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Anti-inflammatory activity of methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages

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ABSTRACT

Nowadays there is a great interest in the use of edible mushrooms as functional food since they are rich in bioactive compounds. Although their immunomostimulant activity has been largely demonstrated, their potential anti-inflammatory activity has been scarcely explored. We have investigated the anti-inflammatory activity of methanolic extracts from different edible mushrooms species: *Agaricus bisporus*, *Boletus edulis*, *Cantherellus cibarius*, *Cratarellus cornucopioides*, *Lactarius deliciosus* and *Pleurotus ostreatus*, in activated macrophages. The species that exhibited higher anti-inflammatory activities were *A. bisporus*, *C. cibarius* and *L. deliciosus*, inducing inhibition of NO production and iNOS, IL-1β and IL6 mRNAs expression in response to LPS stimulation. *C. cornucopioides* only induced inhibition of NO production and iNOS expression, and the other species did not present anti-inflammatory effects. Therefore, some edible mushrooms species have a potential anti-inflammatory capacity *in vitro*, suggesting that they could be regarded as a potential source of natural anti-inflammatory agents.

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1. Introduction

Chronic inflammation is implicated in the pathogenesis of a variety of diseases, such as atherosclerosis, obesity, metabolic syndrome, diabetes (Pradhan, 2007), neurodegenerative diseases (Lee, Han, Nam, Oh, & Hong, 2010), and even several types of cancers (Coussens & Werb, 2002). Therefore in recent years, efforts are focussing on the finding of natural products that show anti-inflammatory properties (Haddad, Azar, Groom, & Boivin, 2005).

Macrophages play a crucial role in the inflammatory response through the release of a variety of factors, such as nitric oxide (NO), prostaglandin mediators and proinflammatory cytokines (TNF-α, IL-1β, IL-6), in response to an activating stimulus, e.g. lipopolysaccharide (LPS). Production of these mediators has been demonstrated in several inflamed tissues, along with enhanced expression of their mRNAs.

Recently natural health products (NHPs) are generating renewed interest, particularly in the prevention and treatment of several chronic diseases. Several compounds have shown anti-inflammatory activity; among them, phenolic compounds have attracted great attention due to both their large distribution among dietary components and the variety of biological activities that they display (García-Lafuente, Guillamón, Villares, Rostagno, & Martínez, 2009). In this regard, mushrooms seem to be a good source of anti-inflammatory natural products (García-Lafuente et al., 2010).

Medicinal mushrooms have a well established history of use in traditional oriental therapies, and fungal metabolites are increasingly being used to treat a wide range of diseases (Guillamón et al., 2010; Lindequist, Niedermeyer, & Jülich, 2005). Moreover, edible mushrooms should not be considered only as simple food, as some of them have been shown to be rich in bioactive compounds (Barros, Ferreira, Queirós, Ferreira, & Baptista, 2007). Mushrooms contain different substances, such as polysaccharides, phenolic compounds, proteins (fungal immunomodulating proteins FIPs, lectins, glycoproteins and non-glycosylated proteins and peptides), polysaccharide–protein complexes, lipid components (ergosterol), and terpenoids, which provide a great assortment of biological properties, e.g. antioxidant (Ferreira, Barros, & Abreu, 2009; Puttaraju, Upparahalli, Dharmesh, Urs, & Somasundaram, 2006), antitumor/anticancer (Moradali, Mostafavi, Ghods, & Hedjaroude, 2007), antimicrobial (Barros, Baptista, Estevinho, & Ferreira, 2007), immunomodulatory (Borchers, Krishnamurthy, Keen, Meyers, & Gershwin, 2008), anti-inflammatory (Padilha et al., 2009), antiatherogenic (Mori, Kobayashi, Tomita, Inatomi, & Ikeda, 2008) and hypoglycemic actions (Hu, Wang, Lien, Liaw, & Lee, 2006). Among them, their anti-inflammatory properties have been scarcely studied.

Some ethanolic and methanolic extracts from medicinal mushrooms, such as *Ganoderma lucidum* (Woo et al., 2005), or *Inonotus*

obliquus (Kim et al., 2007), have been shown to inhibit some macrophage functions by decreasing the production of inflammatory mediators, e.g. NO, prostaglandins (PGE_2), and cytokines. Regarding edible mushrooms, a few trials, carried out with extracts or isolated compounds from *Agaricus bisporus* (Kohno et al., 2008) and some species of *Pleurotus* (Jose, Ajith, & Janardhanan, 2004), have demonstrated *in vitro* or *in vivo* anti-inflammatory activity.

Therefore, in the present work, we investigated the anti-inflammatory activity of methanolic extracts from different edible mushrooms species: *A. bisporus*, *Boletus edulis*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Lactarius deliciosus* and *Pleurotus ostreatus* in LPS-activated macrophages.

2. Materials and methods

2.1. Materials and reagents

Six edible wild mushroom species, *B. edulis*, *C. cibarius*, *C. cornucopioides* and *L. deliciosus*, and the cultivated mushrooms, *A. bisporus* and *P. ostreatus*, were used. All of them were purchased from the local market and they came from different regions in Spain. The mushrooms were lyophilised (Telstar Cryodos), finely milled, and kept at 4 °C in hermetically vacuum sealed plastic bags (Tecnotrip) prior to extraction.

Dulbecco's modified Eagle's minimum essential medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, Griess reagent, DMSO, MTT reagent and lipopolysaccharide (LPS) (*Escherichia coli*, serotype 0127:B8), were obtained from Sigma–Aldrich Co. (Saint Louis, MO, USA). The enzyme immunoassay (EIA) kits for TNF-α, were obtained from R&D Systems (Minneapolis, MN, USA), and all reagents for RNA extraction, retrotranscription, and real time PCR from Applied Biosystems (Foster City, CA, USA).

2.2. Methanolic mushroom extracts

Mushroom powder was extracted by stirring with methanol under reflux at 65 °C for 24 h, and then it was centrifuged at 3000 rpm for 10 min. The residue was re-extracted twice (2 × 24 h) and the methanolic extracts were combined, to give a concentration of 200 mg of dried mushroom/ml, and then diluted with culture media to reach 2 mg/ml concentration. This extract was filtered through a 0.45 μm membrane filter (Millipore, Bedford, USA) and stored as a stock solution.

2.3. Cell line

RAW 264.7, a macrophage-like cell line, was obtained from the European Collection of Cell Cultures (ECACC) and cultured in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml of penicillin and 100 μg/ml of streptomycin and maintained at 37 °C in a 5% CO_2 humidified atmosphere (CO_2 incubator, Heal Force). The cells were treated with the methanolic mushroom extracts at different concentrations during 1 h and stimulated with LPS (1 μg/ml) for the indicated period.

2.4. MTT assay for cell viability

Cells were seeded at a density of 10^4 cells/well in 96-well plates overnight, followed by the pre-treatment with different concentrations of the extracts (2, 1, 0.5 mg/ml) for 1 h before the addition of the LPS. RAW 264.7 cells viability was measured after 24 h of exposure to the tested extracts with a colorimetric assay, based on the ability of mitochondria in viable cells to reduce MTT (Ferrari, Fornasiero, & Isetta, 1990). A 0.5 mg/ml concentration of MTT solution was added to each well and, after 3 h of incubation at 37 °C, the medium was discarded and the formazan blue formed in the cells was dissolved in dimethyl sulfoxide (DMSO). Optical density at 570 nm was determined with a microplate reader FluoStar Omega (BMG Labtech). The optical density of the formazan formed in LPS-treated cells was taken as 100% of viability.

2.5. Nitrite determination

Cells were seeded onto 96-well plates with 2×10^5 cells/well and allowed to adhere overnight. Then, medium was removed and replaced with 0.2 ml of fresh medium alone or containing 0.5 mg/ml of extracts. After 1 h of incubation, LPS stimulation was performed. LPS was added at a concentration of 1 μg/ml for 24 h. The cell-free culture medium was collected, 50 μl were used for NO determination and the remainder was stored at −20 °C prior to performing cytokine determinations. The nitrite accumulated in culture medium was measured as an indicator of NO production, based on the Griess reaction (Green et al., 1982). Briefly, 50 μl of cell culture medium were mixed with an equal volume of Griess reagent (equal volumes of 1 % (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphthylethylenediamide–HCl), incubated at room temperature for 10 min, and then the absorbance was measured at 540 nm, using a microplate reader FluoStar Omega (BMG Labtech). The amount of nitrite present in the samples was calculated by means of a standard curve generated using serial dilutions of $NaNO_2$ in fresh culture medium.

2.6. Cytokine determinations

TNF-α level in the culture medium was determined by a Duo Set mouse TNF-α ELISA kit, according to the manufacturer protocols (R&D Systems. Minneapolis, MN, USA). TNF-α produced by LPS-treated cells was taken as 100%.

2.7. Real time reverse transcription PCR (RT-PCR) of mRNA inflammatory mediators

2.7.1. General

The levels of iNOS, TNF-α, IL-1β, and IL-6 mRNA expression were measured by real time quantitative RT-PCR (Overbergh et al., 2003).

2.7.2. RNA extraction and purification

Cells were located onto 6 well plates at a density of 10^6 cells/well and incubated with extracts at the adequate concentration prior to LPS stimulation. After 6 h, cells were lysed and total RNA extraction was performed by using a Ribopure kit from Applied Biosystems. The purified RNA was treated with DNAse (TURBO DNA-free kit from Applied Biosystems) and quantified by spectrophotometry at 260 nm. The purity of extracted RNA was measured by the 260/280 index and it was in all the samples between 1.8 and 2.1. The RNA quality was assessed in each sample by microfluidic capillary electrophoresis by using the Experion™ RNA StdSens Analysis Kit (Bio-Rad Laboratories, USA). The samples were shown to reach an RQI higher than 7.0, to ensure standards meet the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments).

2.7.3. Reverse transcription and real time PCR

cDNA was synthesised from 0.5 μg of total RNA using a High Capacity cDNA Reverse Transcription Kit (AppliedBiosystems) in a Advanced Primus 96 termocycler (PEQLAB).

Quantitative PCR was performed on the Step One System (Applied Biosystems, Foster City, CA, USA), using TaqMan Gene Expression Master Mix and pre-developed TaqMan Assays specific to mouse: iNOS (assay identification number Mm01309902_m1),

TNF-α (Mm99999068_m1), IL-1β (Mm00434228_m1), IL-6 (Mm99999064_m1) and, for the housekeeping gene, ACTB (Mm01205647_g1). PCR was performed with TaqMan MGB probes labelled with FAM reporter dye in a final reaction volume of 20 μl. The amplification conditions were the universal conditions described by the manufacturer. The probes were validated to ensure the amplification efficiency and linearity in preliminary experiments, using different dilutions of the template. Gene expression levels were assessed and normalised by using ACTB as internal control. The fold change in the target genes normalised to ACTB and relative to LPS (RQ) was calculated using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001) by the Applied Biosystems analysis software.

2.8. Statistics

Data are expressed as mean values ± standard errors of the mean (SEM). Comparisons between extracts-treated groups and non-treated group were performed with the Student's *t*-test. Differences were considered significant at $p < 0.05$. The experiments were performed in triplicate and at least three times each.

3. Results

3.1. Effect of methanolic mushrooms extracts on cell viability

To assess the potential anti-inflammatory activity of mushroom methanolic extracts, macrophages RAW 264.7 were used. First, we examined the cytotoxicity of the extracts administered to the cells. Results are shown in Fig. 1 and expressed as percentage of viability with respect to the LPS-treated cells which were taken as 100% viability. The highest amount of methanol in the different diluted extracts was tested to guarantee that the remaining vehicle in the extracts had no effect on cell viability. Among the different assayed concentrations, 0.5 mg/ml was the only one that did not affect the cell viability. Therefore, these extracts were chosen to be used in further experiments.

3.2. Effect of methanolic mushroom extracts on NO production

To evaluate the effects of methanolic mushroom extracts on NO production, RAW 264.7 cells were incubated with 0.5 mg/ml of the extracts for 1 h and then, stimulated with LPS (1 μg/ml) for 24 h more. NO release was measured as the accumulation of the stable metabolite, nitrite, in the culture supernatants. Unstimulated macrophages produced undetectable levels of nitrite, but treatment with LPS induced a high release of NO to the culture medium, which was taken as 100%. Pretreatment with methanolic extracts from *A. bisporus*, *C. cibarius*, *C. cornucopioides* and *L. deliciosus* resulted in an inhibition of the NO production whereas extracts from *B. edulis* and *P. ostreatus* did not show any effect (Fig. 2, panel A).

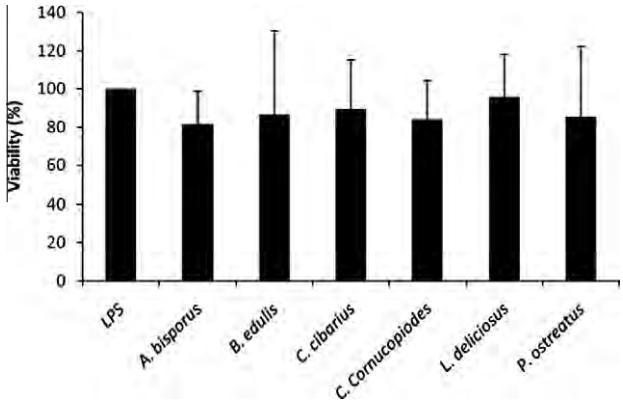

Fig. 1. Effect of methanolic extracts from the mushrooms studied on RAW 264.7 macrophage-like cell viability. Viability of cells treated with LPS alone has been taken as reference (100%). Bars represent the mean and standard deviations from four different experiments performed in triplicate.

3.3. Effect of methanolic mushroom extracts on TNF-α production

The effect of the treatment with methanolic mushrooms extracts on TNF-α production was measured by ELISA in the supernatants of the cells treated with the extracts during 1 h before LPS stimulation. Under basal conditions, macrophages produced low levels of TNF-α, while stimulation with LPS induced a high production of the cytokine (about 165-fold) that was unchanged in the treated groups (Fig. 2, panel B).

3.4. Effect of methanolic mushroom extracts on proinflammatory gene expression by macrophages

To elucidate whether the inhibitory effect observed on NO production by some of the studied extracts was due to the down-regulation of iNOS mRNA expression, real time PCR assays were performed. The level of iNOS mRNA expression was significantly elevated in macrophages treated with LPS. Pretreatment with methanolic extracts from *A. bisporus*, *C. cibarius*, *C. cornucopioides* and *L. deliciosus*, but not from *B. edulis* and *P. ostreatus*, attenuated LPS-induced iNOS expression (Fig. 3, panel A). These results suggest that the inhibitory effect of the extracts on LPS-induced NO production was mediated by the inhibition of iNOS expression.

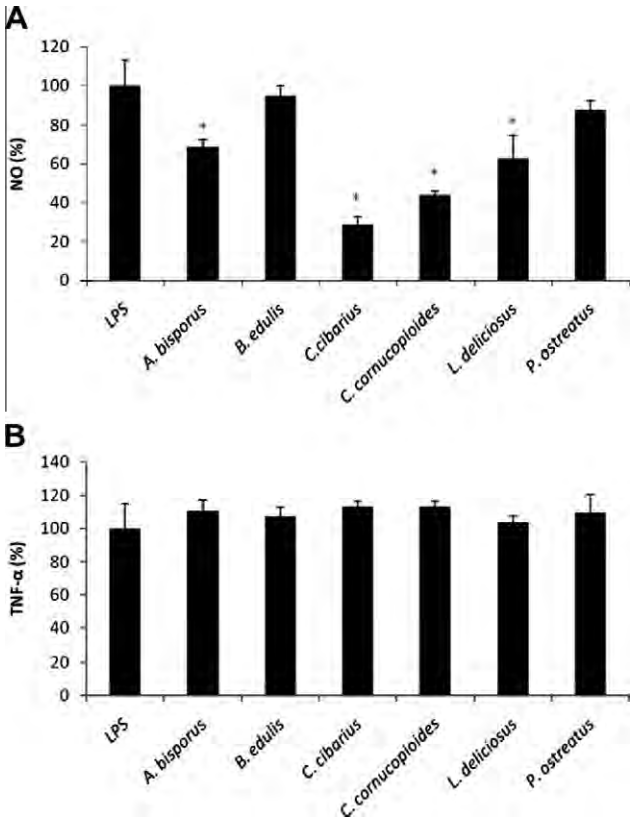

Fig. 2. Effect of methanolic mushroom extracts on LPS-induced inflammatory mediators in RAW 264.7 macrophage-like cells. NO (A) and TNF-α (B) production are expressed as percentages of that of the group treated with LPS alone. Values show the means and standard deviations of four different experiments performed in triplicate. $^{*}p < 0.05$ significantly different from the LPS group.

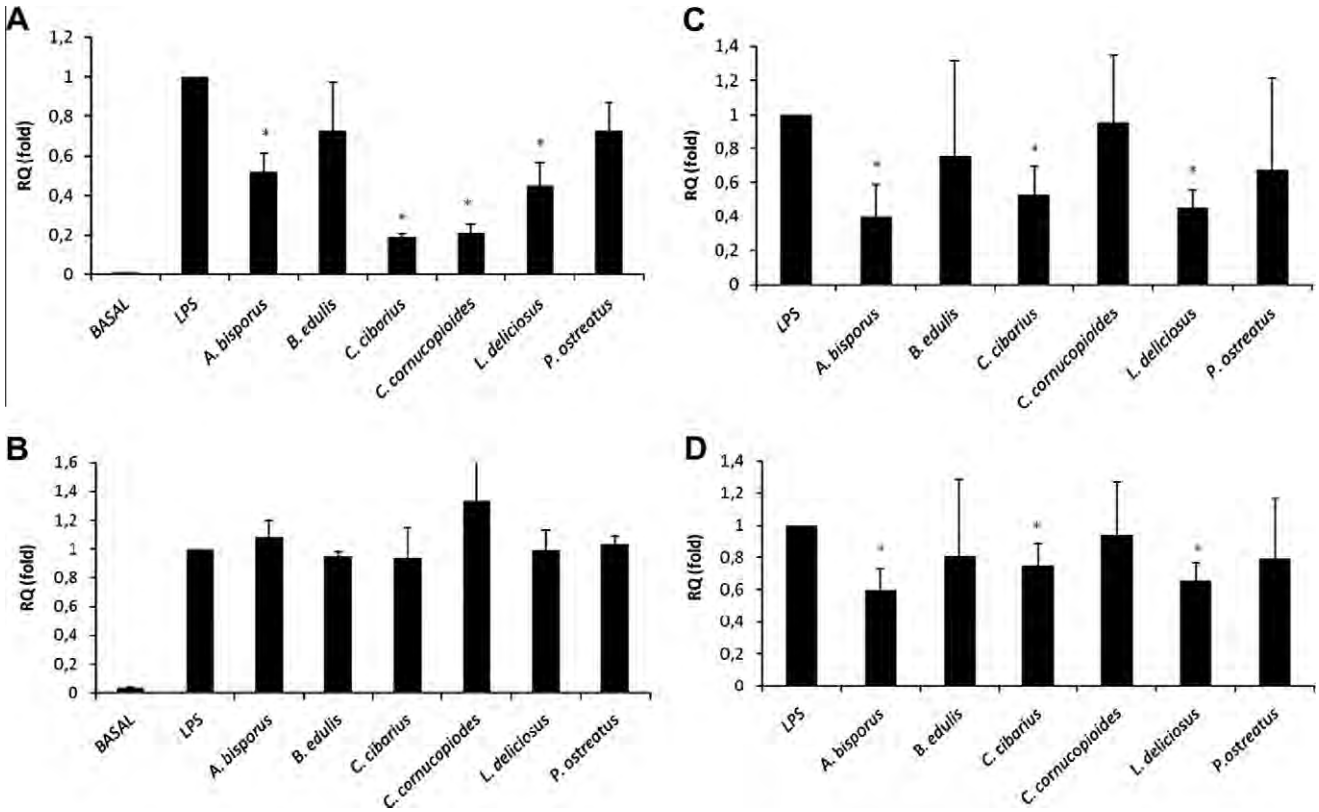

Fig. 3. Effect of methanolic mushroom extracts on pro-inflammatory mRNA expression in LPS stimulated macrophages: iNOS (A), TNF-α (B), IL-1β (C) and IL-6 (D). Results are expressed as fold of change from reference (LPS) treatment. RQ values are calculated relative to the ACTB gene. Values show the means and standard deviations of four different experiments performed in triplicate. $^{*}p < 0.05$ significantly different from the LPS group.

In addition, the mRNA expression of other proinflammatory mediators in activated macrophages was evaluated. LPS treatment caused a marked increase in mRNA expression of all of studied mediators: TNF-α, IL-1β, and IL-6 (Fig. 3, panels B, C and D). Macrophages preincubation for 1 h with mushroom extracts had no effect on TNF-α mRNA expression (Fig. 3, panel B), whereas different effects were observed in IL-1β and IL-6 mRNA expressions with the different mushroom extracts. Thus, the pretreatment with *A. bisporus*, *C. cibarius* and *L. deliciosus* produced a reduction of both IL-1β and IL-6 mRNAs expressions induced by LPS (Fig. 3, panels C and D), being this effect more marked in IL-1β expression. By contrast, the extracts from *B. edulis*, *C. cornucopioides* and *P. ostreatus* failed to inhibit interleukins expression.

4. Discussion

The present study was undertaken to elucidate the anti-inflammatory potential of different edible mushroom species: *A. bisporus*, *B. edulis*, *C. cibarius*, *C. cornucopioides*, *L. deliciosus* and *P. ostreatus*. Indeed, phenolic compounds of different natural origins have demonstrated to elicit potent anti-inflammatory properties (García-Lafuente et al., 2009). Therefore, we prepared phenolic–rich methanolic extracts from mushroom to test their biological effects on the production of inflammatory mediators in macrophages RAW 264.7 upon stimulation with LPS. The results indicated that extracts from some of the studied species inhibited LPS-induced NO production, and the expression of pro-inflammatory cytokines, such as IL-1β, and IL-6. The most efficient species were *A. bisporus*, *C. cibarius* and *L. deliciosus*, while others, such as *B. edulis* and *P. ostreatus*, had no effect.

Macrophages play an important role in both, host-defence mechanisms and inflammation. Activated macrophages secrete a number of different inflammatory mediators, including NO, TNF-α, IL-1β and IL-6. The overproduction of these mediators has been implicated in several inflammatory diseases and cancer (Lin & Karin, 2007). Thus, inhibition of activation of these cells appears to be an important target for the treatment of inflammatory diseases. NO is one ubiquitous cellular mediator of physiological and pathological processes, being largely released at inflammatory sites (Moncada, Palmer, & Higgs, 1992). Stimulation of macrophages with LPS induces a high production of NO by the inducible isoform of the enzyme, nitric oxide synthase (iNOS) (Gupta, Sundaram, Reuter, & Aggarwal, 2010). High levels of NO, produced by iNOS, have been defined as cytotoxic in inflammation and entotoxemia (Kröncke, Fehsel, & Kolb-Bachofen, 1997). In the present work, methanolic extracts from *A. bisporus*, *C. cibarius*, *C. cornucopioides* and *L. deliciosus* effectively inhibited NO production in activated macrophages through inhibition of iNOS mRNA expression, without affecting cell viability. Methanolic extracts from other mushroom species have been found to exhibit similar inhibitory effects. For instance, the methanolic extract from *I. obliquus*, a medicinal mushroom with reported inmunomodulatory and hepatoprotective effects, inhibited LPS-induced NO production in RAW 264.7 macrophages through inhibition of iNOS mRNA expression (Park et al., 2005). Several phenolic compounds, such as quercetin or resveratrol, have been shown to directly inhibit the expression of proinflammatory cytokines and iNOS and COX-2 genes (Hämäläinen, Nieminen, Vuorela, Heinonen, & Moilanen, 2007; Wood, Wark, & Garg, 2010).

The effect of mushroom extracts on the mRNA expression of different proinflammatory mediators, such as TNF-α, II-1β and IL-6, in activated macrophages was studied. The assayed methanolic mushroom extracts were unable to abolish the elevated TNF-α mRNA expression induced by LPS. By contrast, the extracts from

A. bisporus, *C. cibarius* and *L. deliciosus* significantly inhibited the mRNA expression of the other proinflammatory cytokines, IL-1β and IL-6, suggesting an anti-inflammatory effect of these extracts.

Although many substances may participate in the anti-inflammatory activity, phenolic compounds have been largely recognised as natural molecules with anti-inflammatory effects. Positive correlations have been found between total phenolic content and some anti-inflammatory effects of different mushroom extracts (Cheung, Cheung, & Ooi, 2003; Kim et al., 2008). Phenolic compounds comprise a wide range of substances displaying a great diversity of structures; therefore, the biological activity may be strongly influenced by the chemical nature. The content, in the studied mushroom extracts, of different phenolic compounds, has been previously determined by our group and it is summarised in Table 1 (Palacios et al., 2011). Although phenolic acids were the most abundant compounds in the studied species, their content does not correlate with the inhibitory capacity of the extracts, which suggests that the contribution of these kinds of compounds to the anti-inflammatory activity is not relevant. Regarding cinnamic acid derivatives, caffeic acid has been previously described to down-regulate the production of proinflammatory cytokines in CACO-2 cells (Zhao, Shin, Satsu, Totsuka, & Shimizu, 2008). By contrast, in other studies, cinnamic acids derivatives, such as caffeic acid, chlorogenic acid, ferulic acid and *p*-hydroxybenzoic acid, from an ethanolic plant extract, did not affect NO production in LPS-stimulated RAW 264.7 macrophages (Yu, Hsu, & Yen, 2009). In our work, none of the cinnamic acid derivatives present in the mushroom extracts correlates with the anti-inflammatory activity. In contrast, pyrogallol, that is only present in extracts of *A. bisporus*, *C. cibarius*, *C. cornucopioides* and *L. deliciosus* (Palacios et al., 2011), may be partly responsible of the anti-inflammatory activity observed, since these extracts were the most active in terms of NO inhibition. In the same way, pyrogallol from the medicinal plant *Emblica officinalis*, has been identified as one of the active compounds responsible of the anti-inflammatory effect of *Emblica* extracts in bronchial epithelial cells (Nicolis et al., 2008). Synergisms between pyrogallol and other active compounds present in our extracts, such as flavonoids or cinnamic acid derivatives, could explain the differences in activity among the studied extracts.

TNF-α is an important pro-inflammatory cytokine and, like NO, is involved in normal physiological immune and inflammatory processes. However, when inappropriately expressed, TNF-α also plays a role in the development of chronic inflammation and associated diseases (Szekanecz, 2008). RAW 264.7 macrophages stimulated with LPS, produced a high amount of TNF-α. Interestingly, although our mushroom extracts reduced the LPS-stimulated IL-1β, and IL-6 expressions, they were not able to inhibit either the elevated TNF-α production in activated macrophages or the TNF-α mRNA expression. Recently, Feng, Ling, and Duan (2010) have obtained similar results with lycopene, a naturally occurring antioxidant, in RAW 264.7 cells stimulated with LPS (Feng et al., 2010). Lycopene inhibited LPS-induced production of NO and IL-6 with decreased mRNAs of iNOS and IL-6 but it had no effect on TNF-α. The authors investigated the effect of lycopene in the involved signal transduction pathways, and found that lycopene significantly inhibited the effects of LPS by suppressing a key inflammatory pathway related to mitogen-activated protein kinase (MAPK) and nuclear factor κB (NF-Kb). Lycopene significantly inhibited the activation of ERK1/2 and p38 MAPK by LPS, but it had no effect on the activation of JNK (Feng et al., 2010). The fact that lycopene was effective in suppressing the LPS-induced NO, and IL-6 but not TNF-α, supports the hypothesis that the expression of IL-1β and IL-6 is predominantly affected by ERK1/2 whereas that of TNF-α is mainly regulated by JNK, after LPS stimulation (Swantek, Cobb, & Geppert, 1997). In the present work similar results have been obtained with methanolic extracts from the different assayed mushroom species, which suggests that the anti-inflammatory effect observed, could be mediated by the selective inhibition of different upstream factors in macrophage activation by LPS.

The results obtained in the present work suggest that some compounds present in the tested mushroom extracts can down-regulate the cascade of events that takes place in inflammation. The species that demonstrated higher anti-inflammatory activity were *A. bisporus*, *C. cibarius* and *L. deliciosus*, inducing inhibition of NO production and iNOS, IL-1β and IL-6 expression. *C. cornucopioides* was only able to inhibit NO production and iNOS expression, but was not active in terms of interleukin expression inhibition. Finally, *B. edulis* and *P. ostreatus* did not present any anti-inflammatory effect in our conditions. Although some of these species have been investigated, others, such as *C. cibarius*, *B. edulis*, *C. cornucopioides* or *L. deliciosus*, have not been studied yet. As far as we know only scarce reports about the compositions of some of them have been published. This is the first report of anti-inflammatory activity of edible mushroom extracts from species, such as *C. cibarius*, *C. cornucopioides* and *L. deliciosus*.

In conclusion, we have demonstrated, apparently for the first time, anti-inflammatory activity of some edible mushroom species,

Table 1
Concentration of phenolic compounds in methanolic mushroom extracts of edible mushrooms: *A. bisporus*, *B. edulis*, *C. cibarius*, *C. cornucopioides*, *L. deliciosus* and *P. ostreatus*. Data are obtained from Palacios et al. (2011) and are expressed as ng of phenolics per ml of mushroom extract.

	A. bisporus	*B. edulis*	*C. cibarius*	*C. cornucopioides*	*L. deliciosus*	*P. ostreatus*
Cinnamic acid derivatives						
Caffeic acid	7.77	7.55	8.17	n.d.	7.76	n.d.
Chlorogenic acid	31.9	31.4	n.d.	n.d.	31.4	n.d.
p-Coumaric acid	5.19	0.43	n.d.	n.d.	n.d.	5.57
Ferulic acid	8.18	n.d.	5.19	7.01	5.71	10.1
Hydroxybenzoic acid derivatives						
Gallic acid	47.5	107	80.9	59.4	81.2	145
Gentisic acid	n.d.	30.4	27.0	n.d.	28.8	146
p-Hydroxybenzoic acid	7.69	12.0	7.84	3.14	10.7	2.34
Homogentisic acid	1722	1146	158	426	183	315
Protocatechuic acid	8.10	84.2	21.4	2.65	9.32	9.66
Flavonoids						
Myricetin	11.1	8.99	11.6	18.0	10.4	11.0
Catechin	0.25	n.d.	2.91	n.d.	n.d.	n.d.
Others						
Pyrogallol	129	n.d.	45.5	46.2	13.1	n.d.

n.d.: Not detected.

in terms of inhibition of NO production and iNOS, IL-1β, and IL-6 gene expression in activated macrophages. Our study suggests a potential use of edible mushrooms as a source of anti-inflammatory agents and they may also be considered as a functional food.

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