Anti-inflammatory effect of aqueous extracts of spent Pleurotus ostreatus substrates in mouse ears treated with 12-O-tetradecanoylphorbol-13-acetate

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ABSTRACT

Aims: To evaluate the application of spent Pleurotus ostreatus substrates, enriched or not with medicinal herbs, as a source of anti-inflammatory compounds.

Subjects and Methods: P. ostreatus was cultivated on five different substrates: Barley straw (BS) and BS combined 80:20 with medicinal herbs (Chenopodium ambrosioides L. [BS/CA], Rosmarinus officinalis L. [BS/RO], Litsea glaucescens Kunth [BS/LG], and Tagetes lucida Cav. [BS/TL]). The anti-inflammatory activity of aqueous extracts of spent mushroom substrates (SMSs) (4 mg/ear) was studied using an acute inflammation model in the mouse ear induced with 2.5 µg/ear 12-O-tetradecanoylphorbol13-acetate (TPA).

Results: Groups treated with BS/CA, BS/RO, and BS/LG aqueous extracts exhibited the best anti-inflammatory activity (94.0% ± 5.5%, 92.9% ± 0.6%, and 90.4% ± 5.0% inhibition of auricular edema [IAO], respectively), and these effects were significantly different ($P < 0.05$) from that of the positive control indomethacin (0.5 mg/ear). BS/TL and BS were also able to reduce TPA-induced inflammation but to a lesser extent (70.0% ± 6.7% and 43.5% ± 6.6% IAO, respectively).

Conclusions: Spent P. ostreatus substrate of BS possesses a slight anti-inflammatory effect. The addition of CA L. to mushroom substrate showed a slightly synergistic effect while RO L. had an additive effect. In addition, LG Kunth and TL Cav. enhanced the anti-inflammatory effect of SMS. However, to determine whether there is a synergistic or additive effect, it is necessary to determine the anti-inflammatory effect of each medicinal herb.

KEY WORDS: Anti-inflammatory activity, medicinal herbs, Pleurotus ostreatus, spent mushroom substrate

Introduction

Spent mushroom substrate (SMS) is the substrate left over after mushroom harvesting. For every kilogram of mushroom produced, about 5 kg of SMS is generated. SMS has been used as biofertilizer, additive in animal feeding, for recultivation or treating dry bubble disease of mushroom, as well as for soil bioremediation, bioethanol and biogas production, germination, growth of horticultural plants and synthesis of nanoparticles. Recently, we demonstrated the antibacterial activity of an aqueous extract of SMS.

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**Subjects and Methods**

**Materials**

12-O-tetradecanoylphorbol 13-acetate (TPA) and indomethacin were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Herb Material**

The aerial parts (stems and leaves) of Chenopodium ambrosioides L. (CA), Rosmarinus officinalis L. (RO), and Litsea glaucescens Kunth (LG) and aerial parts and flowers of Tagetes lucida Cav. (TL) were purchased in the central market of Pachuca, Hidalgo, Mexico. Taxonomic identification of the material was done by a botanist (Miguel Angel Villavicecio Nieto, Ph.D.). The plants were dried at room temperature and protected from light and the sun. Voucher specimens are deposited at the Herbarium of Biology Research Centre of the Universidad Autónoma del Estado de Hidalgo at Pachuca, Hidalgo, Mexico.

**Spent Substrates**

The spent *P. ostreatus* substrates were obtained from the Ethnobotany Laboratory of Biology Research Centre at the Universidad Autónoma del Estado de Hidalgo at Pachuca, Hidalgo, Mexico. Briefly, *P. ostreatus* (UAEH-003) was cultivated in five different substrates: Barley straw (BS) (*Hordeum vulgare* 100%), and BS combined 80:20 with each medicinal herb (CA L., RO L., LG Kunth, and TL Cav.). After 40 days of culture, *P. ostreatus* fruiting bodies were harvested and spent substrate were labeled as spent *P. ostreatus* substrate of BS, spent *P. ostreatus* substrate of BS/CA L., spent *P. ostreatus* substrate of BS/RO L., spent *P. ostreatus* substrate of BS/LG Kunth, and spent *P. ostreatus* substrate of BS/TL Cav. Afterward, to obtain particles of 5–7 mm, the spent substrates were ground in a blender.

**Preparation of Extracts**

The dried and ground spent *P. ostreatus* substrates (600 g) were extracted by maceration with distilled water in a proportion of 1:3 spent substrate/solvent for 24 h at room temperature. Solvent was eliminated under reduced pressure distillation with a Büchi-Brand rotary evaporator, obtaining yields of 0.56%, 0.73%, 1.46%, 1.08%, and 1.15% of the BS/CA, BS/RO, BS/LG, BS/TL, and BS aqueous extracts, respectively.

12-O-tetradecanoylphorbol 13-acetate-induced Acute Inflammation in Mouse Ears

The anti-inflammatory activity of spent substrates was studied by the method of acute inflammation in mouse ears induced with TPA as described by González-Cortazar *et al.* and Salinas *et al.* with slight modifications.

Adult male CD-1 mice with a body weight of 20–30 g were grouped (five individuals per group). Mice were maintained under standard laboratory conditions at 22°C ± 3°C, 70% ± 5% humidity, 12-h light/dark cycle, and food/water *ad libitum*. The mice were allowed at least 2 weeks to adapt to the laboratory environment before initiating the experiments. Experiments were performed according to the Official Mexican Rule: NOM-062-ZOO-1999 Guidelines (Technical Specifications for the Production, Care, and Use of Laboratory Animals), and the protocol was approved by the Institutional Committee for Ethical Guidelines for the Care and Use of Experimental Animals of the Universidad Autónoma del Estado de Hidalgo.

A negative control group received acetone and other distilled water as a vehicle, and the anti-inflammatory drug indomethacin was used as a positive control. Animal ear inflammation was induced with 2.5 µg TPA dissolved in 20 µL of acetone applied to the internal and external surface of the right ear to cause edema. Sample doses of 4 mg/ear of the extracts were applied topically. Indomethacin was dissolved in acetone and administered at 0.5 mg/ear applied topically. All the samples of the different treatments were dissolved in distilled water and applied topically to the right ear immediately after TPA application; on the left ear, distilled water was applied as the vehicle control. Four hours after application of the doses, the animals of each treatment were sacrificed by cervical dislocation. Circular sections of 6 mm in diameter were taken from both the treated (t) and the nontreated (nt) ears, which were weighed to determine the inflammation.

Percentage of inhibition was determined by using the following equation:

\[
\text{Inhibition} \% = (\Delta w \text{ control} - \Delta w \text{ treatment} / \Delta w) \times 100
\]

Where \( \Delta w = w_t - w_{nt} \), with \( w_t \) being the weight of the section of the treated ear and \( w_{nt} \) being the weight of the section of the nontreated ear.

**Statistical Analysis**

The results obtained from the pharmacological test were submitted to analysis of variance, followed by Tukey tests. *P* < 0.05 was considered significantly different.

**Results**

Aqueous extracts from spent *P. ostreatus* substrates (enriched or not with medicinal herbs) at a dose of 4 mg/ear were evaluated on TPA-induced auricular edema (IAO) model in mice to evaluate the anti-inflammatory activity of spent *P. ostreatus* substrate of Barley straw (BS), as well as the possible synergism between spent *P. ostreatus* substrate of BS and medicinal herbs. All tested extracts had an anti-inflammatory effect which was significantly different (*P* < 0.05) with respect to the negative group control [Table 1].

The group treated with BS/CA aqueous extract exhibited the best anti-inflammatory activity (94.0% ± 5.5% IAO), followed by the groups treated with BS/RO aqueous extract and BS/LG aqueous extract; these effects were not significantly different to each other but were significantly different (*P* < 0.05) to the positive control group, which was treated with indomethacin (0.5 mg/ear). Groups treated with BS aqueous extract and BS/TL aqueous extract were also able to reduce TPA-induced inflammation but to a lesser extent (43.5% ± 6.6% and 70.0% ± 6.7% IAO, respectively); the effect of this latter group was not significantly different to the positive control group [Table 1].

**Discussion**

Many chronic inflammatory diseases, such as rheumatoid arthritis or systemic lupus erythematosus, are becoming common in the aging society worldwide. Among drugs used to
treat different rheumatic diseases, anti-inflammatory agents play an important role in improving the quality of life of these patients. However, the clinical use of the anti-inflammatory drugs for prolonged periods is associated with an increased risk of side effects. Efforts have been made to discover and develop new and promising anti-inflammatory agents from natural sources.\(^\text{[12]}\)

RO L., CA L., and LG Kunth — popularly known in Mexico as romero, epazote, and laurel, respectively — are broadly used in the food industry, and they have several functional properties, such as aromatic, antibacterial, antidepressant, and anti-inflammatory activities.\(^\text{[13-16]}\) TL Cav., which is known in Mexico as pericón, is used as an ornamental plant and has antifungal, antibacterial, larvicidal, and antidepressant activities.\(^\text{[17,18]}\)

SMs is the by-product of edible fungi cultivation, and the massive amounts of waste product can cause environmental problems. Recently, we demonstrated the antibacterial activity of spent \(P.\ ostreatus\) substrate against \(S.\ epidermidis, B.\ subtilis,\) and \(E.\ coli.\)\(^\text{[19]}\)

Several authors have shown different pharmacological effects for the same medicinal herb or source of bioactive compounds. For this reason, in this work, we evaluated the application of spent \(P.\ ostreatus\) substrates, enriched or not with medicinal herbs, as a source of anti-inflammatory compounds using the mouse ear TPA-induced edema model.

Ear edema induced by TPA is used to evaluate anti-inflammatory compounds that can act on the acute phase of inflammation. This model allows inhibitors of the biosynthesis of prostaglandins and leukotrienes, acting on key enzymes in the cascade of biosynthesis of these mediators, cyclooxygenase and lipoxigenase. In addition, TPA induces the expression of the nitric oxide synthase enzyme through the activation of protein kinase C in different tissues and proinflammatory cells.\(^\text{[12]}\) TPA induced an acute inflammatory response, characterized by vasodilatation and edema formation. This response occurs in the first 2 h, followed by increased thickness of the ear. The result is infiltration of polymorphonuclear leucocytes to the tissue.\(^\text{[15]}\) Therefore, extracts or compounds that have anti-inflammatory activity in this model may inhibit the formation of inflammatory mediators.

A previous report demonstrated that the ethanolic extract of RO L. decreased TPA-induced edema by 98.6% in mouse ears at a dose of 1 mg/ear (\(\text{EC}_{50}=128.3\ \mu\text{g/ear}\))\(^\text{[19]}\) while a cream containing 5% ethanolic extract of CA L. inhibited TPA-induced edema in mouse ears mice by 81%.\(^\text{[15]}\)

In this work, the anti-inflammatory effect exhibited by BS aqueous extract was 43.51% ± 6.64% IAO at a dose of 4 mg/ear. Consequently, the anti-inflammatory effect expected, according to the ratio of mixture BS/RO (80:20 BS: RO), is 54.4% IAO, of which 34.8% corresponds to the BS effect and 19.6% corresponds to the RO effect. However, our results showed that the BS/RO aqueous extract exhibited 92.9% ± 0.6% IAO at a dose of 4 mg/ear. Because this effect is greater than expected, we can conclude that there is an additive effect between the SMS and RO L.

In contrast, BS/CA aqueous extract showed a slight synergism because it induced a decrease in TPA-induced inflammation (94.0% ± 5.5%) that was slightly higher than CA L. (81%)\(^\text{[15]}\) and spent mushroom \(P.\ ostreatus\) substrate (BS, 43.5% ± 6.6%) in the same model.

In addition, LG Kunth and TL Cav. addition enhanced the anti-inflammatory effect of SMS; however, it is not possible to determine whether it was an additive or a synergic effect since these medicinal herbs’ anti-inflammatory effect has not been studied individually.

Further studies using a bio-guided chemical separation (by using the TPA-induced mouse ear edema as the monitoring test) to identify the compounds responsible for the pharmacological activity exhibited by these extracts of spent \(P.\ ostreatus\) substrate are in progress.

In summary, our results show that the spent \(P.\ ostreatus\) substrate of BS possesses compounds with a slight anti-inflammatory effect that can be enhanced if a medicinal herb is added to the substrate. The addition of CA L. to mushroom substrate showed a slightly synergic effect while RO L. addition had an additive effect. It is necessary to determine the anti-inflammatory effect of LG Kunth and TL Cav. individually to determine whether these medicinal herbs cause a synergic or an additive effect.

The \textit{in vivo} anti-inflammatory activity displayed by BS/RO, BS/CA, and BS/LG aqueous extracts indicate that spent \(P.\ ostreatus\) substrates are possible candidates for the development of new drugs to treat symptoms associated with inflammatory diseases, such as gout, osteoarthritis, and rheumatoid arthritis.

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Nil.
Conflicts of Interest

There are no conflicts of interest.

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