1.37%) in dogs in a statistically supported manner ($p < 0.05$).

In conclusion, the results indicated the need of individualized immune stimulating therapies, based on species and health status in the studied species, with the prior identification of the most appropriate plant alcoholic extract for each species.

1. Introduction

Healing effects of plants were known for centuries, and are still widely applied, since, unlike conventional drugs, which often mask the problem by aleviating the symptoms, natural remedies eliminate the cause. More recent researches indicated that the plant-based therapy is scientifically founded (Badescu et al., 2015; Valcheva-Kuzmanova and Belcheva, 2006; Pádua et al., 2014). Purified components of plants were proven to show numerous biological effects, among which immunological, antimicrobial and antiinflamatory and also anticancer activities. Furthermore, the side effects so often encountered in classical therapies are missing or very mild for plant extracts used for treatment (Dewick, 1997). Nowadays, physicians, veterinarians and biologists working in different fields show an increased interest for the use of medicinal plants and their extracts as therapeutic means, immunity enhancers and adjuvants for vaccines. Therefore, plant therapy, considered to be known for centuries, is far from being a closed subject.

The combined use of vegetal extractive preparations and classical drugs could increase the innate protective capacity of the individuals through the complex immune-stimulating effects shown by the active components in the plant.

Extracts from various plants were used in experiments aiming to increase the natural resistance to infections or treat infected individuals. Some of those studies proved the efficacy of vegetal extracts in partial restoration of the immune response in

* Corresponding author at: University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Department of Infectious Diseases, 3–5 Mănăştur Street, Cluj-Napoca 400372, Romania.
E-mail addresses: marina.spinu@gmail.com, marinaspinu@yahoo.com (M. Spînu).

http://dx.doi.org/10.1016/j.indcrop.2016.02.060
0926-6690/

immunosuppressed patients (Bauer, 2002; Bezanger-Beauquesne, 1993; Candinas et al., 1996).

Adjuvants are compounds which, added to vaccines, increase the immunogenicity of the antigen, by augmenting the humoral immune response toward it (Mohanna and Nys, 1999). Some of the adjuvants also stimulate the cell-mediated immunity. One of the advantages aimed by including an adjuvant in the vaccine formula was the diminishment of the amount of antigen. Selection of the adjuvant for a certain type of vaccine must be based on knowledge on the nature of antigen, doses and administration routes as well as eventual side effects (Stewart-Tull, 1985, 2003; Laval et al., 1998).

The literature studies concerning immune stimulating effects of vegetal extracts in wild and domestic carnivores are scarce. At our best knowledge, there are no experiments carried out in Silver foxes or mink aiming to prove the effects of medicinal plants on their immune system. Nevertheless, there are several publications concerning immunological and antimicrobial effects of plants in dogs.

It was demonstrated that arabinogalactan treatments increased white blood cell counts, neutrophil and eosinophil numbers but did not affect ($p > 0.05$) serum IgG, IgM or IgA concentrations in dogs (Grieshop et al., 2002). Some experiments using the sap of *Acer okamotoanum*, an Asian plant, suggest that it stimulated neutrophil activity by increasing the *in vivo* and *in vitro* oxidative burst in canine, showing a potential antimicrobial effect in the polymorphonuclear cells (PMNs) of dogs with infections (An et al., 2013). Another traditional Chinese/Japanese medicinal herbal drink Kakkon-to not only increased the body temperature but also enhanced the phagocytic activity of macrophages, suggesting a contribution to the suppression of multiplication of common influenza viruses, which resulted in the improvement of various symptoms during infection (Muraoka et al., 2004).

The aim of this study was to establish the influence of several ethanolic vegetal extracts on *in vitro* cell-mediated reactivity in wild and domestic carnivores and compare the responsiveness of these species under physiological and disease conditions.

2. Materials and methods

2.1. Experimental design

Three experimental designs were applied, including wild carnivores–mink (Order *Carnivora*, Family *Mustelidae*) and foxes (Order Carnivora, Family Canidae) and domesticated carnivores of the same family with the foxes (Order Carnivora, Family Canidae).

In the first experiment, the biological material was represented by comercially farmed mink ($n = 70$), which were diagnosed positive for Aleutian disease by countercurrent immune electrophoresis (CIEF) and circulating immune complexes quantification. The animals were divided in two groups of which group I ($n = 43$) was antigenically primed by use of a 5% sheep red blood cell (SRBC) suspension, injected sucutaneously in a dose of 0.5 ml/animal, twice at two weeks interval. Group II consisted of $n = 27$ untreated, non-primed animals.

Blood was sampled from these animals before and two weeks after the second priming.

The second experiment included healthy young silver foxes ($n = 22$, <12 month), which were similarly distributed into two groups, 16 animals being antigen primed (group I), while the rest of six did not receive antigen (group II). The antigen injected subcutaneously was a thymus dependent antigen, a 5% SRBC suspension, administered following the same protocole as in mink (0.5 ml/animal s.c., twice at two weeks interval). Blood was colected in the same manner as from the foxes.

The third experiment included healthy dogs ($n = 32$) of which 16 were primed by vaccination with a commercial polivalent canine vaccine containing distemper, hepatitis, parvovirus, influenza antigens (group I) while 16 stayed unvaccinated (group II). The thymus dependent antigen was administered according to the manufacturer's protocole, at 7 and 9 weeks of age. Blood was sampled from these animals according to the same scheme used in mink and foxes.

2.2. Leukocyte blast transformation test (Khokhlova et al., 2004)

The leukocyte blast transformation test measures the *in vitro* reactivity of mononuclear cells to sensitizing (*in vivo* encountered) antigens. This test is also useful for evaluating the effects of certain stimulating or inhibiting compounds, such as antibiotics, toxins, or mitogens on the cell-mediated immunity.

Commercial alcoholic extracts for human use of marigold (*Calendula officinalis* Linn., Fam. Asteraceae), leopard's bane (*Arnica montana* L., Fam. Asteraceae), comfrey (*Symphytum officinale* L., Fam. Boraginaceae), purple coneflower (*Echinaceea purpureea* (L.) Moench, Fam. Asteraceae), narrow-leaved purple coneflower (*Echinaceea angustifolia* (DC.) Hell., Fam. Asteraceae) produced by Plantextract, Romania according to the German Homeophatic Pharmacopeia and subject to company quality control, were used to treat the whole blood cultures.

Blood collected from the experimental animals was processed within 4 h after sampling. Each blood sample (400 μl) was diluted with four times the amount of RPMI 1640 supplemented with 5% FCS and antibiotics, pH 7.4, (Sigma-Aldrich, USA). The mixture was distributed in 96-sterile-well plate (200 μl per well). Eight *in vitro* experimental variants were tested in duplicate for each individual animal, namely (1) untreated control culture, (2) phytohaemagglutinin-M (PHA) (1 μ per well) treated culture, (3) 70° alcohol treated culture and (4–8) alcoholic vegetal extracts of *C. officinalis*, *S. officinale*, *A. montana*, *E. angustifolia* and *E. purpurea* (1.5 μl/well).

The quantities of both PHA and antigens were established when using the same technique during preliminary studies (Spînu et al., 2008a,b) as being the most effective *in vitro*. The cultures were incubated for 48 h at 37.5 °C and 5% CO_2 atmosphere. Glucose concentrations, as a growth indicator, were measured in the initial culture medium and in all variants at the end of the incubation period, using a glucose standard (100 mg%), by means of an orto-toluidine colorimetric test. For this, 12.5 μl of the cultural supernatant were transferred to 0.5 ml of orto-toluidine reagent, boiled for 8 min, cooled suddenly in cold water and read in a spectrophotometer at 610 nm wavelength (SUMAL PE2, Karl Zeiss, Jena, Germany), using the reagent as a blank. The cell stimulation/inhibition index (S/I) was calculated as follows: $S/I\% = [(IG–GR)/IG]100$, where S/I = blast transformation index, IG = the initial glucose concentration in the culture medium and GR = glucose residue in the sample after incubation.

2.3. Statistical analyses

Average values and standard error were calculated by use of Excel program. Student's *t*-test was applied to evaluate the statistical significance of the differences.

3. Results and discussion

Plant extracts, as products with increased bioavailability, could provide maximal stimulating and adjuvant activity. Most frequently, their side effects are absent or minimal (Hilton et al., 2002); thus, the use of vegetal extracts as potential enhancers of the immune response to vaccinal antigens or during therapy are