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ORIGINAL PAPER

Antinociceptive and anti-inflammatory properties of α-D-mannan from the yeast *Kluyveromyces marxianus*: evidence for a role in interleukin-6 inhibition

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Abstract

The management of inflammatory states typically involves non-steroidal anti-inflammatory drugs (NSAIDs) and opiates. Understanding the mechanisms underlying the processing of nociceptive information from potential alternatives such as some polysaccharides may enable new and meaningful therapeutic approaches. In this study, α-D-mannan isolated from the *Kluyveromyces marxianus* cell wall produced antinociceptive effects in models of inflammatory pain (formalin and complete Freund's adjuvant tests). Furthermore, α-D-mannan reduced paw edema and interleukin-6 (IL-6) production after carrageenan-induced inflammation. The polysaccharide α-D-mannan was characterized by gas chromatography–mass spectrometry, methylation analysis, and spectroscopic techniques. Moreover, the Doehlert experimental design was applied to find the optimal conditions for biomass production, with the best conditions being 10.8 g/L and 117 h for the glucose concentration and the fermentation time, respectively. These results indicate that α-D-mannan from *K. marxianus* exerts anti-inflammatory and antinociceptive effects in mice, possibly via a mechanism dependent on the inhibition of IL-6 production.

Keywords *Kluyveromyces marxianus* · Polysaccharide · Biological activity · Pain · Inflammation · Anti-inflammatory

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Introduction

Microorganisms are abundant sources of macromolecules with recognized potential for drug discovery with different therapeutic applications (do Nascimento et al. 2015; Wang et al. 2019; Yue et al. 2020) Among these macromolecules, polysaccharides extracted from fungi have been the subject of intense research, due to their high potential for application in treatments of a broad range of disorders.

Mannans are long-chain carbohydrates mainly composed of mannose residues, which can be found in the most diverse sources, such as microorganisms, vegetables, and seeds. In yeast, mannans are an important structural component of cell wall, with a composition directly related with the chemical and environmental conditions prevailing during culture growth (Faustino et al. 2021).

In general, mannans reveal a high potential for use in several applications due to their bioactive properties, such as immunomodulation and prebiotic activity, as well as antioxidant potential. Moreover, the low toxicity of mannans allows for their use in cosmetics, pharmaceutical, and biomedical industries (Faustino et al. 2021; Valasques Junior et al. 2023; Singh et al. 2018). α-D-Mannan from *Pseudozyma* sp. showed analgesic and anti-inflammatory activities (Valasques Junior et al. 2021).

Kluyveromyces marxianus is a fungus that has been isolated from a great variety of habitats. Given this broad range of habitats, this species has great metabolic diversity and potential biotechnological applications in various fields (Fonseca et al. 2008; Lane and Morrissey 2010). In addition, it has also achieved Qualified Presumption of Safely (QPS) and Generally Regarded As Safe (GRAS) status in European Union and United States, respectively, due to its long history in safe association and use with regular dairy products; which makes it particularly suitable to produce pharmaceuticals and food-grade proteins (Karim et al. 2020).

The yeast *K. marxianus* has attracted research interest, due to its characteristics that render it exceptionally suitable industrial application. This yeast can be used to production of enzymes, such as β-galactosidase, β-glucosidase, inulinase, and polygalacturonase (Barnby et al. 1990; Pessoa Jr and Vitolo 1999); single-cell proteins (Schultz et al. 2006); aromatic compounds (Medeiros et al. 2001); and ethanol (Ballesteros et al. 2004; Banat et al. 1996). Other potential applications of the yeast *K. marxianus* include reduction of lactose content in food products (Martins et al. 2002); production of bioingredients from whey cheese (Belem et al. 1997); bioremediation (Aksu and Dönmez 2000), baker's yeast (Caballero et al. 1995); fatty acids (Bilal et al 2022), anticholesterolemic action (Yoshida et al. 2004); a host for heterologous protein production (Panuwatsuk and Silva 2002; van Ooyen et al. 2006); and antioxidant, antiproliferative, and immunostimulatory activities (Maccaferri et al. 2012; Galinari et al. 2017, 2018). In addition, studies have shown that *K. marxianus* modulates the inflammatory process (Stefanova et al. 2010; Romanin et al. 2016).

Due to the promising activities demonstrated by polysaccharides isolated from fungi and the biotechnological potential of *K. marxianus*, this work reports the chemical structure of α-D-mannan isolated from the *Kluyveromyces marxianus* cell wall and their anti-inflammatory and antinociceptive properties in mouse models.

Materials and methods

Microorganism

Kluyveromyces marxianus CCMB 322 was provided from the Collection of Cultures of Microorganisms of Bahia (CCMB, Feira de Santana, Brazil) to investigate the biotechnological potential of the strain. The fungus was isolated from the Bahia semiarid region and maintained on YM agar at 28 °C in an incubator (IGO 150 Cell Life—Jouan).

Culture conditions for *K. marxianus* CCMB 322 growth

The preparation of inoculum and culture conditions for *K. marxianus* CCMB 322 growth was conducted according to procedures described by Valasques Junior et al. (2014).

Doehlert experimental design for biomass production

The Doehlert experimental design was applied to identify the optimal conditions for biomass production. The variables used were fermentation time in hours (h) and glucose concentration in g/L, for a total of nine experiments with a replicate of the central point (C). Each experiment was carried out in triplicate to determine the experimental errors and identify the best culture medium conditions and fermentation time for the maximum biomass production of *K. marxianus* CCMB 322. The glucose concentration was studied at three levels, ranging from 5 to 15 g/L, while the fermentation time was studied at five levels, from 48 to 144 h. Statistic 10.0 software was used to estimate the lack of adjustment and perform analysis of variance (ANOVA).

Fractionation and chemical characterization of yeast polysaccharides

A sample of crude polysaccharides (15 mg/mL) was applied to a Sephacryl S-200 column (48 × 1.2 cm; Amersham Bioscience, USA) pre-equilibrated and developed with sodium phosphate buffer (0.05 mol L^{-1}, pH 7.0). Fractions of 2.5 mL were collected and assayed for total sugar, leading to one symmetrical peak named α-D-mannan. The number-average molecular weight (MWn) and degree of polymerization (DPn) were done the according described in Valasques Junior et al. (2014) using 3,5-dinitrosalicylic acid (DNS) method (DuBois et al. 1956) and the measurement of total carbohydrates using the phenol–sulfuric acid method (Miller 1959 employing the calculation of Vettori et al. (2012).

Chemical

Monosaccharide composition, methylation analysis, and characterization by spectroscopic analysis of the polysaccharide were done the according described in Valasques Junior et al. (2021).

Pharmacological studies

Animals

Experiments were performed using male Swiss Webster mice (25–30 g), under controlled conditions of temperature (22 ± 1 °C) and a 12-h light–dark cycle. All the experiments were performed after approval by the Animal Ethics Committee of State University of Feira de Santana (006/2013) and were conducted in accordance with the International Association for the Study of Pain (IASP) guidelines on the use of animals in pain research (Zimmermann 1983).

Formalin test

The formalin test was conducted according to the methods described by Valasques Junior et al. (2014).

Complete Freund's adjuvant (CFA) test

The CFA test was conducted as previously described by Navarro et al. (2013). Mice were treated with α-D-mannan (10, 30, and 90 mg/kg, i.p.), morphine (5 mg/kg, subcutaneous [s.c.], positive control), or saline (i.p., negative control).

Tail immersion test

The tail immersion test was conducted according to the procedure described by Valasques Junior et al. (2014). The animals were treated with α-D-mannan (10, 30, and 90 mg/kg, i.p.), morphine (5 mg/kg, s.c., positive control), or saline (i.p., negative control).

Hot plate test

Mice were placed on a hot plate (Insight, Ribeirao Preto, SP, Brazil) maintained at 55 ± 1.0 °C. The reaction time was scored when the animal jumped, flinched, or licked its paws. Mice were treated with α-D-mannan (10, 30, and 90 mg/kg, i.p.), morphine (5 mg/kg, s.c., positive control), or saline (i.p., negative control). Measurements were performed before (time 0) and 0.5, 1, 3, and 5 h after treatment, with a cut-off time of 30 s to avoid paw lesions. The antinociceptive index (AI) was calculated according to Eq. 1

$$\mathrm{AI} = \left[(\text{test latency} - \text{baseline latency}) / (\text{off time}(30\ \text{s}) - \text{baseline latency})\right] \times 100. \quad (1)$$

Rotarod and open-field tests

The rotarod and open-field tests were conducted according to the procedures described by Rocha et al. (2019). Mice were treated with α-D-mannan (90 mg/kg, i.p.), saline (i.p., negative control), or diazepam (10 mg/kg, i.p., reference drug) 30 min before the test.

Paw edema test

The paw edema test was conducted according to the method described by Rocha et al. (2019). Thirty minutes before injection of carrageenan (100 µg/paw, i.pl.), the animals were treated with α-D-mannan (10, 30, and 90 mg/kg, i.p.), dexamethasone (2 mg/kg, s.c., positive control), or saline (i.p., negative control).

IL-1β and IL-6 quantification

Three hours after the intraplantar injection of carrageenan, mice were euthanized and the skin tissue of the paw was removed. Samples were homogenized in 100 mL buffer (1× phosphate-buffered saline [PBS], 0.4 M NaCl, 0.05% Tween-20, 0.5% bovine serum albumin [BSA], 0.1 mM phenylmethylsulfonyl fluoride [PMSF], DMSO, 0.1 mM benzethonium chloride, 10 mM ethylenediaminetetraacetic acid [EDTA], and 20 KIU aprotinin), followed by incubation for

1 h at 4 °C. Immediately after, the samples were centrifuged at 10,000 rpm for 25 min and the supernatant was collected. IL-1β and IL-6 levels were estimated using commercially available enzyme-linked immunosorbent assay (ELISA) kits for mouse IL-1β and IL-6 (R&D System, Minneapolis, MN, USA), according to the manufacturer's instructions. Plates were read in spectrophotometer (Spectra Max 190, Molecular Devices, San Jose, CA, USA) at 450 nm and the data analyzed in Softmax 4.3.1 software (Molecular Devices). The concentrations of IL-6 and IL-1β in each sample are expressed as picograms of cytokine per milligram protein (pg/mg).

Statistical analysis

The data are presented as mean ± standard error of the mean for six animals per group. The data were analyzed by one-way ANOVA followed by Tukey's test or repeated-measures two-way ANOVA with Bonferroni's post hoc test. Differences were considered significant when $p < 0.05$.

Results and discussion

Biomass production

Response surface methodology is an efficient statistical technique for the optimization of multiple variables to predict the best conditions with a minimum number of experiments (Box and Behnken 1960). The Doehlert matrix, applied to identify the optimal glucose concentration and the fermentation time for *K. marxianus* CCMB 322 biomass production, is shown in Table 1, together with its real and coded values. Equation 2 shows the relationship between the response (biomass in grams) and the glucose concentration (g L^{-1}), and fermentation time (h)

Table 1 Doehlert experimental design for optimization of *K. marxianus* CCMB 322 biomass production

Experiment	Time (h)	Glucose (g/L)	Biomass (g)
1	72 (−0.5)	15 (+0.866)	0.353
2	120 (+0.5)	15 (+0.866)	0.399
3	48 (−1)	10 (0)	0.353
4C	96 (0)	10 (0)	0.438
5C	96 (0)	10 (0)	0.427
6C	96 (0)	10 (0)	0.447
7	144 (+1)	10 (0)	0.433
8	72 (−0.5)	5 (−0.866)	0.327
9	120 (+0.5)	5 (−0.866)	0.350

(C): central point; coded values are presented in the parentheses

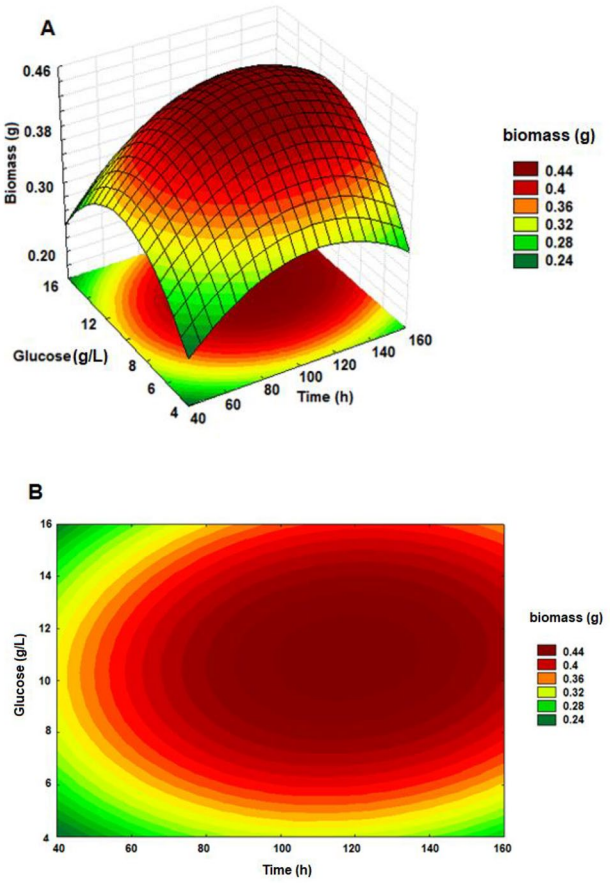

Fig. 1 **A** Surface graph showing the influence of fermentation conditions for the production of *Kluyveromyces marxianus* CCMB 322 biomass. **B** An area chart showing the optimal regions of the fermentation conditions for the production of *K. marxianus* CCMB 322 biomass

$$R = \left[-0.0838 + 0.0040T - 0.00002T^2 + 0.0543C - 0.0028C^2 + (0.000048T \times C)\right]. \quad (2)$$

Figure 1 shows the response surface curve obtained from the applied Doehlert matrix. Based on ANOVA (Table 2),

Table 2 Analysis of variances in the regression model for optimization biomass production from *K. marxianus* CCMB 322

	SS	*df*	MS	Fcal	Tab *F*
Regression	0.01690	5	0.00338	45.93	9.01
Residual	0.00022	3	7.36E-05		
Lack-of-fit	0.00002	1	0.00002	0.20	18.51
Pure error	0.00020	2	0.00010		
Total SS	0.01713	8			

SS sum of squares, *df* degree of freedom, *MS* mean square, *Fcal* calculated *F* value, *Ftab* tabulated *F* value

the quadratic model is well adjusted, with significance in the regression ($F = 45.93 > 9.01$) and without a lack of adjustment ($F = 0.20 < 18.51$). Hence, the errors obtained are random and the results found can be explained by the experimental model within a 95% confidence interval.

The concentrations of glucose greater than 14 and less than 8 g/L caused a decrease in the biomass production. The optimal region for biomass production is between 8 and 14 g/L for the glucose concentration and between 90 and 150 h for the fermentation time, with an optimal point of 10.8 g/L and 117 h for the glucose concentration and the fermentation time, respectively (Fig. 1).

Extraction and fractionation of polysaccharides from the *K. marxianus* CCMB 322 cell wall

The amount of polysaccharide collected in this work (447 mg/g of cells) was higher than those achieved with other *K. marxianus* strains, such as *K. marxianus* R157 (113 mg/g of cells) (Nguyen et al. 1998), *K. marxianus* FII 510700 (200 mg/g of cells) (Lukondeh et al. 2003), and *K. marxianus* CCT7735 (214 mg/g of cells) (Galinari et al. 2017). This variability in the polysaccharide content might be related to different yeast strains, culture medium composition, and growth conditions used in the above-mentioned studies. A sample of polysaccharide extracted was fractionated by means of Sephacryl S-200, leading to one symmetrical peak, which we can be attributed to one type (Fig. 2).

The mannan showed molecular weight (Mw) around 126 kDa of (125,876.15 Da) and DPn of 776.90, which was smaller than the Mw described for other *Kluyveromyces* strains. Galinari et al. (2017) reported mannan (KMM-5) from *K. marxianus* CCT7735 with Mw 203 kDa. Other mannans were reported (KMM-1, KMM-2, KMM-3, and KMM-4) with molecular weight ranging from 7.6 to 75.1 kDa from *K. marxianus* CCT7735 (Galinari et al. 2018). Lukondeh et al. (2003) reported polysaccharides from *K. marxianus* FII 510700 with Mws ranging from 66 to 97 kDa.

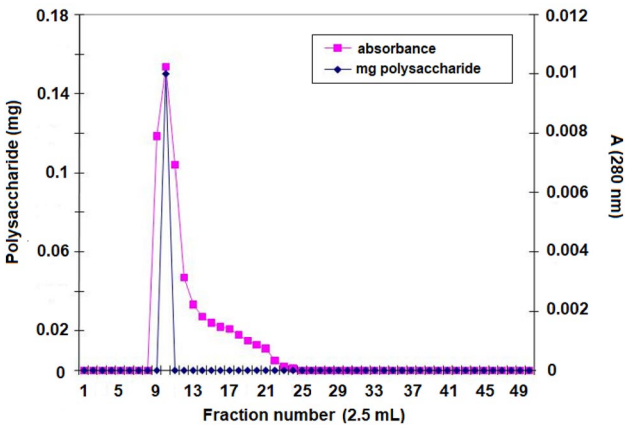

Fig. 2 Elution profile of mannan from Sephadex S-200 with 50 mM sodium phosphate buffer (pH 7.0)

Characterization of the polysaccharide fraction

Following acid hydrolysis (2M TFA at 100 °C for 8 h), the monosaccharide composition of the polysaccharide fraction was determined. The sample of polysaccharide contained a mannan, due to the presence of 92% mannose, as found by Galinari et al. (2017) in the mannans cell of *K. marxianus* CCT7735 and Valasques Junior et al. (2021) in the mannans cell of *Pseudozyma* sp. It also showed small amounts of glucose (4%) and trace amounts of rhamnose, fucose, and galactose.

FT-IR analysis was performed to identify the main functional groups. Figure 3 shows a broad and intense band at 3276 cm^{-1}, characteristic of the stretch vibration of O–H. The signals at 973 and 1050 cm^{-1} suggest the presence of mannose. The band at about 1141 cm^{-1} was assigned to the valent vibrations of the C–O–C bond and glycosidic bridge (Ahmad et al. 2010). The signal at 1638 cm^{-1} was related to the carbonyl group (C=O), while the signal at 1416 cm^{-1} corresponded to the absorbance of CH_2 functional group. In addition, no signals indicating the presence of amines were recorded, which suggests a low level of protein contamination in the samples. Furthermore, no protein was detected using the Bradford method (Bradford 1976).

Methylation analysis was performed and showed that this mannan presented terminal, 2-*O*-, 6-*O*-, and 2,6-*O*-substituted Man*p* units. Although several hydrolysis times were tested, it was not possible to obtain an accurate quantification of the derivatives, because the number of terminal units was higher than the number of branched ones.

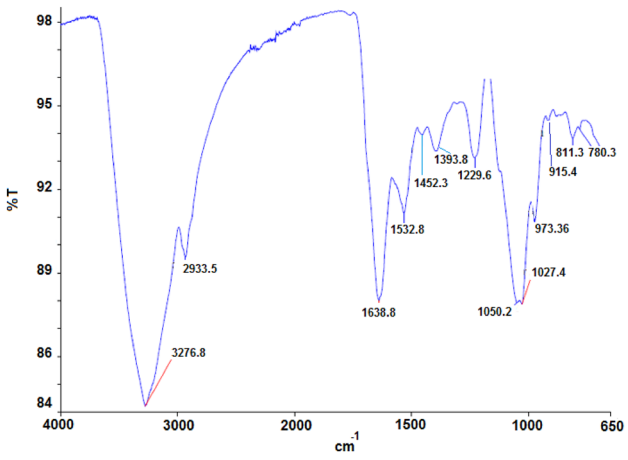

Fig. 3 The FT-IR spectrum of the α-D-mannan fraction. The dried fraction was ground with KBr powder and pressed into pellets for FT-IR

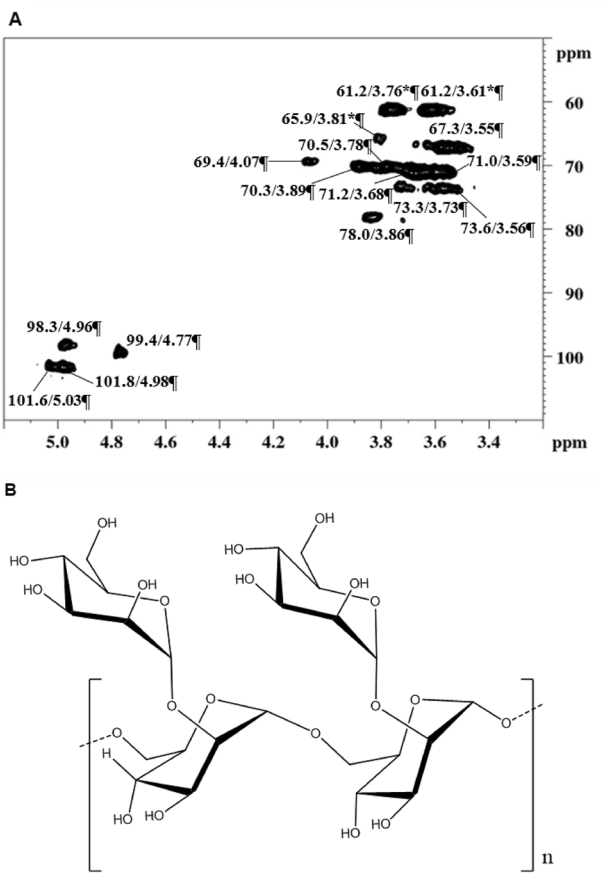

Fig. 4 **A** HSQC correlation map of fraction α-D-mannan, in DMSO-$_{d6}$ at 70 °C; the chemical shifts are expressed as δ ppm. Signals marked with an * were inverted in the DEPT experiment. **B** The possible fragment structure of the cell wall α-D-mannan from *K. marxianus* CCMB 322

The HSQC correlation map (Fig. 4) showed four anomeric signals at δ 98.3/4.96, δ 99.4/4.77, δ 101.6/5.03, and δ 101.8/4.98 (Table 3). The coupling constant ($^1J_{C\text{-}H}$) was measured, giving a value of 170 Hz for all signals, characterizing them as having α anomericity. The signal at δ 78.0/3.86 was attributed to substituted C-2, while the inverted signal in the DEPT experiment at δ 65.9/3.81 was assigned to substituted C-6 from α-D-Man*p* units. Inverted signals at δ 61.2/3.76 and δ 61.2/3.61 are from unsubstituted C-6 from α-D-Man*p* units. ^{1}H NMR, HSQC, and ^{1}H HSQC analyses together with comparison with the literature (Gorin 1973; Gorin 1981; Gorin and Mazurek 1975) allowed the assignment of almost all carbons and hydrogens of the α-mannan present in the sample (Table 3). Signals from 2-*O*-linked Man*p* units, as observed in the methylation analysis, were not observed in the HSQC correlation map, probably due to their low amounts. Unfortunately, the quantification of the different linkages was also not possible by ^{1}H NMR spectroscopy due to overlapping anomeric signals at δ 4.96–4.98.

In summary, the main polysaccharide present is an α-D-mannan, with 6-*O*-linked Man*p* units in the main chain partially substituted at *O*-2 by terminal Man*p* units and small amounts of 2-*O*-linked Man*p* side chains.

Pharmacological studies

The antinociceptive action of the α-D-mannan extracted from *K. marxianus* was investigated using formalin test (Fig. 5A, B). In this test, pain-related behaviors are assessed over two temporally distinct phases (Cowan et al. 1989; Hunskaar et al. 1985). The early phase is caused by direct stimulation of nociceptive neurons (Hunskaar and Hole 1987), whereas the late phase or inflammatory pain is characterized by the release of pro-inflammatory mediators, for example, prostaglandins, histamine, and cytokines (Malmberg and Yaksh 1992; Shibata et al. 1989). Pretreatment with α-D-mannan (30 mg/kg) significantly inhibited only the late phase of the formalin test (Fig. 5B). Thus, this result suggests an anti-inflammatory and antinociceptive potential from α-D-mannan extracted from *K. marxianus.*

Another test used to confirm the antinociceptive action of α-D-mannan was the model of inflammatory pain induced by CFA. The intraplantar CFA injection produces a long inflammatory response that develops early, due to the release of inflammatory mediators, such as prostaglandins and cytokines (Batista et al. 2010). Pretreatment with α-D-mannan (10–90 mg/kg, i.p.) or morphine (5 mg/kg, positive control) produced antinociception in the CFA model (Fig. 5C).

The literature already reported the antioxidant, antiproliferative, and immunostimulatory activities of cell wall

Table 3 NMR assignments of the α-mannan from *K. marxianus* CCMB 322

Units		1	2	3	4	5	6
α-D-Man*p*-(1→	C	101.6/101.8	70.3	71.2	67.3	73.6	61.2
	H	5.03/4.98	3.89	3.68	3.55	3.56	3.76/3.61
→6)-α-D-Man*p*-(1→	C	99.4	73.3	–	67.3	69.4	**65.9**[a]
	H	4.77	3.73	–	3.55	4.07	3.81
→2,6)-α-D-Man*p*-(1→	C	98.3	**78.0**[a]	70.5	67.3	71.0	**65.9**[a]
	H	4.96	3.86	3.78	3.55	3.59	3.92

[a]Indicates *O*-substituted carbon

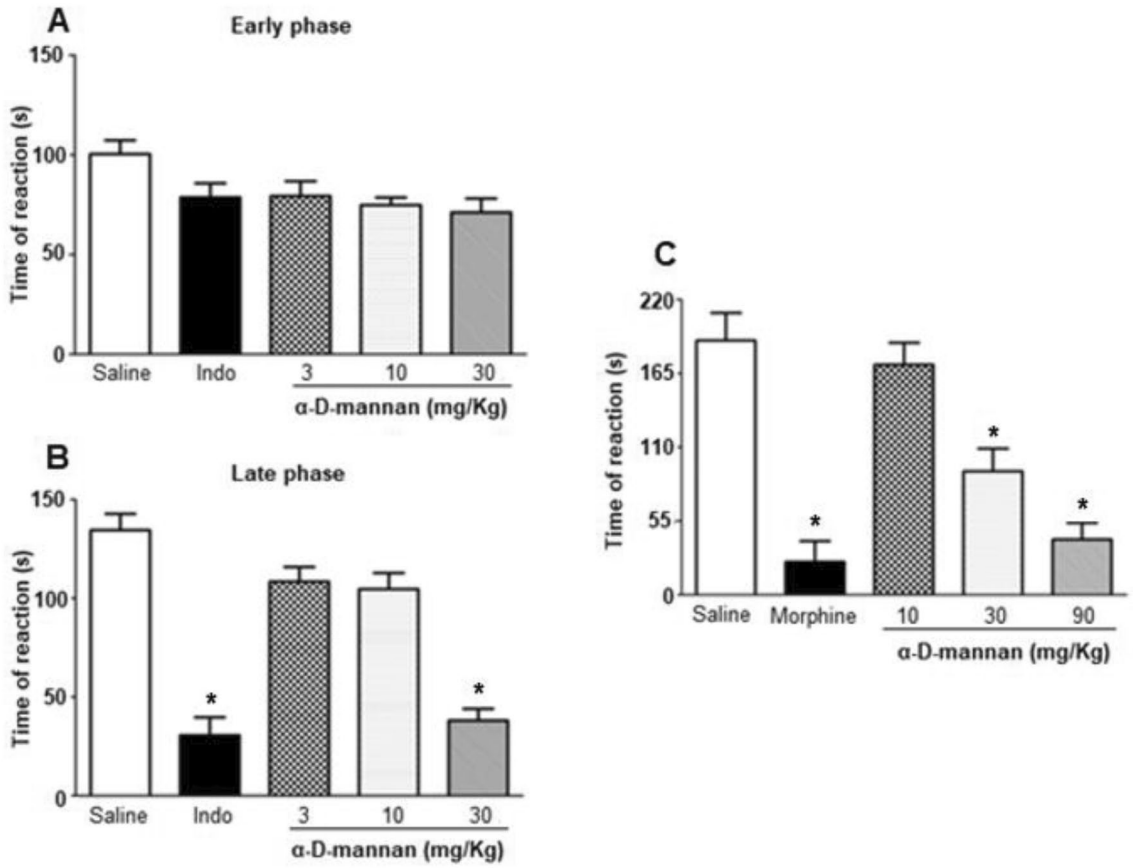

Fig. 5 The effects of intraperitoneal administration of α-D-mannan on nociception induced by formalin and CFA. Animals were treated with α-D-mannan (i.p.) or saline (i.p.) 30 min before i.pl. injection of 20 µL of 2.5% formalin (**A**, **B**) or i.pl. injection of 10 µL CFA (**C**). Indomethacin (Indo, 10 mg/kg, i.p.) and morphine (Mor, 5 mg/kg, s.c.) were used as positive controls. The data are expressed as mean ± standard error of the mean ($n = 6$–7 mice per group). *Significantly different from the control group ($p < 0.05$), as determined by ANOVA followed by Tukey's test

α-D-mannan from *K. marxianus* (Galinari et al. 2018). These results show that α-D-mannan from *K. marxianus* also produces antinociception possibly due to inhibition of inflammation.

The tail-flick and hot-plate tests are well-established assays to evaluate centrally acting analgesics (Langerman et al. 1995; Yaksh and Rudy 1977). Treatment with α-D-mannan (10, 30, and 90 mg/kg, i.p.) did not inhibit thermal nociception in the tail-flick (Fig. 6A) and the hot-plate (Fig. 6B) tests.

The tail-flick response is mediated by a spinal nociceptive reflex, while the hot-plate test measures higher brain functions and is considered to involve a supraspinally organized response (Jinsmaa et al 2004). Therefore, our results suggest that the antinociceptive activity of α-D-mannan does not involve the participation of spinal or supraspinal mechanisms.

Central nervous system depressants can reduce the motor function of animals, as well as the expression of nociceptive behaviors. Therefore, to clarify whether the antinociceptive effect of α-D-mannan is the result of motor deficits, the rotarod and the open-field tests were performed. Treatment with 90 mg/kg α-D-mannan neither modified motor coordination as demonstrated in the rotarod test (Fig. 7A) nor locomotor activity as demonstrated in the open-field test (Fig. 7B). These findings exclude the possibility that the antinociceptive effect of α-D-mannan could be related to disturbances in the mouse motor function. Diazepam, used as positive control, significantly decreased the responses evaluated in both tests (Fig. 7A, B). These results validate the antinociceptive effect of α-D-mannan, as suggested by the experimental pain models.

The effect of pretreatment with α-D-mannan on the paw edema test and cytokine levels

The injection of carrageenan produces local inflammation via the release of inflammatory mediators, which increase blood flow and vascular permeability, resulting in edema (Morris 2003). In this context, the pro-inflammatory

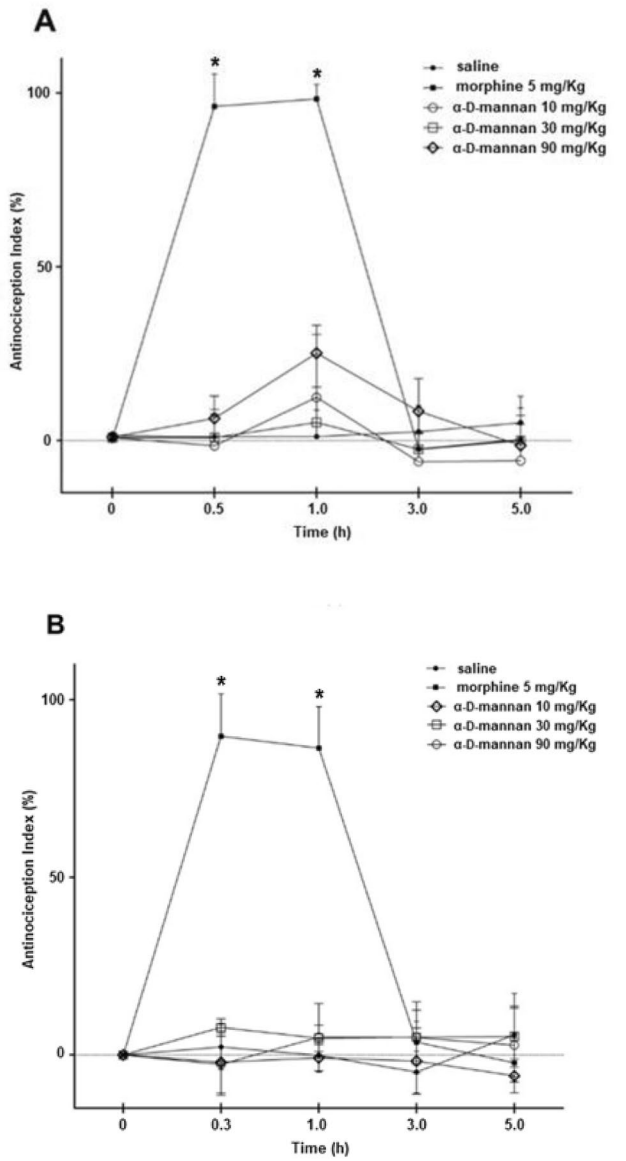

Fig. 6 The effects of intraperitoneal administration of α-D-mannan on the tail-flick and hot-plate tests. The thermal nociceptive threshold was evaluated with the tail-flick (**A**) and hot-plate (**B**) tests before (0), 0.5, 1, 3, and 5 h after administration of α-D-mannan (10, 30, and 90 mg/kg, i.p.), morphine (5 mg/kg, s.c., positive control), or saline (i.p., negative control). The data are expressed as mean ± standard error of the mean ($n = 6$ mice per group). *Significantly different from the control group ($p < 0.001$), as determined by two-way ANOVA followed by the Bonferroni's test

cytokines IL-1β and IL-6 are significant players in the immunological response to infection or injury. To investigate the mechanisms involved with the anti-inflammatory properties of α-D-mannan, the effect of this polysaccharide on local cytokine levels (IL-1β and IL-6) was evaluated.

Pretreatment with α-D-mannan at 30 and 90 mg/kg, but not at 10 mg/kg, reduced in a significant and dose-dependent manner the paw edema induced by carrageenan (Fig. 8A) as found by Valasques Junior et al. (2021), who showed the antiedematogenic effect of α-D-mannan from *Pseudozyma* sp.

The study of Valasques Junior et al. (2021) demonstrates that α-D-mannan has an activity similar to those of non-steroidal anti-inflammatory and glucocorticoid drugs. However, pretreatment with 90 mg/kg α-D-mannan significantly reduced IL-6 levels without changing IL-1β levels in the inflamed paw induced by carrageenan (Fig. 8B, C). Treatment with dexamethasone, the positive control, significantly reduced IL-6 and IL-1β compared with the control group.

Yang et al. (2022) showed that mannan oligosaccharide (MOS) supplementation reduced pro-inflammatory cytokine IL-6 level in neonatal goats. Bezerra et al. (2018) observed that polysaccharide mixture and isolated fractions of mannan from wines (Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc), formed by a sequence of α-D-Manp (1 → 6)-linked and side chains *O*-2 substituted for α-D-mannan (1 → 2)-linked, inhibited the production of inflammatory cytokines in RAW 264.7 cells.

Maccaferri et al. (2012) showed that *K. marxianus* extract inhibited IL-6 but not IL-1β in lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells. Stefanova et al. (2010) observed that superoxide dismutases from *K. marxianus* var. bulgaricus reduced significantly pro-inflammatory cytokines, IL-12, IFN-gamma, IL-6 and TNF-alpha in experimental model—adjuvant-induced arthritis in rodents. Romanin et al. (2016) reported the yeast-treated animals showed a reduced histopathological score ($P < 0.05$) and lower levels of circulating interleukin-6 ($P < 0.05$).

Several pain models have revealed the overexpression of IL-6 and its receptor in the spinal cord and dorsal root ganglia (Murphy et al. 1995; DeLeoet al. 1996; Bao et al. 2001; Zhou et al 2016) Furthermore, the administration of IL-6 could cause mechanical and thermal hypernociception, and treatment with anti-IL-6 or anti-IL-6R neutralizing antibody attenuates these pain-related behaviors (Arruda et al. 2000). Thus, inhibitors of IL-6 or its receptors may represent new therapeutic instruments and beneficial for the management of inflammatory pain.

The literature reported that tocilizumab, an anti-IL-6R monoclonal antibody, improves signs and symptoms of rheumatoid arthritis (Choy et al. 2002; Nishimoto et al. 2004) and presented a short-term analgesic effect in patients with discogenic low back pain (Sainoh et al. 2016). Therefore, treatment with α-D-mannan induces anti-inflammatory and antinociceptive properties in mice possibly by a mechanism dependent on inhibition of IL-6 production.

In summary, this study demonstrated that the polysaccharide α-D-mannan extracted from *K. marxianus* presents antinociceptive and anti-inflammatory activity, which probably occur through inhibition of IL-6. These findings support further investigation of the therapeutic potential of α-D-mannan to control pain and inflammation.

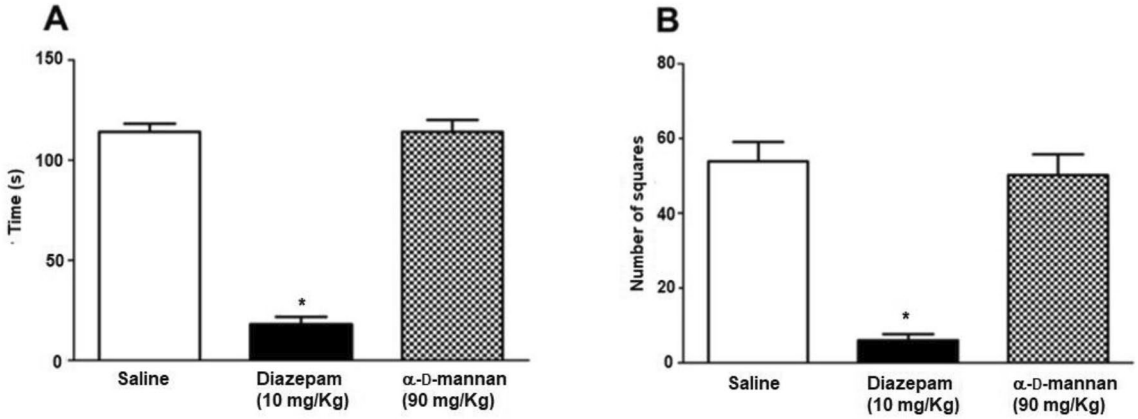

Fig. 7 The effects of intraperitoneal administration of α-D-mannan on the rotarod and open-field test. The animals were treated with α-D-mannan (90 mg/kg, i.p.), diazepam (10 mg/kg, *i.p*. positive control), or saline (i.p., negative control) 30 min before the rotarod (**A**) and open-field test (**B**). The data are expressed as mean ± standard error of the mean ($n = 6$ mice per group). *Significantly different from the control group ($p < 0.05$), as determined by ANOVA followed by Tukey's test

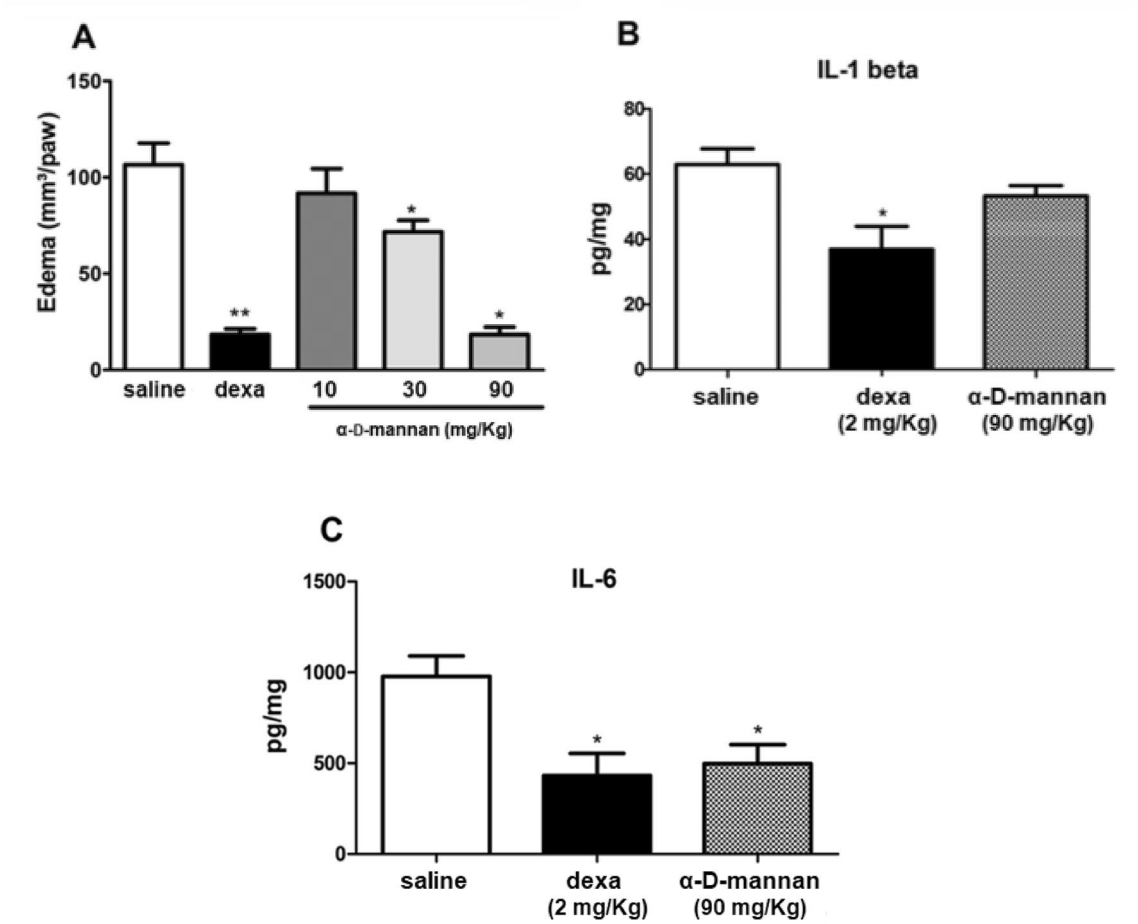

Fig. 8 The effects of intraperitoneal administration of α-D-mannan on carrageenan-induced paw inflammation. Mice were treated with α-D-mannan (10, 30, and 90 mg/kg, i.p.), dexamethasone (2 mg/kg, s.c., positive control), or saline (i.p., negative control) 30 min before the intraplantar injection of carrageenan (100 µg/paw). The edema was measured before and 3 h after the carrageenan injection (**A**). The samples of subcutaneous plantar tissue were collected 3 h after carrageenan injection and IL-1β (**B**) and IL-6 (**C**) levels were determined by ELISA; they are expressed as picograms of cytokine per milligram of protein. The data are expressed as mean ± standard error of the mean ($n = 6$ mice per group). *Significantly different from the control group ($p < 0.05$), as determined by ANOVA followed by Tukey's test

Conclusions

It can be concluded that the method for polysaccharide extraction from *K. marxianus* CCMB 322 was effective and α-D-mannan formed by α-(1 → 6) mannopyranose units in the main chain, partially substituted at *O*-2 position by α-D-Man*p* non-reducing terminals was obtained. This mannan shows peripheral antinociceptive activity, which is probably through inhibition of IL-6.

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Data availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval All the experiments were performed after approval by the Animal Ethics Committee of State University of Feira de Santana.

Consent for publication Not applicable.

Consent to participate Not applicable.

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