ORIGINAL ARTICLE

Received: Mar 08, 2024

Revised: Jul 03, 2024

Approved: Aug 09, 2024

Schizophyllan Polysaccharide from *Schizophyllum commune* demonstrates antinociceptive effect on preclinical models of acute pain

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Declaration of interests: Nothing to declare

Funding: FAPESC, FAP Univille

ABSTRACT

Introduction: Twenty-five to 29% of the global population experiences pain that motivates seeking emergency services. **Objective:** To evaluate the effect of schizophyllan glucan polysaccharide (SPG), an isolated β-(1→3),(1→6) glucan polysaccharide of *Schizophyllum commune*, in acute pain and neuromuscular performance on pre-clinical models. **Methods:** Male adult Swiss mice (20-30g, 60 days) were acclimated for a week in groups of 7 per cage before the experiment. SPG was administered, intraperitoneally, at doses of 0.1, 1.0, 3.0, 5.0, 10.0, 30.0, and 100.0 mg/Kg for the writhing test, and 1.0, 10.0, and 30.0 mg/Kg for the formalin and Rotarod tests, respectively. Statistical analysis was performed using one-way ANOVA, followed by the Duncan post hoc test, respectively, as appropriate ($p<0.05$). **Results:** Regarding the abdominal writhing test, SPG doses of 1.0, 5.0, 10.0, 30.0, and 100.0 mg/Kg promoted a significant reduction in writing of, respectively, 90.6%, 86.6%, 83.0%, 86.6%, and 76.2%. In the formalin test, the dose of SPG 30 mg/Kg reduced phase II nociception time by 78.0%. Relevant sedation was observed only to SPG 100 mg/Kg in the Rotarod test. **Conclusion:** SPG showed significant analgesic effects on acute inflammatory pain without causing concomitant central nervous system depression.

Keywords: acute pain; fungal polysaccharides; *Schizophyllum*; models, animal; analgesia; anti-inflammatory agents.

INTRODUCTION

According to the Montreal Declaration signed by the International Pain Summit (IPS) of the International Association for the Study of Pain (IASP), access to pain treatment should be recognized as a human right[1]. 25% to 29% of the global population experiences pain, which is the primary reason for seeking emergency services. 78% of emergency service consultations are related to pain, with one-third of those individuals reporting intense pain[2].

Despite the global popularity of nonsteroidal anti-inflammatory drugs (NSAIDs) as one of the highest-selling drugs, with an annual market value of around 25 million dollars, there are several side effects associated with their extensive use, which include indigestion, stomach ulcers, dizziness, nephrotoxicity, allergic reactions, headache, and others. Hence, the medical and scientific community remains intrigued by the ongoing pursuit of effective pharmacological monitoring for specific types of pain[3].

Various polysaccharides derived from fungi have been documented in the literature for their therapeutic potential. Among them, β-(1→3)-glucans are known to modulate the immune system, making them a promising alternative for reducing acute pain symptoms[4].

Schizophyllum commune is a macrofungus typically found in decaying wood during rainy seasons in tropical and subtropical forests. While it is primarily known for being edible, compounds with significant activity against cancers, tumors, and immunomodulation have been unequivocally identified in recent studies conducted on this species[5-7].

Based on the above, there is a growing focus on identifying new molecular targets for pain management. One of the most important stages in developing safe and effective therapies is pre-clinical research, which helps to identify potential pathways and minimize risks.

Given the previously proposed anti-inflammatory, antioxidant, and analgesic properties of fungal polysaccharides[8,9] and the importance of controlling acute pain, substances derived from *S. commune* may be an effective and safe alternative to alleviate it.

Thus, this study aims to evaluate the effect of schizophyllan glucan polysaccharide (SPG), an isolated β-(1→3),(1→6) glucan polysaccharide of Schizophyllum commune, in acute pain and neuromuscular performance on pre-clinical models.

METHODS

Production, extraction, purification, and characterization of the polysaccharide

The strain used to produce exopolysaccharides was *Schizophyllum commune* 227E.32, collected from the Atlantic Forest biome (28°58'08.33'S; 50°28'58.08'W 'W 'W). The Institute of Biotechnology at the University of Caxias do Sul partnered in cultivating *S. commune* to obtain its exopolysaccharides. The subsequent extraction and purification processes were also conducted through this partnership.

The purification and characterization of polysaccharides concerning monosaccharide composition, types of glycosidic bonds, and structure was developed in partnership with the Department of Chemistry at the Federal University of Catalão - GO.

To conduct this work, a 3 g sample of the polysaccharide was used to prepare experimental solutions. Production, purification, and characterization were previously described by Vanin *et al.*[10], confirming the structure β-(1→3)-glucan partially substituted in 0-6 by non-reducing glucose residues as β-(1→3)-(1→ 6)-glucan.

Animals

Before experimentation, adult Swiss mice of the Mus musculus species were allowed a week to acclimate. These male mice weighed between 20 and 30 grams and were 60 days old. They were housed in groups of seven in a temperature-controlled environment with a 12-hour light/12-hour dark cycle. Additionally, they were provided with free access to food

and water. All these conditions met the recommended protocols outlined in the "Guide for the Care and Use of Laboratory Animals, 1996". And according to the provisions of the Brazilian Federal Law 11,794 the 2008, and the Normative Resolutions of the National Council for the Control of Animal Experimentation – CONCEA in 2008. The research project was conducted only after its approval by the Ethics Committee on Animal Use (CEUA) of the University of the Region of Joinville (UNIVILLE) (Protocol # 001/2021).

Acute pain models

Writing test

The nociceptive response was induced by intraperitoneal (i.p.) injection of 0.9% acetic acid (0.1 mL/10 g, i.p.) in mice that were given SPG thirty minutes before (0.1, 1, 3, 5, 10, 30 and 100 mg/kg, i.p.) to screen the most effective dose. Control group animals received only 0.9% saline solution followed by acetic acid (0.1 mL/10 g, i.p.). The other control groups received all SPG doses before a 0.9% saline solution.

The animals were observed for 20 minutes, and the number of abdominal writhings as an arching of back, extension of hind limbs, and contraction of abdominal musculature was registered[11].

Formalin test

Animals were treated with SPG 1, 10, and 30 mg/kg (i.p.) half an hour before intraplantar injection of formalin (2.5%, 40 µL) or intraplantar saline solution (40 µL) in the left paw. Immediately after, the animals were observed for 40 minutes to monitor the time of nociceptive behavior. Control of animals received 0.9% saline solution (40 µL, intraplantar) in the left paw[12,13]. The other control group received 0.9% saline solution (40 µL, intraplantar) after formalin (2.5%, 40 µL) without receiving SPG.

The nociceptive behavior was observed in 10 blocks of 5 minutes, and the results were expressed as the time (seconds) that the animals performed a nociceptive behavior (paw shaking, licking, and paw elevation).

Rotarod Test

The Rotarod test[14] is one of the oldest used to assess the effects of a drug on animal behavior. It provides a quick estimate of whether a substance affects neuromuscular coordination. The Rotarod consists of a circular rod rotating at a constant or increasing speed[15]. Drugs that alter neuromuscular coordination, such as benzodiazepines, reduce the time that animals can remain on the pole[16]. Diazepam is normally used as a reference when performing the test[17].

The test was performed with mice treated once daily with SPG (1, 5, 10, 30, and 100 mg/kg, i.p.) after 12 and 17 days of exposition. The number of falls for three minutes was registered one hour after the last SPG administration for each period of exposition.

Statistical analysis

Statistical analyses were performed using the IBM Statistical Package for Social Sciences (SPSS) for Windows, version 20.0, using a PC-compatible computer (IBM Corp. Armonk, NY, USA). The Kolmogorov-Smirnov normality test was performed to confirm a parametric distribution; data were then analyzed by one-way ANOVA, followed by Duncan's *posthoc* test when the F-test was significant. Values of $p<0.05$ were considered significant. Results are expressed as means ± SD for seven independent experiments (animals).

RESULTS

Effect of SPG on the writhing test

Animals given saline or SPG dose thirty minutes before receiving saline did not demonstrate a significant writhing response. Thirty minutes after the administration of saline, induced significant writing in comparison to controls ($p<0.001$), which were significantly reduced by all doses of SPG. A 0.1 mg/Kg SPG *dose* promoted a significant reduction of writing (21.8%), but its intensity was statistically lower than the 1 mg/Kg onward dose (Figure 1). Reduction of writing were, respectively, 90.6%, 86.6%, 83.0%, 86.6%, and 76.2% for 1, 5, 10, 30, and 100 mg/kg doses of SPG.

Effect of SPG in the formalin test

SPG was ineffective in reducing the nociception time of the first phase of the formalin test (Figure 2A). The second phase was not changed by SPG 1 and 10 mg/Kg. However, 30 mg/Kg SPG promoted a significant reduction of 78.0% (Figure 2B) of nociception time. No SPG doses promoted significant nociception time in animals that received intraplantar saline solution.

Effect of chronic administration of SPG on the rotarod test

Chronic administration of SPG 100 mg/kg promoted statistically significant sedation on the 12th (Figure 3A) and 17th days of exposition (Figure 3B). All other SPG doses promoted no significant sedation during the whole exposition period.

DISCUSSION

This study investigated the effect of SPG in preclinical acute pain models and motor performance. The potential of SPG in the biological assays described in this work is related to

the anti-inflammatory activity of β-1,3-glucans substituted in O-6 by D-Glcp. The significant reduction of writing and formalin second phase tests indicates an antinociceptive effect that may be related to a possible anti-inflammatory activity. Acetic acid-induced abdominal writhing in mice was first described by Siegmund[18]. This test is highly sensitive to the presence of analgesic activity, fast and simple to perform, and, therefore, widely used to screen anti-inflammatory properties, since acetic acid promotes and induces local inflammatory process, whose mediators result in sensitization of nociceptors and consequent expression of the stereotypical behavior of pain by the animals[19,20].

It is also important to mention that the administration of SPG 1 mg/kg promoted a maximum analgesic effect, once higher doses did not differ significantly from that, evidencing the consistency of the SPG effect. No dose of the SPG promoted writhing at a relevant intensity in animals that received saline as a control of acetic acid, so the SPG did not tend to cause significant local inflammation.

The formalin test evaluates the amount of time the animals spend expressing nociceptive behavior directed to the injected paw as an algic response. The model evaluates two phases of nociceptive behavior: the initial phase lasting the first five minutes and the late phase lasting 20 to 40 minutes after formalin injection. A primary afferent C-fiber drive is linked to both phases of the behavioral response, which is expected to initiate and maintain activity-dependent sensitization at the spinal level[12,21,22].

Phase one of the formalin tests is characterized by nociceptive pain, mediated by direct depolarization of nociceptors due to the stimulus caused by the intraplantar application of formaldehyde, which promotes tissue damage. The early phase is short, followed by a brief period of relative dormancy. This phase is attenuated by drugs that act on opioid receptors since they promote the hyperpolarization of nociceptors or by antagonistic compounds of ionotropic

receptors for glutamate, which are ionic channels permeable to ions that trigger action potentials in nociceptive neurons[23].

The second phase of the formalin test is due to the recruitment of the inflammatory response because of tissue damage induced by formalin, which is mediated by prostaglandins and, therefore, is attenuated by compounds with anti-inflammatory action, such as non-steroidal anti-inflammatory drugs and steroids[24]. Thus, the reduction of the second phase time of nociceptive behavior of the formalin test suggests that the compound has an anti-inflammatory effect and, therefore, analgesic action for pain arising from this nature: SPG may act by modulating the synthesis of pro-inflammatory mediators, such as histamine, serotonin, bradykinin, and prostaglandins, that are recruited after tissue injury caused by formalin.

In a recent study using a model for periodontal disease induced by lipopolysaccharides from *Aggregatibacter actinomycetemcomitans*, it was found that β-(1,3)-glucans from *S. commune*, substituted in O-6 by D-Glcp, were responsible for upregulating IL-10. This response was stimulated by the activation of the Syk protein (splenic tyrosine kinase). The binding of the polysaccharide to dectin-1 in murine macrophages led to an upregulation of IL-10 expression and production, which occurred via pathways that are dependent on mitogen and stress-activated protein kinases (MSK1) and cAMP response element-binding protein (CREB). This resulted in an enhanced anti-inflammatory effect and response that could be potentially utilized to develop interventions for the treatment of inflammation caused by bacterial infections[25].

The protective effects of total polysaccharides are lower when compared to purified β-glucans that contain only polysaccharides with β-linkages. This may be attributed to the lower amount of β-glucan in the total extracts, which is well-known for its significant antioxidant properties. Fungal β-glucans exhibit diverse biological activities, and our objective was to establish a correlation between their chemical structure and anti-inflammatory and antinociceptive activities[26].

Smiderle *et al*.[27] indicated the antinociceptive and anti-inflammatory action of the β-(1→3), (1→6) glucan isolated from *Pleurotus pulmonarius*, a macrofungus from the same class as *S. commune*, in acute pain models (acetic acid-induced writhing and formalin test), that promoted a significant reduction of 96±4% in the nociception time with a 30 mg/Kg dose.

Ruthes *et al*.[28] also demonstrated that *Amanita muscaria* fucomannogalactan and β-D-glucan were successful in protecting mice against the inflammatory pain caused by formalin-induced nociception, reaching a reduction of 91 ± 8% to a 30 mg/Kg dose. Abreu et al.[29] demonstrated in their work about *Pholiota nameko* that the β-D-glucan at 0.3, 1.0, and 3.0 mg/Kg significantly inhibited the inflammatory pain in 24.8%, 56.9%, and 82.3%, respectively, in the inflammatory phase of the formalin-induced nociception in mice. Interestingly, the same pattern was also observed in this study.

Purified mannogalactan from *Pleurotus sajor-caju* was effective in reducing acetic acid (54,2%), second-phase formalin (37,0%) nociception, and carrageenan-induced edema (70%) in mice[30]. These tests revealed that the anti-inflammatory action of the mushroom β-D-glucans, which approaches that of nonsteroidal anti-inflammatory medicines, is likely related to the analgesic effect[27,28]. Although these studies are not specific to *S. commune* β-glucans, they support the analgesic effects observed in inflammatory pain models of mushroom-derived structures.

It was found that the production of pro-inflammatory mediators (nitric oxide, IL-6, and tumoral necrosis factor-α), as well as the expression of induced nitric oxide synthase and cyclooxygenase-2, were all in varying degrees reduced by polysaccharides linked to β-glucan, the triple-helical polysaccharides with β-(1→3),(1→6) linked glucose units exhibited stronger anti-inflammatory activity, within a range of concentration that did not result in cell death toxicity[31].

Yelithao *et al.*[32] reported that the primary immune response to fungal polysaccharides is triggered through complement (CR-3) and Toll-like (TLR-4) receptors, and other studies involving basidiomycetes have shown an upregulation of gene expression for anti-inflammatory cytokines like interleukin (IL) -10 and IL-12[32,33].

Baggio *et al.*[35] evidenced that the β-(1→3),(1→6) glucan isolated from *Pleurotus pulmonarius* carried significant anti-inflammatory and analgesic (antinociceptive) properties related to a mechanism of antinociception involving interleukin-1 pathways, protein kinase C inhibition, transient receptor potential channels, and ionotropic glutamate receptors in acute (glutamate, NMDA, AMPA, kainite and interleukin-1 intrathecally induced-nociceptive pain were reduced, respectively, in 67%, 89%, 74%, and 75%) and neuropathic pain (sciatic nerve ligation mechanical allodynia was reduced 45-60%) models in mice.

The Rotarod test described by Dunham and Miya[14] is one of the earliest methods for evaluating how drugs affect animal behavior, providing a quick, straightforward assessment of whether a chemical impacts neuromuscular coordination. Animals stay on the rod for a shorter period while under the influence of benzodiazepines, typically used as a positive control for CNS depression[16,17]. Despite a small number of falls observed in animals who were given SPG therapeutic doses, those were not statistically significant, which means the animals were not sedated to the point of being prevented from manifesting the nociceptive behaviors monitored in the other pain models. Although the motor performance test was conducted using a different protocol, specifically chronic treatment rather than acute, no motor impairments were observed in the animals following each treatment. These findings from the chronic study further support this conclusion.

Conclusion

The SPG promoted a significant analgesic effect on inflammatory acute pain, suggesting a potential ability to decrease pain perception without causing concomitant CNS depression. Additional research is necessary to confirm these encouraging preclinical results and SPG toxicity to develop better and safer therapeutic options for pain.

ACKNOWLEDGMENTS

The authors thank the valuable support of the following collaborators: Ana Paula Vanin, Marli Camassola, for supplying the purified polysaccharide.

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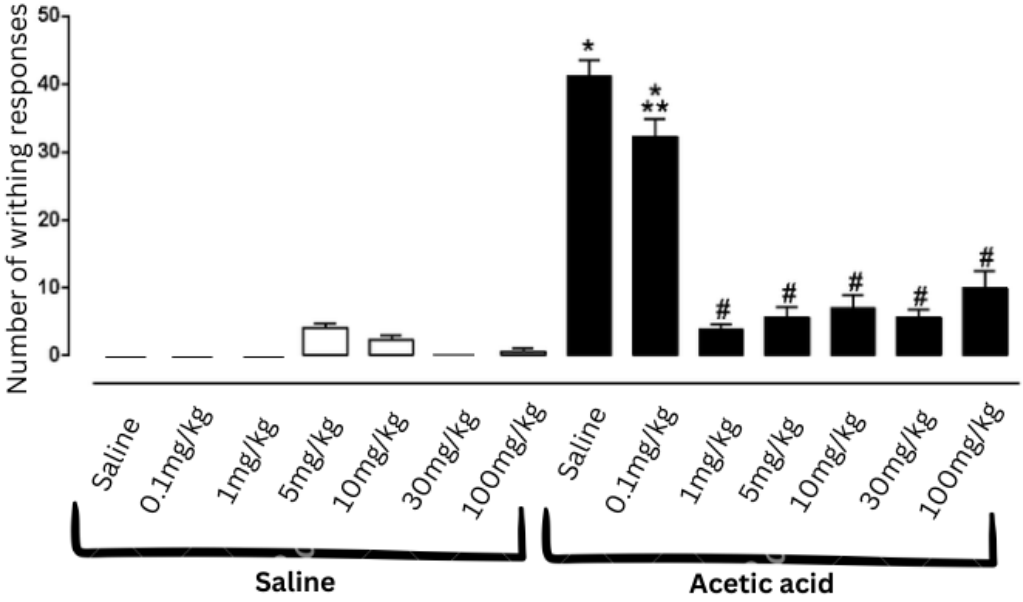
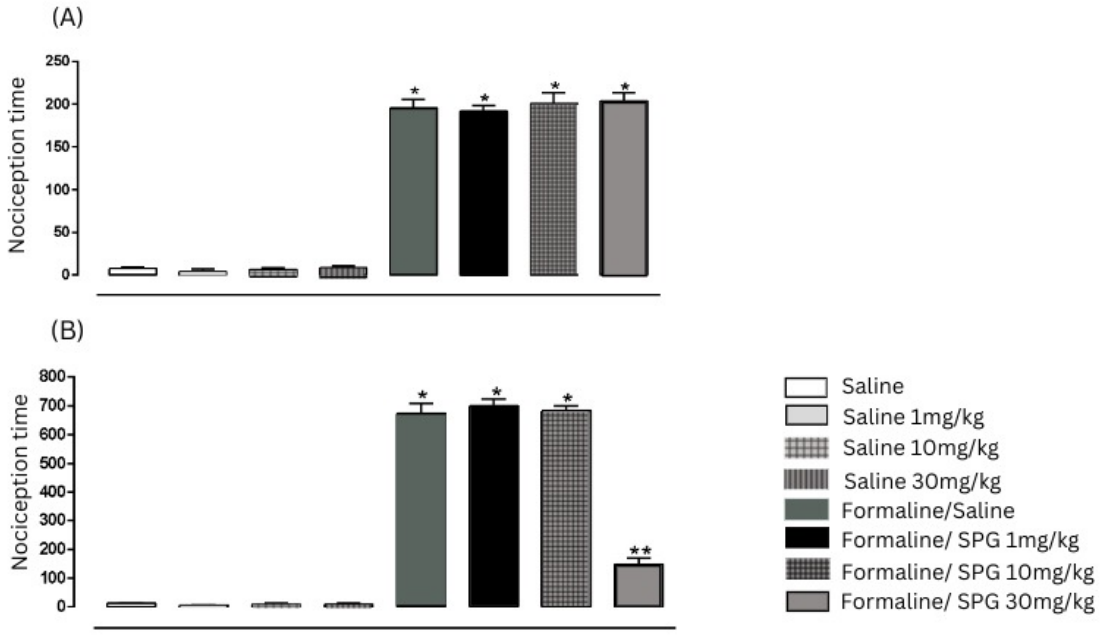
FIGURES

Figure 1: Writhing test in mice exposed to different doses of SPG.



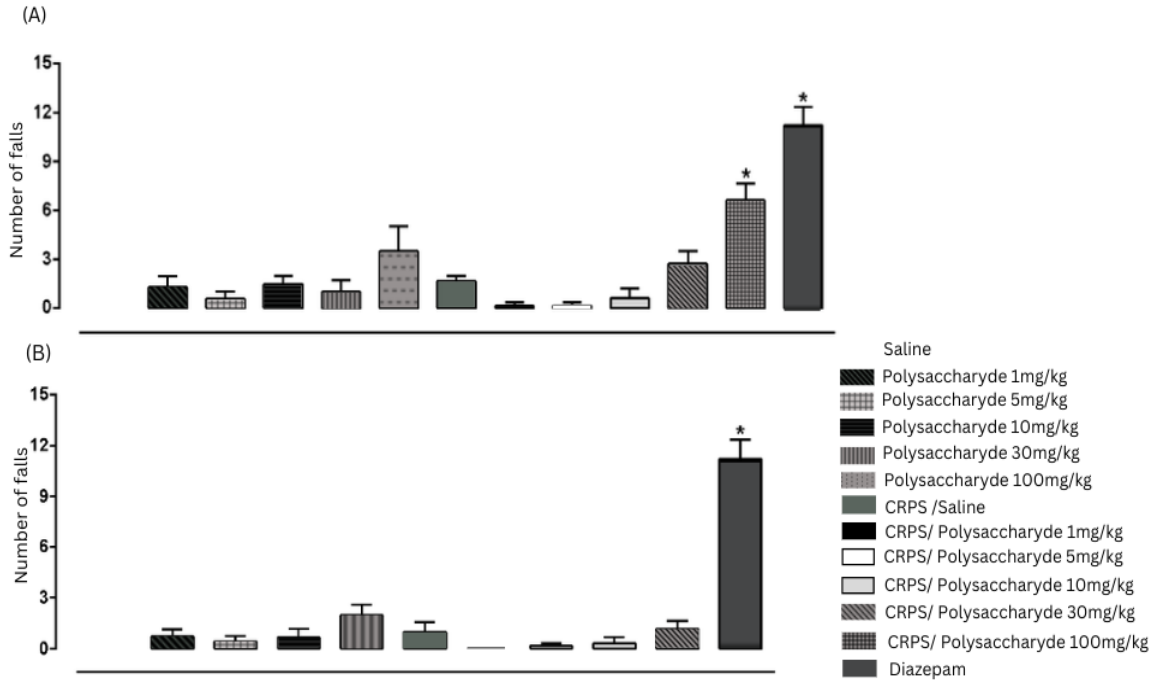
*Statistically significant difference compared to saline and SPG control groups ($p < 0.001$). **Statistically significant difference between the 0.1mg/kg SPG group compared to the acetic acid and saline group ($p<0.001$). #Statistically significant difference compared to 0.1 mg/Kg SPG and acetic acid group ($p<0.001$).

Figure 2: Effect of different doses of SPG on the first (A) and second phases (B) of the formalin test.



*Statistically significant differences about the saline-saline group ($p<0.001$). **Statistically significant difference between the saline-formalin, 1mg/kg-formalin, and 10mg/kg-formalin groups ($p<0.001$).

Figure 3: Rotarod test in animals exposed to different doses of SPG for twelve (A) and seventeen days (B).



*Statistically significant difference between saline and SPG groups ($p<0.001$).