

# Modulation of murine macrophages phagocytic activity by polysaccharide extract from *Chenopodium bonus-henricus*

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## Abstract

*Chenopodium bonus-henricus* (popularly known as Good King Henry or Perennial Goosefoot) is a spontaneous plant used for its cicatrisation, anti-arthritis and anti-cough properties and also for antimicrobial, antifungal, vermifuge, anti-inflammatory, diuretic and immunomodulating effects. Despite these, it is not recognized as an important medicinal plant in the international pharmacopeias, probably because of the few existent researches. In this context our study was made to assess the effect of the polysaccharides extract of *Chenopodium bonus-henricus* herb on cellular immunity. Murine peritoneal macrophages were incubated with 20, 30, 60 and 80 µg/ml of the extract. In order to evaluate the phagocytosis response, sheep red blood cells were co-cultivated with the treated macrophages. Results showed that polysaccharide extracts increase the phagocytic activity of murine macrophage depending on the concentration. The highest values of the phagocytic index and mean phagocytosis were obtained at 30 µg/ml concentration. Considering mice the main mammalian model organism for studying certain human diseases, *Chenopodium bonus-henricus* might have clinical applications due to its immunomodulatory properties.

**Key words:** polysaccharide extract, *Chenopodium bonus-henricus*, murine, phagocytic activity.

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## Introduction

The genus *Chenopodium* includes more than 200 species spread in Europe, Asia and both North and South America. Although many of these possess therapeutic and edible properties, the medicinal uses of *Chenopodium* are not widely known [1]. *Chenopodium bonus henricus* L., named the shepherd's spinach (in Romania), or Good King Henry, Perennial Goosefoot and God's herb (in other countries), is a spontaneous, nitrofil plant. Its medicinal properties and nutritional qualities have been known to people since ancient times [2]. This plant is used in folk medicine for cicatrisation, anti-arthritis and anti-cough properties and also for antimicrobial, antifungal, vermifuge, anti-inflammatory, diuretic and immunomodulating effects. Its nutritional value is due to the high content of proteins and iron, considered to be a good food plant.

According to Kokanova-Nedialkova *et al.* [1], the genus *Chenopodium* includes 379 compounds of diverse chemi-

cal nature: minerals, primary metabolites – carbohydrates, amino acids, nonpolar constituents, proteins, aromatic cytokinins, hormones and secondary metabolites – flavonoids, saponins, terpenes, sterols, alkaloids and vitamins. In the case of *bonus henricus*, there were found some active principles such as polyphenol, saponins, tannins, ecdysteroids, polysaccharides [3] and vitamin C [4].

There are a lot of researches that show immunomodulatory effects of certain vegetal compounds, most of them including polysaccharides and carotenoid pigments. Nonspecific immunomodulatory activity of aqueous and hydroalcoholic extracts of under-ground parts of *Rhodiola quadrifida* had been demonstrated in pigs, rats and mice in *in vitro* and *in vivo* experimental models [5, 6]. Concerning immunopharmacology, carbohydrate macromolecules represent nonspecific immunostimulatory or para immunity inducers [7]. Polysaccharide fractions (with molecular weights between 25000 and 50000) isolated from the water

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or water-alkaline extract of *Echinacea*, *Eupatorium*, *Chamomilla*, *Calendula*, *Baptisia*, *Achyrocline*, *Arnica*, *Sabal* and *Eleutherococcus* proved to have good immunomodulatory effects in the carbon clearance test [8]. Pharmacological studies have shown that polysaccharide extracts and saponins obtained from *Chenopodium bonus-henricus* are not toxic and are efficient in treating high blood-lipid content [9].

The aim of this experiment was to determine the effect of different concentrations of the polysaccharide extract from *Chenopodium bonus-henricus* on the phagocytic activity of peritoneal murine macrophages.

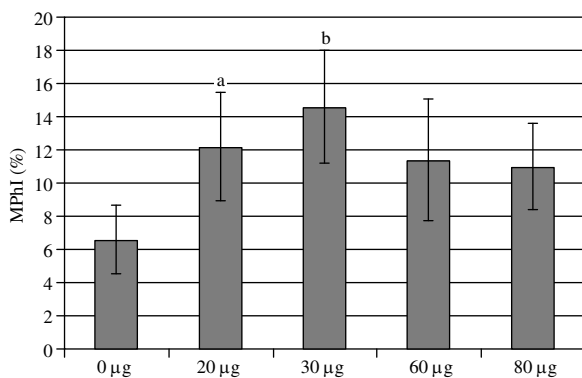
## Material and methods

The chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA) and the antibiotics from Antibiotice (Iași, Romania). Polysaccharides were isolated from *Chenopodium bonus-henricus* herb using hot water extraction, ethanol and acetic acid precipitation and then sterilized by microfiltration according to the previously described method by Hanganu *et al.* [10]. Experiments were performed using five male Swiss white mice and were approved by the local Ethical Committee (No. 14618/27.09.2012). Resident peritoneal macrophage cultures were obtained following a previously reported method [11]. The mononuclear cell fractions were washed twice in Dulbecco phosphate buffer solution pH 7.4 (DPBS – Sigma, D1408) and collected in RPMI 1640 medium (Sigma, R8758) supplemented with 5% heat-inactivated fetal calf serum (FCS – Sigma, F0392), 100 IU/ml penicillin G (Antibiotice) and 100 µg/ml streptomycin sulphate (Antibiotice). Cell density and viability have been assessed by the trypan-blue exclusion test (viability min 95%) and consequently cell suspension was adjusted to  $3 \times 10^6$

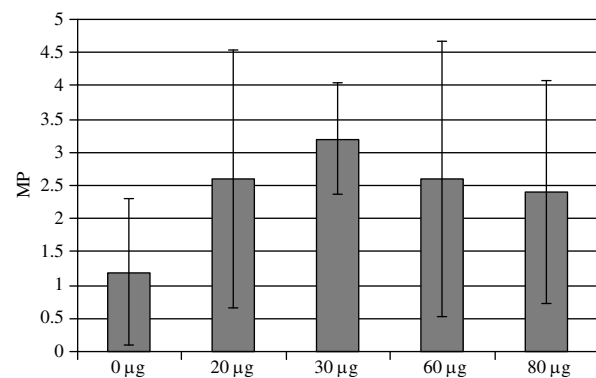
macrophages/ml. One milliliter of this cell suspension was dispensed into each of the four compartments of a Petri dish containing one sterile glass coverslip. The culture dishes were incubated at 37°C in a humidified incubator in the presence of 5% CO<sub>2</sub>. After 24 h of incubation, the coverslips were washed with DPBS to remove non-adherent cells. For the next 48 h washed coverslips were transferred to new Petri dishes containing fresh growth medium. After that, the macrophage cultures were exposed (in duplicates) to DMSO (control group, final concentration 0.03% v/v) (Sigma, D2650) and to experimental trials of polysaccharide extracts in 20, 30, 60 and 80 µg/ml concentrations for 2 h incubation.

Phagocytic activity of peripheral macrophage cells for sheep red blood cells (SRBC) was determined as previously reported [12]. Briefly, freshly collected SRBC were washed three times in sterile phosphate buffer saline pH 7.2 (PBS, Sigma, P5493) and 5% SRBC suspension was extemporaneously prepared in RPMI 1640. The culture medium was removed and 1.0 ml of the 5% SRBC suspension was added to each coverslip sample. After 1 h of incubation, the coverslips were washed to remove free SRBC, then fixed in methanol for 10 min, stained with May-Grünwald-Giemsa and mounted on clean glass slides. Two hundred macrophages per coverslip were scored microscopically at 100× magnification. The following parameters were defined: phagocytic index (MPhI%) as the percent of phagocytic macrophages and the mean phagocytosis (MP) as the number of SRBC per macrophage. The experiment has been repeated five times.

The results were statistically analyzed using one-way ANOVA. When ANOVA presented considerable differences, we used Dunnett Multiple Comparisons Test. All statistical analyses were made using GraphPad InStat.



**Fig. 1.** Effects of polysaccharide concentrations of *Chenopodium bonus-henricus* herbal extract on phagocytic index (MPhI%). The values between the control group and labeled columns differ significantly: a)  $p < 0.05$ ; b)  $p < 0.01$ . Statistical analysis was performed by one-way ANOVA and Dunnett Multiple Comparison Test



**Fig. 2.** Effects of polysaccharide concentrations of *Chenopodium bonus-henricus* herbal extract on mean phagocytosis (MP). The values between control and experimental groups did not differ significantly by one-way ANOVA analysis

## Results

The herbal extracts of *Chenopodium bonus-henricus* have shown a stimulatory effect on phagocytic capacity of mouse macrophages (Fig. 1). In all experimental trials with different concentrations of polysaccharides, SRBC uptake increased ( $12.3 \pm 3.29\%$ ) in comparison with the control group ( $6.6 \pm 2.07\%$ ). MPhI% was significantly higher at 20  $\mu\text{g/ml}$  ( $12.2 \pm 3.27\%$ ;  $p < 0.05$ ) and 30  $\mu\text{g/ml}$  ( $14.6 \pm 3.36\%$ ;  $p < 0.01$ ) in comparison with the control group. The same distribution of the results was observed in the case of MP (Fig. 2), with a lower value in the control group ( $1.2 \pm 1.09\%$ ) and a higher value in the 30  $\mu\text{g/ml}$  group ( $3.2 \pm 0.83\%$ ).

The immunomodulatory effect of polysaccharides from *Chenopodium bonus-henricus* herb was also tested on adult hens by Hanganu *et al.* [10]. Using the carbon clearance test, they have found that phagocytic indexes are variable according to polysaccharide concentrations, and increase at 40 and 60  $\mu\text{g/ml}$ .

## Discussion

Mousavi *et al.* [4] found that co-administration of *Chenopodium album* and CpG oligonucleotides extract reverses IgG2a/IgG1 ratios and increases IFN- $\gamma$  and IL-10 production in the murine model of asthma; these components can be used with other allergens in order to prevent inflammatory conditions. Another study indicated that water-soluble components from *Alternanthera tenella* are able to modulate effectively the immunological interactions [13].

Nose *et al.* [14] investigated the effects of crude polysaccharide fractions obtained from the shoot and hairy root of *Glycyrrhizae* sp. on the murine peritoneal macrophage function, in order to clarify whether plants grown under aseptic conditions produce immunomodulatory polysaccharides. Their results indicated that all crude polysaccharide fractions induced nitric oxide production by murine peritoneal macrophages *in vitro* and suggested that shoot and hairy root have biosynthesized polysaccharides that could have stimulated macrophages *de novo*. In other studies, crude polysaccharides from *Plantago lanceolata*, *Rudbeckia fulgida*, *Salvia officinalis* and *Valeriana officinalis* exhibited similar significant immunomodulatory activities using *in vitro* mitogenic and comitogenic rat thymocyte tests, with a particularly high adjuvant activity in the case of the *Rudbeckia* and *Salvia* polysaccharides [15, 16]. The pectic polysaccharide-rich complex from *Valeriana* and *Opilia celtidifolia* also stimulated the immune function of bone marrow cells and macrophage activity [15, 17]. Polysaccharides fraction isolated from *Ginkgo biloba* folium has a good immunomodulatory activity rate [18], while fraction C from *Caltha palustris* extract can change the percentage and absolute number of T cell subsets in thymus, and T and B lymphocytes in the spleen and mesenteric lymph nodes [19].

Results of immunological assays have shown that the alkaline soluble polysaccharides of *Ganoderma lucidum* do

not have any noticeable effect on monocyte phagocytosis and immune organ (spleen, thymus) weight of immunocompromised mice at the tested dosages [20]. Both *in vitro* and *in vivo* studies of Xu *et al.* [21] suggested that anti-tumor activities of *Ganoderma lucidum* polysaccharides are mediated by their immunomodulatory, anti-angiogenic, and cytotoxic effects. *Ganoderma lucidum* polysaccharides affect immune cells and immune-related cells including B lymphocytes, T lymphocytes, dendritic cells, macrophages, and natural killer cells. Although polysaccharides from many Chinese medicine herbs have been indicated to have immunomodulatory and anti-tumor activities [22-24], their immunoregulatory mechanisms are still not fully understood.

In conclusion, in this study we assessed *in vitro* the immunomodulatory effect of *Chenopodium bonus-henricus* polysaccharides on murine resident peritoneal macrophage cultures. The results showed an increase of phagocytic activities (phagocytic index and mean phagocytosis) due to polysaccharide treatments. For a better immunomodulatory effect, our study indicated a 30  $\mu\text{g/ml}$  concentration of the polysaccharide extract from *Chenopodium bonus-henricus*.

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*The authors declare that they have no conflict of interest concerning this article.*

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