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Original Article

Anti-inflammatory activity of a novel lectin isolated from *Pleurotus eryngii* var. *ferulae* mushroom*

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ABSTRACT

Background and Aims: *Pleurotus* species are edible mushrooms with important economic and medicinal value. Many pharmacological properties of these species, such as anticancer, immunomodulating, and anti-inflammatory activities, have been attributed to lectin. The aim of this study was to purify a novel lectin from *Pleurotus eryngii* var. *ferulae* (PEFL) and investigate its anti-inflammatory activity.

Methods: PEFL was purified by 80% ammonium sulphate fractionation, diethylaminoethyl (DEAE)-Sepharose-4B anion exchange, and Sephadex G-100 gel filtration chromatography. The molar mass of the purified lectin was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions. Inhibition of lectin-induced haemagglutination by several carbohydrates and one glycoprotein (ovalbumin) was also performed using the haemagglutination inhibition activity test. The anti-inflammatory effect of PEFL was tested in a lipopolysaccharide (LPS)-induced inflammation model in mouse macrophage cells (RAW 264.7). The levels of prostaglandin (PG)-E2, tumour necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), and interferon-gamma (IFN-γ) were determined using enzyme-linked immunosorbent assay (ELISA) kits. Furthermore, the expression of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) was estimated by western blot analysis.

Results: PEFL was obtained in 16% yield. The specific activity of PEFL was calculated as 1280 haemagglutinating units (HU)/mg protein and was inhibited only by D-galactose. The molecular weight was determined to be 46 kDa. PEFL showed an anti-inflammatory effect by reducing the production of pro-inflammatory PGs through COX-2 inhibition, as well as reducing iNOS expression. Furthermore, our findings reported that PEFL has a protective effect on inflammation by decreasing the production of pro-inflammatory cytokines and increasing the production of the anti-inflammatory cytokine (IL-10).

Conclusion: The results suggest that PEFL can be considered as a potential therapeutic agent in the development of new therapeutic strategies for inflammatory diseases.

Keywords: Cytokines, Lectin, Lipopolysaccharide, Mushroom, *Pleurotus eryngii* var. *ferulae*, RAW 264.7 macrophage

INTRODUCTION

In recent years, lectins have begun to be investigated more extensively because it is understood that these proteins are invaluable tools for the structural and functional examination of complex carbohydrates, especially glycoproteins, specific drug targeting, bioadhesion applications, diagnostics, and cancer therapy (Santos et al., 2014; Dan, Liu & Ng, 2015). It has been discovered that lectins play a role as recognition molecules in many biological events such as signal transduction, regulation of glycoprotein synthesis and intracellular trafficking, cancer and metastasis, attachment of infecting agents to host cells, directing leukocytes to inflammation sites, immunomodulation, and regulation of cell growth and apoptosis (Sharon & Lis, 2004; Muramoto, 2017; Coelho et al., 2017).

Today, scientific researches are focussed on drug production from pure substances obtained from natural sources. Mushrooms have been used as food for centuries as well as medicine for the treatment of many diseases. Mushrooms can be considered as a healthy food, rich in polysaccharides, proteins, and other functional components with many bioactivities that

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increase immunity, reduce the risk of cancer, prevent the proliferation of tumour cells, and protect the nervous system. Overall, the spectrum of beneficial effects of mushrooms is heterogeneous, and it has been suggested that mushrooms can be consumed daily as one of the best acceptable nutraceutical foods (Ma et al., 2018).

Lectins isolated from medicinal mushrooms are used in the prevention and treatment of many diseases, and scientific studies on this subject have been increasing rapidly all over the world. It has been reported that lectins isolated from mushrooms have mitogenic, antiviral, antitumor, immunomodulatory, and anti-HIV-1 reverse transcriptase activities (Singh, Bhari & Kaur, 2010; Hassan, Rouf, Tiralongo, May & Tiralongo, 2015). Medicinal mushroom lectins can be considered as potential therapeutic agents in the development of new therapeutic strategies for various cancers and inflammatory diseases because of their anti-inflammatory and immunomodulatory effects (Lull, Wichers & Savelkoul, 2005; Muszyńska, Grzywacz-Kisielewska, Kała & Gdula-Argasińska, 2018). Many mushroom lectins have been reported to date. Lectins from higher fungi have been reviewed by Guillot and Konska (1997) and Wang, Ng, and Ooi (1998). Singh et al. (2010) published a comprehensive review of 336 mushroom lectins. A complete list of lectins identified in mushrooms and their carbohydrate and/or glycoprotein specificity was provided by Hassan et al. (2015).

Pleurotus species are edible mushrooms with important economic and medicinal value (Khan & Tania, 2012). Studies have reported that *Pleurotus* species have antitumor, antioxidant, antiviral, antibacterial, hypocholesterolemic, hypoglycaemic, immunomodulatory, and anti-HIV-1 reverse transcriptase effects and reduce blood pressure and blood lipid levels (Li, Liu, Wang & Ng, 2008; Patel, Naraian & Singh, 2012; Anjana & Savita, 2017). In addition, their antiviral activities against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been recently investigated (Elhusseiny et al., 2022).

Pleurotus eryngii (DC. ex Fr.) Quel. var. *ferulae* Lanzi (PEF) is an edible mushroom of the family Pleurotaceae and the order Agaricales (Jo et al., 2019). PEF is frequently consumed and distributed in the Eastern Anatolia Region of Turkey (Akyüz & Kırbağ, 2007). PEF extracts have been reported to have antioxidant (Hu, Lien, Hsieh, Wang, & Chang, 2009), antiatherosclerotic (Mori, Kobayashi, Tomita, Inatomi & Ikeda, 2008; Choi, Kim, Kim & Kim, 2017), antitumor, and immunostimulatory (Choi, Cha, Kang & Lee, 2004; Yuan et al., 2017a; Sun, Hu & Li, 2017) effects.

New sources should be explored to isolate novel lectins with potential therapeutic properties. The aim of this study was to elucidate the anti-inflammatory activity of lectin purified to homogeneity from PEF mushroom.

MATERIALS AND METHODS

Materials and Chemicals

A glass column (Pharmacia Fine Chemicals, Upsala, Sweden), diethylaminoethyl (DEAE)-Sepharose ion exchange resin (DCL6B100, Sigma-Aldrich, St. Louis, MO, USA), and Sephadex G-100 (17-0061-01, Pharmacia) were used during the purification processes. RAW 264.7 mouse macrophage cells (ATCC TIB-71) were obtained from the American Type Culture Collection. Dulbecco's Modified Eagle Medium (DMEM) (319-005 CL, Wisent, Montreal, QC, Canada), penicillin-streptomycin (450-201-EL, Wisent, Montreal, QC, Canada) and fetal bovine serum (FBS) (FBS-11A, Capricorn, Ebsdorfergrund, Germany) were used for cell culture. All ELISA kits were obtained from Thermo-Invitrogen (Carlsbad, CA, USA). Anti-iNOS (ab15323, Abcam Cambridge, MA, USA), anti-COX-2 (NB100-689SS, Novus Bio, Littleton, CO, USA), anti-beta-actin (MO1263, BOSTER, Beijing, China), anti-rabbit IgG H&L (HRP) (ab205718, Abcam Cambridge, MA, USA), anti-mouse IgG (HRP) (sc-2005, Santa Cruz, CA, USA) antibodies and protein ladder (LC5615, Thermo-Invitrogen, Carlsbad, CA, USA) were used for western blot analysis. Lipopolysaccharide (LPS) (L2654), bicinchoninic acid (BCA) kit (BCA1), and phenazine methosulfate (PMS) (P9625) were obtained from Sigma (St. Louis, MO, USA), and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (G1112) was obtained from Promega (Madison, WI, USA).

Mushroom material

PEF mushrooms were collected and dried by Dr. Hevidar Alp at Munzur University, Tunceli Province, Turkey, in April-May-June 2020. The identification of the species was performed by Prof. Dr. Abdulnasır Yıldız from Dicle University, Faculty of Science, Department of Biology, Turkey.

Purification of lectin from *Pleurotus eryngii* var. *ferulae* (PEFL)

30 g of dried PEF were crushed with a blender until it became a powder, and then incubated overnight with 500 mL PBS (phosphate-buffered saline), pH 7.4, in a refrigerator. The supernatant was collected by centrifugation at 10,000 rpm for 20 min in a refrigerated centrifuge to obtain the crude extract. Proteins in the crude extract were precipitated by the addition of $(NH_4)_2SO_4$ to 80% saturation. The precipitate, which was obtained by centrifugation at 10,000 rpm for 20 min at 4°C, was dissolved in PBS. After centrifugation at 10,000 rpm for 10 min to remove unabsorbed materials, the supernatant was named the 80% NH_4SO_4 fraction and dialysed against the same buffer, and then applied to a DEAE-Sepharose-4B (1x15 cm) pre-equilibrated with 6 mM PBS. The column was eluted with

a linear gradient from 25 to 1000 mM NaCl in PBS. The fractions were collected in 1 mL volumes at a flow rate of 30 mL/h. The fraction showing haemagglutinating activity was concentrated by ultrafiltration through a Millipore CX-10 membrane with a stirred cell (model; Amicon, Inc., Beverly, Mass.) and dialysed against PBS. The concentrated and dialysed fraction was then applied to a Sephadex G-100 column, which had previously been equilibrated with 6 mM PBS and eluted with the same buffer at a flow rate of 30 mL/h. The elutes were collected in 1 mL fractions, assayed at 280 nm for protein content, and tested for haemagglutinating activity. The fraction with the highest haemagglutinating activity was concentrated and stored at -20°C for analysis.

Determination of the protein concentration

Protein concentration was determined by measuring at 280 nm and using the BCA kit. The BCA method was performed by optimising the kit protocol recommended by the manufacturer using bovine serum albumin as a standard.

Evaluation of the stability of the haemolysin activity in crude mushroom extract

To inhibit the haemolytic activity of haemolysin found in PEF, which causes the haemolysis of erythrocytes and prevents the interpretation of haemagglutination activity tests, 1 mL of crude extract was heated at 37°C and 50°C for 30 min, and the haemolytic activity was examined. In addition, serial dilutions of 50 mM Na_2CO_3, Na_3PO_4, $FeCl_2$, and $CuCl_2$ salt solutions were added to equal volumes of the crude extract in separate experiments to examine the effect of some salts on the haemolytic activity of PEF haemolysin.

Measurement of PEFL haemagglutinating activity

The test was performed by making serial 2-fold dilutions of PEFL solution in 0.9% NaCl solution using U-bottom microplates. To each well 25 µL of 4% human (A Rh+ blood group) erythrocyte suspension was added, and after incubation at room temperature for 30 min, haemagglutination was determined both visually and microscopically. Haemagglutination titre was expressed as the reciprocal of the dilution exhibiting detectable agglutination, according to Lis & Sharon (1972). The results were expressed as a haemagglutinating unit (HU), which is the minimum amount of sample capable of inducing agglutination. Specific activity was defined as the number of haemagglutinating units per mg of protein (HU/mg).

Haemagglutination inhibition test: determination of the specific carbohydrate unit of lectin

To investigate the inhibition of lectin-induced haemagglutination by several carbohydrates and one glycoprotein (ovalbumin) at different concentrations, the haemagglutinating inhibition test was performed in a manner similar to the haemagglutination test. Serial 2-fold dilutions of sugar samples were prepared in PBS and mixed with an equal volume (25 µL) of a PEFL solution containing four haemagglutination units. The mixture was incubated for 30 min at room temperature and then mixed with 25 µL of a 4% erythrocyte suspension.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The purity and molecular mass of the protein were determined by SDS-PAGE according to the Laemmli method (Laemmli, 1970) and visualised by 0.2% Coomassie blue R-250 staining. The molecular weight was determined by comparing the electrophoretic mobility of lectin with that of marker proteins from Invitrogen (LC5615).

Cell culture

RAW 264.7 cells (ATCC TIB-71) were cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin, followed by incubation under a humidified environment containing 5% CO_2 at 37°C. For enzyme-linked immunosorbent assay (ELISA) and western blot analysis, $3x10^6$ cells suspension was cultured in 100-mm Petri dishes and incubated for 24 h at 37°C and 5% CO_2. After incubation, cells were treated with different concentrations (100, 50, 25, 12.5, and 6.25 µg/mL) of PEFL and then stimulated with LPS (1 µg/mL) (Yoon et al., 2009) for 24 h. To determine the effect of LPS, one group of cells was treated with LPS only; as a negative control, non-treated cells with any LPS or lectin were used. All Petri dishes were incubated for a further 24 h in a 5% CO_2 incubator at 37°C. After incubation for 24 h, the cells were harvested and separated into two groups to be evaluated for cytokine production and western blot analysis.

Cytotoxic activity of PEFL in macrophages

RAW 264.7 cells ($2x10^4$) were seeded into 96-well plates and incubated overnight. The next day, PEFL was added to the wells at different concentrations (100, 50, 25, 12.5, and 6.25 µg/mL) and incubated for a further 24 h. After incubation, cell viability was assessed using the MTS assay according to the manufacturer's instructions. Briefly, 20 µL of MTS/PMS mixture was added and incubated for 2-4 h at 37°C. The absorbance was then read on the microplate reader device (Eon Biotek, Winooski, VT, USA) at 490 nm. Cell growth inhibition percentages were

calculated as Cell growth inhibition (%) = [1-(Absorbance of sample/Absorbance of control)]x100

Enzyme-linked immunosorbent assay (ELISA)

The harvested cells were sonicated by ultrasonic homogenizer (Art-MICCRA D-1, Heitersheim, Deutschland) with a 10-15 second interval, then centrifuged at 10,000 g for 5 min in a refrigerated centrifuge, and the supernatants were collected. Commercial ELISA kits were used for tumour necrosis factor-alpha (TNF-α), prostaglandin-E2 (PG-E2), interferon-gamma (IFN-γ), interleukin-1-beta (IL-1β), interleukin-6 (IL-6), and interleukin-10 (IL-10) levels determination, and the test was performed according to the manufacturer's instructions.

Western blot analysis

The harvested cells were washed with cold PBS and lysed in radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate and 0.1% SDS). The samples were centrifuged at 24,000 g for 45 min in a refrigerated centrifuge at 4°C, and the supernatants were used to determine cyclooxygenase-2 (COX-2) and nitric oxide synthase (iNOS) protein levels by western blot analysis. For western blot analysis, an equal amount of cellular protein (20 µg/mL) was separated by SDS-PAGE. The separated proteins were transferred to a polyvinylidene difluoride membrane using the Trans-Blot Turbo™ transfer system (Bio-Rad, Hercules, CA, USA). The membrane was then blocked with 5% non-fat milk blocking buffer and consecutively incubated with primary antibodies [iNOS (1:500), COX-2 (1:20000), and beta-actin (1:10000)] and HRP-conjugated secondary antibodies [anti-mouse (1:10000) and anti-rabbit (1:20000)]. In the last step, the membrane was incubated with a chemiluminescent substrate for 5 min and visualised by the imaging system (Vilber Lourmat, Fusion FX5, Marne-la-Vallée, France).

Statistical analysis

The data are presented as mean ± standard deviation. GraphPad Prism 9 (GraphPad Software, La Jolla, CA, USA) and SPSS 20.0 (SPSS Inc, Chicago, IL, USA) were used to evaluate the differences between the tested items. A p-value of <0.05 was set as the limit of significance.

RESULTS

Purification of lectin from *Pleurotus eryngii* var. *ferulae* (PEFL)

Elution of the PEFL active 80% ammonium sulphate fraction through a DEAE-Sepharose-4B column with a stepwise NaCl gradient resulted in the appearance of four protein peaks, with one peak showing haemagglutinating activity (eluted with PBS containing 0.2 M NaCl) (Figure 1). The peak with haemagglutinating activity was further purified by gel filtration chromatography on Sephadex G-100. The chromatogram showed one peak with haemagglutinating activity, as shown in Figure 2.

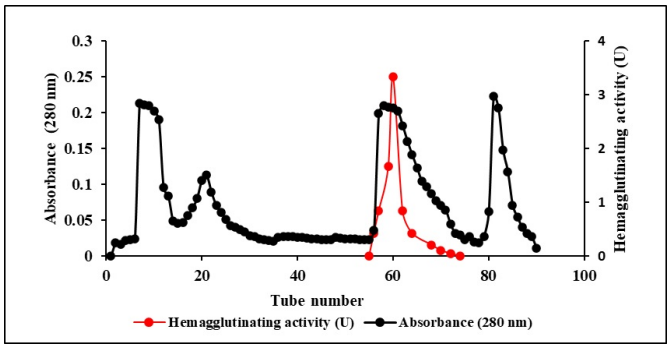

Figure 1. DEAE-Sepharose-4B ion exchange chromatography elution profile of the 80% ammonium sulfate fraction of PEF crude extract.
Column: 15 x 1 cm, sample volume: 12 mL (28.8 mg protein), flow rate: 1 mL/min, the lectin was eluted with a linear gradient of 0.25-1 M NaCl in 6 mM PBS buffer.

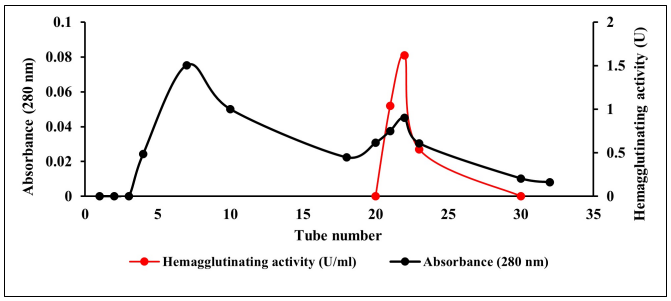

Figure 2. Elution profile of the elute obtained by DEAE-Sepharose-4B on the Sephadex G-100 column.
Column dimensions: 10 x 1 cm, sample volume 1.6 mL (5.4 mg protein), flow rate: 1 mL/min. Buffer used: 6 mM PBS.

PEFL was purified approximately 16-fold over the crude extract. The specific activity of the purified lectin was 1280 HU/mg. The results of the purification of PEFL are summarised in Table 1.

It examined the stability of the haemolysin activity in the crude mushroom extract. It was found that the haemolytic activity of the haemolysin was not inhibited by heating the crude extract at 30°C and 50°C for 30 min. Furthermore, it was observed that among all the salts examined, only Na_3PO_4 inhibited the haemolytic activity of the lectin at a concentration of 25 mM, but the haemolytic activity recovered after 30 min. The haemagglutinating activity of the lectin was determined within 30 min.

Among the glycoproteins and various carbohydrates tested, D-galactose was the only carbohydrate that could inhibit the haemagglutinating activity of PEFL (Table 2).

Table 1. Purification of PEFL (30 g powder).

Step	Volume (mL)	Protein (mg/mL)	Total protein (mg)	Haemagglutinating activity (HU)	Specific activity (HU/mg)	Purification fold
Crude extract	400	3.5	1400	112000	80	1
80% $(NH_4)_2SO_4$	12	2.4	28.8	4608.1	160	2
DEAE-Sepharose-4B	3.4	1.6	5.4	3481.2	644	8
Sephadex G-100	2	1	2	2560	1280	16

Table 2. Haemagglutination inhibition test of PEFL by various sugars and one glycoprotein (initial haemagglutinating activity is 4 haemagglutinating units).

Carbohydrate/glycoprotein	Concentration (mM)							
	100	50	25	12.5	6.25	3.12	1.56	0.78
D- Lactose	+	+	+	+	+	+	+	+
D- Glucosamine hydrochloride	+	+	+	+	+	+	+	+
Methyl-α-D-mannopyranoside	+	+	+	+	+	+	+	+
D- Fucose	+	+	+	+	+	+	+	+
D- Mannose	+	+	+	+	+	+	+	+
N-Acetyl-D-galacosamine	+	+	+	+	+	+	+	+
N-Acetyl-D-galactosamine	+	+	+	+	+	+	+	+
4-Nitrophenyl-β-D-glucopyranoside	+	+	+	+	+	+	+	+
4-Nitrophenyl-α-D-glucopyranoside	+	+	+	+	+	+	+	+
D- Raffinose	+	+	+	+	+	+	+	+
Ovalbumin	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
D- Galactose	-	-	-	-	-	-	-	-

+: haemagglutinating activity; –: no haemagglutinating activity.

PEFL showed a single band with a molecular weight of 46 kDa determined by SDS–PAGE under denaturing conditions (Figure 3).

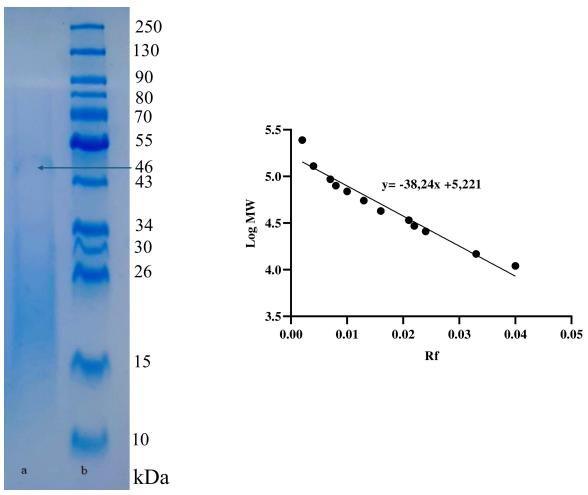

Figure 3. Molecular weight determination based on SDS-PAGE.
The monomeric subunit molecular weight of the lectin was calculated from the calibration curve and estimated to be 46 kDa as a single monomeric subunit using 10% SDS-PAGE (Coomassie blue stained). a) Sephadex G-100 chromatography output. b) Protein molecular mass ladder.

Cytotoxic activity of PEFL

To evaluate the cytotoxic effects of PEFL on macrophage cells, we examined macrophage cell growth inhibition in the presence of different concentrations of PEFL using the MTS assay. As shown in Figure 4, PEFL showed low cytotoxic activity at 100, 50, and 25 µg/mL and high cytotoxic activity at 200 µg/mL concentration. The remaining concentrations were non-toxic against macrophage cells.

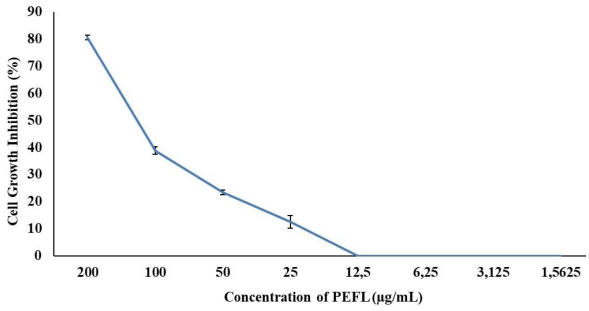

Figure 4. Effect of PEFL on cell growth inhibition in RAW 264.7 cells.
Data were expressed as mean ± standard deviation (n=3). Values represent cell growth inhibition (% inhibition) resulting from 24 h incubation with PEFL.

Anti-inflammatory effects of PEFL

Effect of PEFL on cytokine profile

The anti-inflammatory effects of PEFL were tested on lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 macrophages. In the LPS-treated group, prostaglandin-E2 (PG-E2) and cytokine levels were significantly ($p < 0.05$) higher than those in the control group. It was observed a significant reduction in PG-E2, tumour necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β), interleukin-6 (IL-6), and interferon-gamma (IFN-γ) levels, as well as a significant increase in interleukin-10 (IL-10) levels in the LPS+PEFL treated groups ($p < 0.05$), compared with the only LPS treated group (Figure 5). No inhibitory effects were detected at concentrations of 12.5 and 6.25 µg/mL.

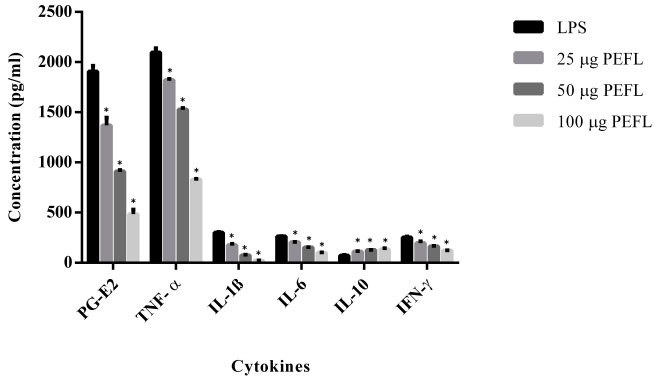

Figure 5. Cytokines levels in the supernatants of cell groups (Mean ± standard deviation). * p<0.05

Western blot analysis

In western blot analysis, nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were markedly expressed in the LPS-treated groups, whereas in the LPS+PEFL-treated groups, PEFL significantly inhibited the expression of these proteins in a concentration-dependent manner. iNOS protein expression was 100% inhibited by the application of PEFL at concentrations of 100 µg/mL and 50 µg/mL. Furthermore, inhibition of 61.81% was observed even at the lowest concentration (6.25 µg/mL). Considering the COX-2 protein level, it was determined that COX-2 protein expression decreased by 69.4% and 5.94% after the application of PEFL at concentrations of 100 µg/mL and 25 µg/mL, respectively (Figure 6).

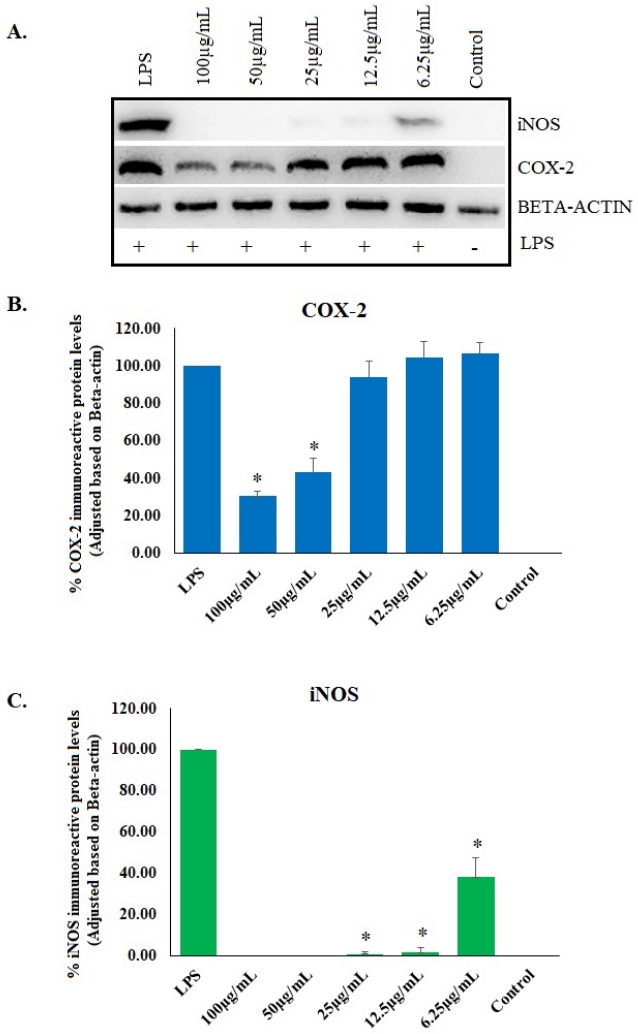

Figure 6. The effects of PEFL on iNOS and COX-2 protein expressions in LPS-induced RAW 264.7 cells.
A: The effects of different doses of PEFL on iNOS and COX-2 protein expressions in a model of LPS-induced inflammation (LPS 1 µg/mL).
B: Effect of PEFL on COX-2 protein expression in a model of LPS-induced inflammation. * p<0.05
C: Effect of PEFL on iNOS protein expression in a model of LPS-induced inflammation. * p<0.05

DISCUSSION

There is an increasing interest in lectins because of their unique carbohydrate-binding properties. The ability of lectins to specifically bind to glycoconjugates present on the cell surface has made them essential tools in diverse applications. Taking advantage of this specific interaction, it is possible to design drugs to combat many diseases such as infection, inflammation, and cancer (Coelho et al., 2017). Lectins have been obtained from different sources such as mammalian tissues, viruses, bacteria, insects, plant seeds and roots, and fungi (Hassan et al., 2015). Mushrooms are a rich source of lectins (Singh et al., 2010). It is well established that many mushroom-

extracted compounds are commonly used as immunomodulators or biological response modifiers (Zaidman, Yassin, Mahajna & Wasser, 2005).

The present study was based on the findings of Yuan et al. (2017b), in which four different protein fractions (DE1, DE2, DE3, and DE4) were obtained from *Pleurotus eryngii* and a protein (named PEP) with anti-inflammatory properties was isolated from the DE3 fraction. These results suggest that one of these four fractions may be lectin. In view of the importance of lectins, the present investigation was undertaken to purify a lectin from *Pleurotus eryngii* var. *ferulae* (PEFL) using a three-step procedure of $(NH_4)_2SO_4$ precipitation, ion exchange chromatography on DEAE-Sepharose-4B, followed by gel filtration chromatography on a Sephadex G-100 column. In addition, our results were in agreement with the work of Xu et al. (2014), who isolated and purified a D-galactose-specific lectin with strong proliferative and haemagglutination activity against mouse splenocytes from *P. ferulae* extract by applying 75% (NH4)2SO4 precipitation, DEAE-cellulose ion exchange, and Sepharose-6B affinity chromatography. A novel lectin with potent antitumor, mitogenic, and HIV-1 reverse transcriptase inhibitory activities from fresh fruiting bodies of the edible mushroom *P. citrinopileatus* (Li et al., 2008) and an N-acetylglucosamine-specific lectin from fresh sclerotia of the edible mushroom *P. tuber-regium* (Wang & Ng, 2003) were isolated using similar protocols.

The lectin's haemagglutinating activity underwent a decline because of the presence of haemolysin (Ngai & Ng, 2006; Shibata et al., 2010), which caused haemolysis of erythrocytes and prevented determination of haemagglutinating activity of the lectin. Lectins isolated from *Pleurotus* species have been identified with varying sugar specificities towards melibiose, galactose, lactose, raffinose, (*P. ostreatus*), inulin, maltose, ortho-nitrophenyl-β-D-galactopyranoside, ortho/para-nitrophenyl-β-D-glucopyranoside (*P. citrinopileatus*), N-acetylglucosamine (*P. tuber-regium*), N-acetylgalactosamine (*P. serotinus*), lactose (*P. spodoleucus*), methyl-α-D-galactoside, galactosamine, mannosamine, asialofetuin (PEL) (*P. eous*), and asialo-mucin (*P. cornucopiae*) (Hassan et al., 2015). The D-galactose specificity of the pure lectin obtained in this study was similar to that of lectin isolated from *P. ostreatus*.

The molecular weights of fungal lectins vary between 12 and 68 kDa (Singh et al., 2015). The lectin isolated from the fresh fruit body of *P. ostreatus* was reported to be a heterodimer, with molecular weights of 40 and 41 kDa for each subunit (Wang, Gao & Ng, 2000). The lectins of *P. citrinopileatus* (Li et al., 2008) and *P. tuber-regium* (Wang & Ng, 2003) were reported to be homodimers with a subunit molecular mass of 32 kDa. The molecular weight of the lectin from *P. ferulae* was determined to be 35 kDa and was found to be a homodimer with a subunit molecular mass of 17.5 kDa (Xu et al., 2014). In this study, the monomeric subunit molecular weight of PEFL was estimated to be 46 kDa, which is similar to that of *P. ostreatus* lectin.

Lectins have attracted attention because of their mitogenic, antitumor, antiproliferative, and immunomodulatory activities. Owing to their specificity in binding to surface receptors, certain lectins are potent immunomodulators (Zaidman et al., 2005). It has been reported that lectins have anti-inflammatory effects by decreasing the secretion of pro-inflammatory cytokines through I-kappa-B (IkB)/nuclear factor kappa B (NF-κB) transduction and increasing the production of anti-inflammatory cytokines (Coelho et al., 2017). In addition, it has been reported that they show anti-inflammatory effects by reducing the production of proinflammatory prostaglandins (PG) through the inhibition of cyclooxygenase-2 (COX-2) whose expression is frequently increased in many premalignant tissues and malignant tumours. It has been observed that lectins reduce the secretion of proinflammatory cytokines by suppressing the increased expression of induced nitric oxide synthase (iNOS) during inflammatory processes (Muszyńska et al., 2018).

There are several reports of *Pleurotus* extracts showing anti-inflammatory activities. The methanolic extract of *P. florida* was found to reduce carrageenan-induced and formalin-induced paw acute inflammation in mice (Jose, Ajith & Janardhanan, 2004). Jedinak, Dudhgaonkar, Wu, Simon, & Sliva (2011) reported that *P. ostreatus* concentrate demonstrates its anti-inflammatory effect by inhibiting lipopolysaccharide (LPS)-induced interleukin-6 (IL-6), interleukin-12, prostaglandin-E2 (PG-E2), and nitric oxide (NO) production and modulating the downstream regulation of iNOS and COX-2 expression via the suppression of NF-κB activation in RAW 264.7 cells. Tanaka, Nishimura, Sato, Sato, and Nishihira (2016) found that oyster mushroom extract stimulates macrophages by interferon-gamma (IFN-γ) which results in the activation of the immune system.

Nowadays, the researches are focussed on the isolation and characterisation of the pure active constituents of mushrooms, including mainly polysaccharides (in particular β-D-glucans), polysaccharopeptides (Lull et al., 2005) and proteins like lectins, fungal immunomodulatory proteins, among others (Motta, Gershwin & Selmi, 2021), which demonstrate immunomodulatory activities. An insoluble beta-glucan (pleuran) isolated from *P. ostreatus* was reported to suppress inflammation in a model of acute colitis in rats (Bobek, Nosálová & Cerná, 2001). The extract and polysaccharide isolated from *P. ostreatus* were reported to be effective in stimulating natural killer cells cytotoxic effect with induction of IFN-γ against lung and breast cancer cells, enhanced in the presence of interleukin-2 (El-Deeb et al., 2019). The anti-inflammatory effect of glucan isolated from *P. pulmonaris* was examined by acetic acid-induced writhing reaction in mice, which is a typical model to measure inflammatory pain, and it was observed that the glucan

inhibits leukocyte migration to damaged tissues (Smiderle et al., 2008). Minato, Laan, van Die & Mizuno (2019) reported that the *P. citrinopileatus* polysaccharide can inhibit the secretion of pro-inflammatory cytokines and chemokines in LPS/IFNγ activated macrophages and promote the expression of the anti-inflammatory cytokine interleukin-10.

Some immunomodulator compounds have been isolated from *P. eryngii*. A glycosphingolipid isolated from *P. eryngii* induces the secretion of IFN-γ and interleukin-4 from T cells (Nozaki et al., 2008). Sun et al. (2017) showed that a polypeptide isolated from *P. eryngii* mycelium is a good antioxidant with antitumor and immunostimulatory activities. In another study (Yuan et al., 2017b), a protein (PEP) isolated from P. eryngii showed strong anti-inflammatory effects by inhibiting pro-inflammatory mediators, including NO, interleukin-1-beta, and IL-6, and deactivating NF-κB and mitogen-activated protein kinase pathways. Although there is a possibility that the protein is a lectin, the study has not provided any explanation regarding this anti-inflammatory protein being a lectin. Therefore, there are currently no reports on the anti-inflammatory effectiveness of lectins isolated from *Pleurotus* species. In the present study, a lectin with potent anti-inflammatory activity that may be used as a prospective immunotherapeutic molecule in the treatment of some immunodeficiency diseases was isolated and purified to homogeneity from *P. eryngii* crude extract. Our study was in agreement with other studies showing that edible mushrooms possess anti-inflammatory activity (Muszyńska et al., 2018).

CONCLUSION

It was concluded that PEFL may play a role in the regulation of the inflammatory process by suppressing COX-2 and iNOS expressions and reducing the production of pro-inflammatory cytokines. Moreover, because of its anti-inflammatory and immunomodulatory effects, it can be considered as a potential therapeutic agent in the development of new therapeutic strategies for various cancers and inflammatory diseases. The present study is the first to show that the anti-inflammatory effect of *P. eryngii* is due to its lectin.

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