

A.E. PARVU¹, M. PARVU², L. VLASE³, P. MICLEA⁴, A.C. MOT⁴, R. SILAGHI-DUMITRESCU⁴

ANTI-INFLAMMATORY EFFECTS OF *ALLIUM SCHOENOPRASUM* L. LEAVES

¹Department of Pathophysiology, Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; ²Department of Biology, Faculty of Biology and Geology, "Babes-Bolyai" University, Cluj-Napoca, Romania; ³Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; ⁴Department of Chemistry, Faculty of Chemistry and Chemical Engineering, "Babes-Bolyai" University, Cluj-Napoca, Romania

Allium schoenoprasum has antimicrobial and antifungal properties and is used to relieve pain from sunburn and sore throat. The aim of the present study was to evaluate the anti-inflammatory effects of the extracts from *A. schoenoprasum* leaves. A 1:1 (w:v) extract was prepared by a modified Squibb repercolation method. The total phenolic content of 68.5±2 g gallic acid equivalent (GAE)/g plant was determined using the Folin-Ciocalteu method. The *in vitro* antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl bleaching method (6.72±0.44 g/mg DPPH) and the trolox equivalent antioxidant capacity (132.8±23 g trolox eq./g plant) assay. Analysis of the extracts using the hemoglobin ascorbate peroxidase activity inhibition assay or the electron spin resonance did not yield signals above the detection limit. The anti-inflammatory effects of three extract concentrations (25%, 50%, 100%) were evaluated *in vivo* on a model turpentine oil-induced inflammation in rats. These three extracts were also evaluated *in vitro* for the ability to inhibit phagocytosis, the accumulation of total nitrites and nitrates in the serum, the total oxidative status, the total antioxidant response and the oxidative stress index. Pure extracts (100% concentration) had the best inhibitory activity on phagocytosis and oxidative stress. In conclusion, these results support the hypothesis that extracts from *A. schoenoprasum* leaves exert anti-inflammatory activities by inhibiting phagocytosis through the reduction of nitro-oxidative stress.

Key words: *Allium schoenoprasum*, antioxidant, inflammation, phagocytosis, nitric oxide, oxidative stress, nitrite, nitrate

INTRODUCTION

Inflammation is associated with many common diseases worldwide. Phagocytic cells, and the oxidative stress generated by these cells play an important role in the inflammatory response. There are two primary chemical pathways from which oxidative stress is derived *in vivo*: reactive oxygen species (ROS) and reactive nitrogen oxide species (RNOS). The resulting nitro-oxidative stress may lead to diseases, such as cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts, inflammation, and aging (1).

At the cellular and organism level, antioxidant protection is provided by numerous endogenous enzymatic and non-enzymatic antioxidants. Because chronic medical conditions, often involving persistent inflammation, afflict more than half of the population numerous studies have searched for effective exogenous antioxidants. In recent decades, interest in natural plant products with antioxidant properties has grown rapidly, as these products are assumed to be less toxic than synthetic antioxidants (2).

The *Allium* L. genus belongs to the *Liliaceae* family, and includes approximately 500 species that are widespread around the world. Ethnobotanical studies from Romania mention 32 wild and cultivated species of *Allium* L. (3). These plants have been valued

for many centuries for their use in seasoning food, medicinal properties, and in some parts of the world, their use in religious rites. *Allium schoenoprasum* L. (chive) is an herbaceous perennial plant that is easily grown. It is cultivated for its leaves, which have both culinary and medicinal uses. In food chives are used as a condiment that provides a milder flavor than other *Allium* species. The medical properties of chives are similar to those of garlic, but weaker. Chives are used to lower blood pressure, relieve sunburn and sore throat pain, and as antimicrobial (4) and antifungal agents. One mechanism by which *A. schoenoprasum* produces these effects depends upon their antioxidant activity (5).

It is thought that the combinations of different natural antioxidants present in medicinal plants work better than individual antioxidants (6,7). The antioxidant activity of *Allium* the species is due to a variety of sulphur-containing compounds and their precursors, in addition to other bioactive compounds such as polyphenols, dietary fibre and microelements (8).

Therefore, the aim of the present study was to evaluate the anti-inflammatory effects of extracts from *A. schoenoprasum* leaves. To this end, a model of experimental inflammation was used to determine the inhibitory effects of phenolic compounds found in *A. schoenoprasum* extracts on phagocytosis and nitro-oxidative stress.

MATERIAL AND METHODS

Chemicals

Sulphanilamide (SULF), N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDD), vanadium (III) chloride (VCl_3), methanol, diethylether, xylenol orange [o-cresosulfonphthalein-3,3-bis(sodium methyliminodiacetate)], ortho dianisidine dihydrochloride (3-3'-dimethoxybenzidine), ferrous ammonium sulphate, hydrogen peroxide (H_2O_2), sulphuric acid, hydrochloric acid, glycerol, trichloroacetic acid (TCA), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and Folin-Ciocalteu reagent were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (Taufkirchen, Germany). Analytical grade chemicals were used exclusively.

Plant material

Fresh *A. schoenoprasum* leaves (fragments of 0.5–1.0 cm) were purchased from the Agrobotanical Garden of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, and extracted with 70% ethanol (Merck, Bucuresti, Romania) in the Mycology Laboratory of Babes-Bolyai University, Cluj-Napoca, Romania, by a modified Squibb repercolation method, producing a 1:1 (w:v) extract (9). Plants were identified and voucher specimens (CL 659561/14.05.2007) were deposited at the Herbarium of "A. Borza" Botanical Garden, "Babes-Bolyai" University of Cluj-Napoca, Romania.

Phytochemical analysis

The total phenolic content (PhC) of the extract from *A. schoenoprasum* leaves was determined spectrophotometrically using the Folin-Ciocalteu method as previously described (10, 14). Briefly, extracts from *A. schoenoprasum* leaves (20 ml) were added to distilled water (640 mL) and Folin-Ciocalteu reagent (200 mL), followed by incubation in the dark for 5 min. After incubation, 150 mL of 20% sodium carbonate solution was added. After 30 min incubation in the dark, the absorbance was measured at 725 nm. A calibration curve was constructed using the gallic acid standards (Pearson correlation coefficient: $R=0.996$). The PhC for each sample was determined in terms of gallic acid equivalents. Measurements were done in triplicate.

In vitro antioxidant activity

The *in vitro* antioxidant activity of the *A. schoenoprasum* extract was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) bleaching method (11), the trolox equivalent antioxidant capacity (TEAC) assay (12), and the hemoglobin ascorbate peroxidase activity inhibition (HAPX) assays (13). The influence of the extract on hydroxyl radical ($HO\cdot$) formation was studied by electron spin resonance (ESR) (14).

*In vivo anti-inflammatory effects**Experimental design*

The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of the Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca.

The experiments were performed on adult male Wistar-Bratislava albino rats, weighing 200–250 g, that were bred in the Animal Facility of Iuliu Hatieganu University of Medicine and Pharmacy. The animals were randomly assigned to six groups ($n=8$). Group I, rats were injected intramuscularly (i.m.) with 0.9% saline solution as a negative control. Inflammation was

induced by i.m. injection of turpentine oil (6 mL/kg BW) in groups II–VI (15). Animals were housed under controlled conditions (12 h light/dark cycle, at an average temperature of 21–22°C and humidity of 50–55%), and had free access to standard pellet (Cantacuzino Institute, Bucharest, Romania) basal diet and water *ad libitum*. After the i.m. injection, the animals received the following intraperitoneal (i.p.) injections: groups I and II received 1 mL of a 0.9% saline solution; groups III, IV and V received 5 mL/kg BW of *A. schoenoprasum* plant extract diluted in distilled water to concentrations of 25% (0.25), 50% (0.5) and 100% (1) respectively; group VI received 20 mg/kg BW indomethacin (16). Twenty-four hours after the incubation of inflammation, the rats were anesthetized using a combination of 50 mg/kg BW ketamine and 20 mg/kg BW xylazine (17), and blood was withdrawn by retro-orbital puncture. Blood collected for use in the phagocytosis test was harvested on ethylenediaminetetraacetic acid (EDTA) while blood harvested for use in the nitro-oxidative stress test was collected without anticoagulant. Coagulated blood was centrifuged and the separated serum was stored in $-80^\circ C$ until use. The total nitrites and nitrates (NOx), total oxidative status (TOS), total antioxidant response (TAR) and oxidative stress index (OSI) calculation was measured in the serum.

The experiments were performed in triplicate. At the end of the experiments the animals were sacrificed by cervical dislocation.

In vitro phagocytosis test

Phagocytic activity was determined as previously described with minor modifications (18). The blood samples that were harvested on EDTA were incubated with an *E. coli* suspension (4×10^6 bacteria/mL, in 0.9% saline solution in the ratio of 0.2 mL of blood/20 μL *E. coli* suspension) in a silicon tube at 37°C for 30 min. May-Grünwald-Giemsa stained smears were then prepared and counted by light microscopy using an Olympus microscope). We used two parameters to assess phagocytic capacity: the phagocytic activity (PA) which was the number of the *E. coli* bacteria that were phagocytized by 100 leukocytes, and the phagocytic index (PI) which was the percentage of leukocytes that phagocytized at least one bacterium.

Oxidative stress evaluation

First serum samples were passed through 10-kDd filters (Sartorius AG, Goettingen, Germany) and contaminant proteins were removed by extraction with a 3:1 (v:v) solution of methanol/diethyl ether. The sample methanol/diethyl ether ration was 1:9 (v:v) (22). The Griess reaction was used to indirectly determine NO synthesis (NOx). In brief, 100 μL of 8 mg/mL VCl_3 was added to 100 μL of filtered and extracted serum supernatant in order to reduce nitrate to nitrite, followed by the addition of the Griess reagents, 50 μL of SULF (2%) and 50 μL of NEDD (0.1%). After 30 min incubation at 37°C, the sample absorbance was read at 540 nm. The concentration of serum NOx was determined using a sodium nitrite-based curve, and expressed as nitrite $\mu mol/L$ (19).

The total oxidative status (TOS) of the serum was measured using a colorimetric assay (20). This assay measures the oxidation of ferrous ion to ferric ion in the presence of various reactive oxygen species in an acidic medium. The ferric ion are detected by reaction with xylenol orange. Assay measurements were standardized using hydrogen peroxide (H_2O_2) as the oxidative species, and the assay results are expressed in $\mu mol H_2O_2$ Equiv./L.

The total antioxidant response (TAR) was measured in serum using a colorimetric assay (21). In this assay the rate of hydroxyl radical production by the Fenton reaction was monitored by following the changes in the absorbance of coloured dianisidyl radicals. Upon addition of a serum sample, the hydroxyl radical-initiated oxidative reactions are suppressed by antioxidant

present in the serum. Inhibition of dianisidyl oxidation prevents the subsequent colour change, thereby effectively measuring the total antioxidant capacity of the serum. This assay is calibrated using trolox and results are expressed as mmol trolox Equiv/L.

The ratio of the TOS to the TAR represents the oxidative stress index (OSI), an indicator of the degree of oxidative stress (22): OSI (Arbitrary Unit) = TOS (mol H₂O₂ Equiv/L) / TAR (mmol trolox Equiv/L).

All of the spectroscopic measurements were performed using a Jasco V-530 UV-Vis spectrophotometer (Jasco International Co., Ltd., Tokyo, Japan).

Statistical analysis

All results were expressed as the mean±S.E.M. for normally distributed data. Otherwise, the median and first quartile (Q1) and third quartile (Q3) were reported. Statistical comparisons between two independent groups were performed using the Student's *t*-test (with equal and unequal variances, depending upon to the results of the *F*-test) for normally distributed data. Mann-Whitney's test was used for non-parametric data. Pearson and Spearman's correlation analyses were used to calculate statistical relationships between parameters. A *p* value <0.05 was considered as statistically significant. Analyses were performed using SPSS 16.0 for Windows (SPSS Inc, USA).

RESULTS

Phytochemical analysis of the phenolic content

The total PhC of the *A. schoenoprasum* extracts, expressed as gallic acid equivalents (GAE), were small, amounting to approximately 68.5±2 g GAE/g leaves.

The in vitro antioxidant effect

The DPPH assay is widely used to investigate the free radical scavenging activities of several natural compounds. Extracts from *A. schoenoprasum* leaves exhibited low antioxidant activity with an EC₅₀ value of 6.72±0.44 g/mg DPPH (Table 1, Fig. 1). The TEAC assay was used to evaluate the total oxidant-scavenging activity of the leaf extracts. The mean TEAC value of the *A. schoenoprasum* extracts was 132.8±23 µg trolox eq./g leaves (Table 1, Fig. 2). Unfortunately, EPR and HAPX analysis did not yield signals that were above the detection limit.

The in vivo anti-inflammatory effects

In vitro phagocytosis test

In the turpentine-induced rat inflammation model there was a significant increase in both the PI (51.2±2.12%) and the PA

(177±12.01 *E. coli*/100 leukocytes) (*p*<0.001) compared to control rats. Indomethacin exerted a strong anti-inflammatory effect (*p*<0.001) decreasing both the PI (17±2.22%) and the PA (18±2.84 *E. coli*/100 leukocytes). Only highest *A. schoenoprasum* concentration reduced the PI (27±3.18%) and the PA (52±2.21 *E. coli*/100 leukocytes) significantly (*p*<0.001), but the effect was not as pronounced as that of indomethacin (*p*<0.01). Importantly, the changes in PI and PA were correlated (*r*=0.97) at the highest *A. schoenoprasum* concentration tested. *A. schoenoprasum* extracts at concentrations of 0.25 and 0.5 did not affect the PI (50±3.95% and 48±1.43%, respectively) and the PA (102±11.50 *E. coli*/100 leukocytes and 98±8.48 *E. coli*/100 leukocytes, respectively). These results were not significantly different from those of the turpentine-induced inflammation group (*p*>0.05) (Fig. 3).

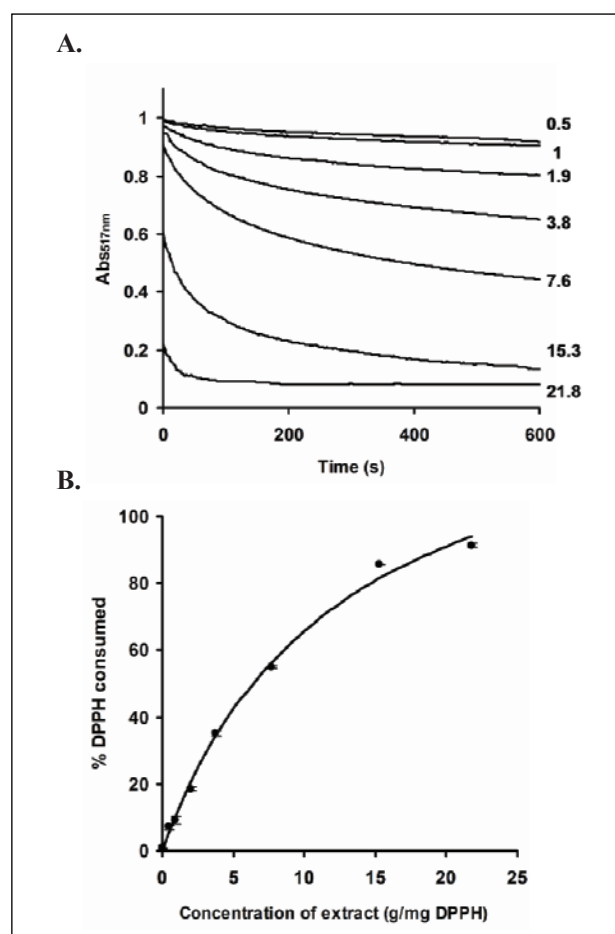


Fig. 1. Assays conducted using *A. schoenoprasum* leaf extracts obtained by DPPH bleaching: (A): DPPH bleaching profiles for *A. schoenoprasum* at several concentrations (g dried plant/mg DPPH); (B): The plot of % DPPH consumed in 10 minutes vs. extract concentration used for the EC₅₀ determination.

Table 1. A comparison of the DPPH bleaching and TEAC assays on the contents of *A. schoenoprasum* leaf extracts.

Type of assay	Parameter	Value
DPPH bleaching assay	EC ₅₀ (g/mg DPPH)	6.72±0.44
	T _{EC50} (min)	6.60
	AE (×10 ²)	225
TEAC	QF (µg quercetin eq./g plant)	16.4±2.3
	TEAC (µg trolox eq./g plant)	132.8±23

DPPH = 1,1-diphenyl-2-picrylhydrazyl; TEAC = trolox equivalent antioxidant capacity; values are expressed as mean ± S.E.M. of three determinations.

The evaluation of oxidative stress

In order to determine the anti-inflammatory mechanisms of *A. schoenoprasum* extracts, markers of nitro-oxidative stress, including NOx, TOS, TAC and OSI was evaluated in the serum from turpentine oil-treated rats. Serum NOx was significantly elevated in the turpentine oil-treated group ($p < 0.001$) compared to serum from the control rats. Importantly, indometathacin decreased NO production ($p < 0.001$). Treatment of inflamed rats with extracts from *A. schoenoprasum* leaves reduced NO

synthesis in a concentration-dependent manner, with the highest concentration having the greatest inhibitory effect ($p < 0.001$). In addition, we found that 0.25 *A. schoenoprasum* had comparable efficiency ($p < 0.001$), but that 0.5 ($p < 0.001$) and 1 ($p < 0.001$) had greater inhibitory activity on NO synthesis compared to indometathacin. When we compared the three *A. schoenoprasum* dilutions, we found that the highest concentration had the greatest inhibitory activity ($p < 0.001$ for 0.25 vs. 1; $p < 0.001$ for 0.5 vs. 1) (Table 2). The *A. schoenoprasum*-induced NOx decrease correlated with the PA ($r = 0.86$) and the PI ($r = 0.79$).

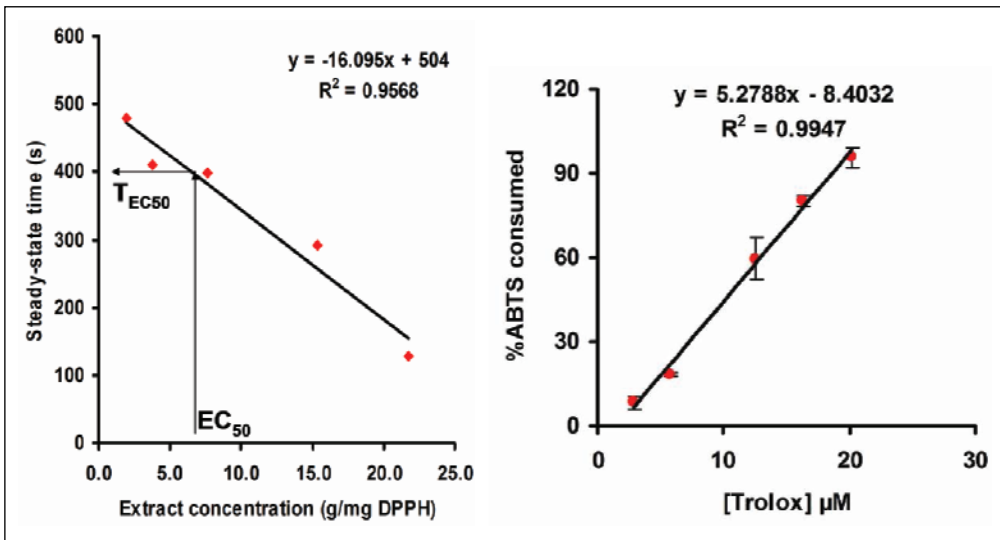


Fig. 2. The determination of the TEC₅₀ for the *A. schoenoprasum* leaf extracts (left) and the calibration curve used in TEAC assay (right).

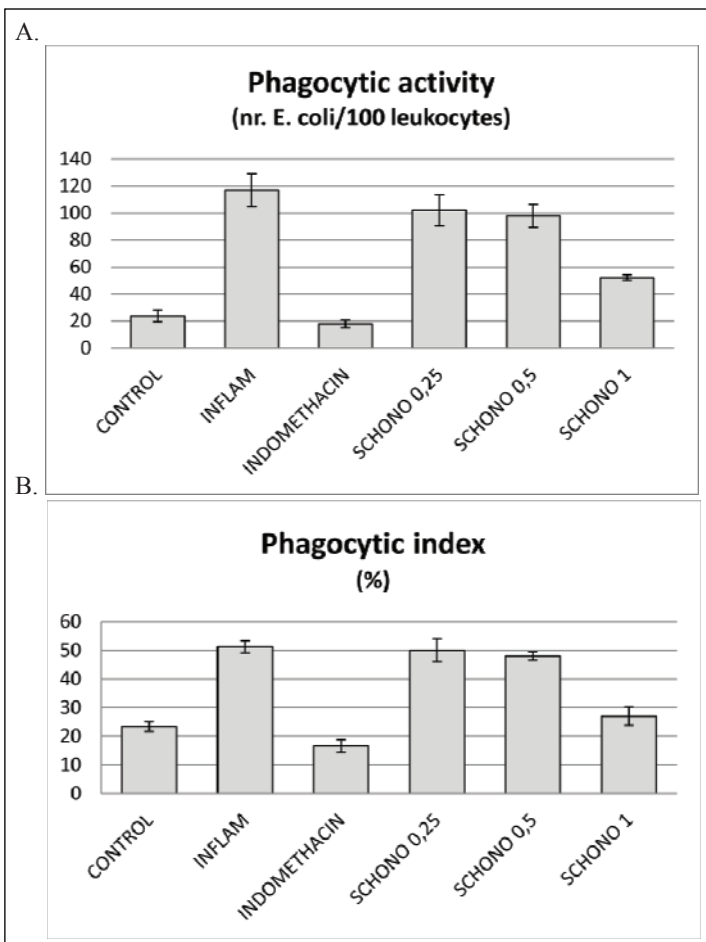


Fig. 3. The *in vitro* assessment of the effects of *A. schoenoprasum* leaf extracts on the phagocytic activity of blood leukocytes: (A): The phagocytic activity (PA = the number of the *E. coli* bacteria phagocytized by 100 leukocytes); (B): The phagocytic index (PI% = percentage of leukocytes that phagocytized at least one bacterium). SCHONO = *A. schoenoprasum* leaf extract. Values are expressed as mean ± S.E.M. of three determinations.

Table 2. The effects of *A. schoenoprasum* leaf extracts on parameters of nitro-oxidative stress.

	CONTROL	INFLAMM.	INDOMETHACIN	SCHONO 0.25	SCHONO 0.5	SCHONO 1
NOx ($\mu\text{mol/L}$)	4.24 \pm 1.41	69.79 \pm 2.94	39.86 \pm 5.68	40.34 \pm 4.08	38.22 \pm 5.06	32.40 \pm 2.81
TOS ($\mu\text{mol Equiv. H}_2\text{O}_2/\text{L}$)	15.20 \pm 3.12	167.26 \pm 6.60	81.41 \pm 9.34	80.90 \pm 7.58	72.54 \pm 7.28	58.33 \pm 8.62
TAR (mmol Equiv TROLOX/L)	1.32 \pm 0.05	0.23 \pm 0.07	1.1 \pm 0.01	1.10 \pm 0.06	1.10 \pm 0.09	1.10 \pm 0.10
OSI	0.12 \pm 0.05	7.26 \pm 0.39	0.73 \pm 0.23	0.74 \pm 0.16	0.68 \pm 0.14	0.54 \pm 0.16

NOx = total nitrites and nitrates; TOS = total oxidative status; TAR = total antioxidant reactivity; OSI = oxidative stress index; SCHONO = *A. schoenoprasum* leaf extract; values are expressed as mean \pm S.E.M. of three determinations.

Turpentine-induced inflammation significantly increased ($p < 0.001$) and treatment with indomethacin significantly reduced ($p < 0.001$) the TOS. Importantly, the TOS was also significantly reduced by the treatment with any concentration of the *A. schoenoprasum* extract ($p < 0.001$). The inhibitory effects were strongest for the highest plant extract concentration ($p < 0.001$ for 0.25 vs. 1; $p < 0.01$ for 0.5 vs. 1). Indomethacin treatment had a similar effect on TOS inhibition ($p < 0.01$) compared to treatment with either the 0.25 ($p < 0.01$) or the 0.5 ($p < 0.01$) *A. schoenoprasum* extract. However, the *A. schoenoprasum* extract was a better TOS inhibitor than indomethacin ($p < 0.001$) (Table 2).

Inflammation reduced the TAR in the experimental model ($p < 0.001$), and indomethacin treatment significantly increased the TAR ($p < 0.001$). The decrease in the TAR was prevented by the treatment with the 0.2, 0.5 and 1 *A. schoenoprasum* ($p < 0.001$) extracts, and all dilutions were equally efficacious ($p < 0.001$). All tested *A. schoenoprasum* dilutions had a reduced antioxidant effect compared to indomethacin treatment ($p < 0.001$) (Table 2). The decrease in NOx was negatively correlated with the increase in the TAR ($r = -0.67$).

In the inflammation group, the OSI was significantly elevated ($p < 0.001$), and indomethacin treatment decreased the OSI ($p < 0.001$). The 0.25, 0.5 and 1 extracts of *A. schoenoprasum* leaves induced a significant decline in OSI ($p < 0.001$). The 0.25 extract of *A. schoenoprasum* had similar effect ($p > 0.05$), but the 0.5 and 1 extracts were better at reducing the OSI ($p < 0.001$). The 1 extract of *A. schoenoprasum* leaves was the most effective ($p < 0.001$) (Table 2). The OSI correlated with the TOS ($r = 0.92$) and NOx ($r = 0.81$).

DISCUSSION

The extracts of *A. schoenoprasum* leaves that we prepared proved to have anti-inflammatory activity through a mechanism involving the inhibition of phagocytosis and a reduction in oxidative stress.

Our results documenting the *in vitro* antioxidant activity of *A. schoenoprasum* extracts were in accordance with other studies that investigated the same effects of the bulbs, leaves and stalks of cultivated *A. schoenoprasum* plants. These studies indicated that all parts of the *A. schoenoprasum* plant exhibit some antioxidant activity, particularly the leaves. When tests of a crude extract of *A. schoenoprasum* were performed in a tissue culture model, it was noted that the roots exhibited the highest antioxidant activity (14). In general, the amount of natural antioxidants present in the plant material varies with cultivar, soil composition, climate, geographic origin, cultivation practices and exposure to diseases, such as fungal infections (7). The antioxidant activity of *A. schoenoprasum* leaf extracts derived

using the DPPH bleaching method were four orders of magnitude lower than those obtained using the TEAC method. Extracts obtained using the TEAC method had greater antioxidant activity than other previously tested extracts (5).

Many studies have indicated that polyphenols in herbs possess anti-inflammatory activities, that manifested through ROS scavenging, metal chelation, and modulation of antioxidant enzyme activity, as well as effects on cell signaling pathways and gene expression (23). The total PhC of the tested *A. schoenoprasum* leaf extracts was found to be low, as we (25, 26) and others (24) have reported.

Not only does the PhC of every plant differ from every other plant, but it also differs depending upon the particular part of a given plant that is analysed. The analysis of *A. schoenoprasum* leaf extracts that was previously performed determined the presence of 19 polyphenolic compounds ($\mu\text{g}/100$ g leaves). A non-hydrolysed sample contained *p*-coumaric acid (149.59 \pm 1.05), ferulic acid (188.06 \pm 1.51), sinapic acid (44.91 \pm 0.39), isoquercitrin (363.78 \pm 1.89), quercetol (58.38 \pm 0.62) and kaempferol (129.83 \pm 1.03). Conversely, the hydrolysed sample contained only *p*-coumaric acid (163.71 \pm 1.35), ferulic acid (542.33 \pm 1.93), sinapic acid (44.91 \pm 0.39), quercetol (200.48 \pm 1.74) and kaempferol (1563.46 \pm 2.96) (25, 26). The total amounts of polyphenols were higher in the hydrolysed samples compared to the non-hydrolysed samples, suggesting that polyphenols are present as both unbound and bound glycosides and/or esters (25, 26). These phytochemical analyses suggested the possible anti-inflammatory and antioxidative effects of the *A. schoenoprasum* polyphenols.

Turpentine oil is like carrageenan, a non-antigenic inflammatory stimulus (27), which activates phagocytosis as part of the cellular acute phase response associated with inflammation. There are two professional phagocytes: polymorphonuclear granulocytes (PMNs) and mononuclear cells. After recognizing a target, a phagocytic cell is activated. Activation results in the ingestion of the target by the phagocyte and destruction of the target *via* the intracellular activity of reactive oxidants and hydrolytic enzymes (18).

In our study, we evaluated the anti-inflammatory activity of blood leukocytes. Only the total *A. schoenoprasum* leaf extract nitrate reduced the PI and the PA nitrate and this effect was smaller than that of indomethacin.

Our previous phytochemical analysis of the *A. schoenoprasum* leaf extract revealed high amounts of allicin (320.0 \pm 1.71 mg/100 g vegetal product), β -sitosterol (25.09 mg/100 g vegetal product) and campesterol (7.21 mg/100 g vegetal product) (25). Allicin is thought to be the main active component in the antimicrobial, antifungal (it is more effective than nystatin) and antiviral activity of *Allium* species. The high allicin content of *A. schoenoprasum* leaf extracts may explain the anti-inflammatory effects. Furthermore, phytosterols have

anti-inflammatory and immunomodulatory properties (29). Therefore, we concluded that β -sitosterol and campesterol contained in the extracts of *A. schoenoprasum* leaves might play an anti-inflammatory role as well.

Over the last couple of decades, the dual role of NO as a deleterious as well as a beneficial agent has been widely explored. Besides the essential role as signalling molecule, NO reacts with oxygen as well as with superoxide, to generate RNS. These RNS inevitably react with biological targets, and may lead to cell death. On the other hand, the protective effects of NO have been noted, and the proposed mechanism involves an antioxidant activity. The balance between nitrosative and oxidative chemistry therefore depends upon the relative concentration of NO. Thus, at low NO fluxes, these reactions would tend to lead to the oxidation of substrates, whereas at higher levels of NO, nitrosation reactions would predominate (30). In our turpentine-induced rat inflammation model, we observed a large release of NO. Treatment with *A. schoenoprasum* extracts reduced NOx activity in a concentration-dependent manner, the higher being the stronger inhibitor. This effect may be linked to the PhC, because other studies showed that natural phenols are efficient scavengers of nitrogen dioxide (NO₂), a peroxynitrite (ONOO⁻) intermediate (31). Furthermore, the diluted *A. schoenoprasum* extracts had effects comparable to indomethacin, and the undiluted *A. schoenoprasum* extract was a better NOx inhibitor than indomethacin. These results suggest that inhibition of NO synthesis is an important mechanism in the anti-inflammatory effects of the *A. schoenoprasum* leaf extracts.

The methods of quantifying oxidative damage, often called fingerprinting, involve analysing a range of specific ROS-derived biological end-products, such as lipid, protein, and DNA modification, as well as low-molecular-weight antioxidants (32). The presence of these end-products serves as proof of the prior existence of ROS. The measurement of TOS reflects the additive oxidative effect of different molecules (20). The reduction in TOS after *A. schoenoprasum* treatment indicated that the anti-inflammatory effects of *A. schoenoprasum* were due to, in part, to reduction in oxidative stress.

Currently, golden standard methods for measuring antioxidant capacity in biology do not exist. Assays of total antioxidant capacity like TAR, instead of individual antioxidants, have been developed since *in vivo* antioxidant systems work together and not in isolation. As such, interactions between hydrophilic and lipophilic antioxidants and undiscovered antioxidant species risk being ignored (21). By increasing TAR the *A. schoenoprasum* treatment had an anti-inflammatory effect. These data show that *A. schoenoprasum* leaf extracts different *in vitro* and *in vivo* antioxidant effects.

The oxidative/antioxidative balance was also evaluated by calculating the OSI (22). The parallel TOS decrease and TAR increase after *A. schoenoprasum* treatments reduced the OSI to a greater extent than indomethacin treatment.

Numerous reports show that nitrosating intermediates have the greatest affinity for thiols such as glutathione (GSH), suggesting that thiols are a primary target for nitrosative stress. Glutathione-depleted cells were dramatically more susceptible to toxic nitrosative stress. Furthermore, several studies show that the formation of S-nitrosothiol-protein adducts inhibits variety of enzymes, including antioxidant enzymes (20, 30). These mechanisms may explain the correlations observed between increased NOx, a reduced antioxidant effect, and increased TOS and OSI in inflamed rats. These processes may also be involved in the biological effects of the *A. schoenoprasum* plant extracts, since NOx activity correlated with the TAR.

In summary, this study supports the hypothesis that extracts of *A. schoenoprasum* leaves exert anti-inflammatory effects in the rat turpentine oil induced-inflammation model. This effect

might be due to inhibition of phagocytosis, which occurs through the reduction of nitro-oxidative stress. It is possible that the total phenolic constituents, as well as allicin, β -sitosterol and campesterol may contribute to the anti-inflammatory activity of *A. schoenoprasum* leaf extracts. Based on these observations, adjunctive administration of an *A. schoenoprasum* leaf extract may be a useful host-modulatory therapy in inflammatory diseases.

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; ESR, electron spin resonance; GAE, gallic acid equivalent; HAPX, hemoglobin ascorbate peroxidase activity inhibition; NOx, total nitrites and nitrates; OSI, oxidative stress index; PA, phagocytic activity; PhC, total phenolic content; PI, phagocytic index; RNOS, reactive nitrogen oxide species; ROS, reactive oxygen species; TAR, total antioxidant response; TEAC, trolox equivalent antioxidant capacity; TOS, total oxidative status;

Acknowledgments: This work was supported by CNCIS-UEFISCSU, projects PNII-IDEI-2008-2272 and PN-II-PT-PCCA-2011-3.1-0914.

Conflict of interest: None declared.

REFERENCES

- Liao JC, Deng JS, Chiu CS, *et al.* Chemical compositions, anti-inflammatory, antiproliferative and radical-scavenging activities of *Actinidia callosa* var. *ephippioides*. *Am J Chin Med* 2012; 40: 1047-1062.
- Chen W, Sudji IR, Wang E, Joubert E, van Wyk BE, Wink M. Ameliorative effect of spalathin from rooibos (*Aspalathus linearis*) on acute oxidative stress in *Caenorhabditis elegans*. *Phytomedicine* 2013; 20: 380-386.
- Ciocarlan V. Illustrated Flora of Romania. Pteridophyta et Spermatophyta. Bucharest, Ceres Publishing House, 2009, pp. 915-921.
- Rattanachaiakunsopon P, Phumkhachorn P. Diallyl sulfide content and antimicrobial activity against food-borne pathogenic bacteria of chives (*Allium schoenoprasum*). *Biosci Biotechnol Biochem* 2008; 72: 2987-2991.
- Stajner D, Popovic BM, Calic-Dragosavac D, Malencic D, Zdravkovic-Korac S. Comparative study on *Allium schoenoprasum* cultivated plant and *Allium schoenoprasum* tissue culture organs antioxidant status. *Phytother Res* 2011; 25: 1618-1622.
- Liu Y, Flynn TJ, Ferguson MS, Hoagland EM. Use of the Combination Index to determine interactions between plant-derived phenolic acids on hepatotoxicity endpoints in human and rat hepatoma cells. *Phytomedicine* 2013; 20: 461-468.
- Bruno G, Sparapano L. Effects of three esca-associated fungi on *Vitis vinifera* L:V. Changes in the chemical and biological profile of xylem sap from diseased cv. Sangiovese vines. cv. Sangiovese vines. *Physiol Molec Plant Pathol* 2007; 71: 210-229.
- Nencini C, Cavallo F, Capasso A, Franchi GG, Giorgio G, Micheli L. Evaluation of antioxidative properties of *Allium* species growing wild in Italy. *Phytother Res* 2007; 21: 874-878.
- Parvu M, Rosca-Casian O, Puscas M, Groza G. Antifungal activity of *Allium fistulosum* L. *Contrib Bot* 2009; 44: 125-129.
- Mot AC, Damian G, Sarbu C, Silaghi-Dumitrescu R. Redox reactivity in propolis: direct detection of free radicals in basic medium and interaction with hemoglobin. *Redox Rep* 2009; 14: 267-274.

11. Mot AC, Silaghi-Dumitrescu R, Sarbu C. Rapid and effective evaluation of the antioxidant capacity of propolis extracts using DPPH bleaching kinetic profiles, FT-IR and UV-vis spectroscopic data. *J Food Compos Anal* 2011; 24: 516-522.
12. Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. *Methods Enzymol* 1994; 234: 279-293.
13. Cooper CE, Silaghi-Dumitrescu R, Rukengwa M, Alayash AI, Buehler PW. Peroxidase activity of hemoglobin towards ascorbate and urate: a synergistic protective strategy against toxicity of hemoglobin-based oxygen carriers (HBOC). *Biochim Biophys Acta* 2008; 1784: 1415-1420.
14. Tamokou J de D, Chouna JR, Fischer-Fodor E, et al. Anticancer and antimicrobial activities of some antioxidant-rich Cameroonian medicinal plants. *PLoS One* 2013; 8: e55880.
15. Razani-Boroujerdi S, Behl M, Hahn FF, Pena-Philippides JC, Hutt J, Sopori ML. Role of muscarinic receptors in the regulation of immune and inflammatory responses. *J Neuroimmunol* 2008; 194: 83-88.
16. Adham KG, Al-Humaidhi EM, Daghestani MH, Aleisa NA, Farhood MH. Protective role of indomethacin on lipopolysaccharide-stimulated fever induction and cerebral catecholamine biosynthesis in Wistar rat. *Neuro Endocrinol Lett* 2012; 33: 713-721.
17. Kieling CO, Backes AN, Maurer RL, et al. The effects of anesthetic regimen in 90% hepatectomy in rats. *Acta Cir Bras* 2012; 27: 702-706.
18. Moldovan CM, Oniga O, Parvu A, et al. Synthesis and anti-inflammatory evaluation of some new acyl-hydrazones bearing 2-aryl-thiazole. *Eu J Med Chem* 2011; 46: 526-34.
19. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* 2001; 5: 62-71.
20. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005; 38: 1103-1111.
21. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem* 2004; 37: 112-119.
22. Harma M, Harma M, Erel O. Increased oxidative stress in patients with hydatidiform mole. *Swiss Med Wkly* 2003; 133: 563-566.
23. Soobrattee MA, Neergheena VS, Luximon-Ramma A, Aruoma OI, Bahoruna T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutat Res* 2005; 579: 200-213.
24. Kucekova Z, Mlcek J, Humpolicek P, Rop O, Valasek P, Saha P. Phenolic compounds from *Allium schoenoprasum*, *Tragopogon pratensis* and *Rumex acetosa* and their antiproliferative effects. *Molecules* 2011; 16: 9207-9217.
25. Vlase L, Parvu M, Parvu AE, Toiu A. Chemical constituents of three *Allium* species from Romania. *Molecules* 2013; 18: 114-127.
26. Parvu M, Toiu A, Vlase L, Parvu AE. Determination of some polyphenolic compounds from *Allium* species by HPLC-UV-MS. *Nat Prod Res* 2010; 24: 1318-1324.
27. Bukhari IA. The central analgesis and anti-inflammatory activities of the methanolic extract of *Carthamus oxyacantha*. *J Physiol Pharmacol* 2013; 64: 369-375.
28. Bouic PJ. Sterols and sterolins: new drugs for the immune system? *Drug Discov Today* 2002; 15: 775-778.
29. Thomas DD, Ridnour LA, Isenberg JS, et al. The chemical biology of nitric oxide. Implications in cellular signaling. *Free Rad Biol Med* 2008; 45: 18-31.
30. Flores-Santana W, Moody T, Chen W, et al. Nitroxide derivatives of non-steroidal anti-inflammatory drugs exert anti-inflammatory and superoxide dismutase scavenging properties in A459 cells. *Br J Pharmacol* 2012; 165: 1058-1067.
31. Olas B, Wachowicz B, Nowak P, et al. Studies on antioxidant properties of polyphenol-rich extract from berries of *Aronia melanocarpa* in blood platelets. *J Physiol Pharmacol* 2008; 59: 823-835.
32. Hamlaoui S, Mokni M, Limam N, et al. Protective effect of grape seed and skin extract on garlic-induced erythrocyte oxidative stress. *J Physiol Pharmacol* 2012; 63: 381-388.

Received: August 13, 2013

Accepted: February 26, 2014

Author's address: Dr. Marcel Parvu, Department of Biology, Faculty of Biology and Geology, "Babes-Bolyai" University, 42 Republicii Street, Cluj-Napoca, RO-400015, Romania.
E-mail: mparvucluj@yahoo.com