Original article

Ocimum basilicum Seeds lectin (OBSL) Relieves Pain through Central and Probably Peripheral Antinociceptive Mechanisms

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Abstract

In Sudan, *Ocimum basilicum* is reported to be used by locals as an analgesic and antimicrobial. This study evaluated the analgesic and antinociceptive potential of *Ocimum basilicum* seeds lectin (OBSL) in Swiss mice. Thermal (hotplate) and chemical (acetic acid) pain induction models were employed with different doses of OBSL. Intraperitoneal administration of OBSL significantly increased the latency time of the thermal threshold as well as acetic acid-induced writhing. These data suggest that OBSL has antinociceptive activity associated with peripheral and probably central antinociceptive mechanisms in facilitating the effect. Additionally, this investigation may provide a rationale for the frequent use of *O. basilicum* as an analgesic and may pave the way for further analysis for broader pharmacological future applications.

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1. Introduction

According to the International Association for the Study of Pain (IASP), pain is defined as *"An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*."(Merskey 1979). Pains are of two types nociceptive and neuropathic; nociceptive pain is caused by tissue injuries resulting from physical factors such as trauma and burn. At the same time, neuropathic pain is due to direct damage that affects the nerves (Nicholson 2006). Generally, the most employed pharmacotherapies drugs used for pain relief are nonsteroidal anti-inflammatory such as diclofenac, aspirin, ibuprofen, acetaminophen, mefenamic acid, etc. (Day and Graham 2013). These drugs cause many side effects, some of which are serious, especially when administered to patients suffering from acute illness of liver cirrhosis and kidney problems (Bancos et al. 2009). Therefore, the need for safe and effective natural remedies has become urgent. Herbal medicine and dietary supplements may provide a safer and usually effective treatment for pain relief, particularly for long-term use (Nunes et al. 2020).

Lectins are a group of sugar-binding and cell-agglutinating proteins or glycoproteins of non-immune origin found almost in entire life kingdoms ranging from viruses to humans (Sharon 2008). These lectins are synthesized during seed development together with other major storage proteins; although their functions are not well understood, strong evidence relating them to the defense mechanism of plants is widely discussed (Lannoo and Van Damme 2014; Nascimento et al. 2012; Vandenborre, et al. 2011). Depending on their properties and distribution in tissues and their abilities to recognize other molecules in different ways make them relevant in research involving purification, structural analysis, and applications in

immunology, pharmacology, medicine, molecular and cell biology (Santos et al. 2014).

Ociumum is a genus of an aromatic plant belonging to Lamiaceae, native to tropical zones. The genus comprises more than 100 species disseminated worldwide. Fourteen are grown in Sudan as ornamental plants and are locally known as *raihan* (Abduelrahman et al. 2009). *Ocimum sanctum* and *O. basilicum* are among the species that are widely cultivated in Khartoum State. The aqueous crude leaf extract of *Ociumum gratissimum, O. Sanctum and O. kilimandscharicum* are known for *their* antinociceptive properties. However, no details are given on the active ingredients responsible for this effect (Khanna and Bhatia 2003; Mwangi et al. 2012; Tanko et al. 2008). Since many plant lectins are endorsed for their analgesic and antinociceptive properties (Campos et al. 2016; de Freitas Pires et al. 2013) and reviewed in ref (Konozy, et al. 2022), it was tempting to purify lectin from *O. basilicum* and test its analgesic effect using an animal model.

2. Materials and Methods

2.1 Materials and Reagents

Good quality *Ocimum basilicum* seeds were collected locally and used for the isolation of lectin and purification. Typed human blood cells (A) were obtained from healthy donors. All other drugs and reagents were purchased from SDFCL India and KRKA Slovenia. The study was conducted at the laboratory of glycobiology and proteomics, Africa city of technology, Khartoum, Sudan.

2.2 Experimental Animals

Male Swiss albino mice (20±5g) purchased from the Faculty of Medicine University of Khartoum were used. Animals were housed in environmentally controlled conditions (25±2°C) with a 12 h light/dark cycle for at least one week and fed on a standard pellet diet and water ad libitum. Before the assays, animals were fasted for 24 h with free water access.

2.3 Ethical Statement

The experimental protocols followed the standard international ethical guidelines for the use of animals. Ethical clearance Ref. BioMed/29/2021 was obtained from the respective department.

2.3 Extraction and Isolation of OBSL

OBSL was purified to homogeneity, as described earlier (Dafalla et al. 2022). Briefly, 1g: 30mL of defatted *O. basilicum* seeds powder was extracted for 4 hrs under a cold condition with 50mM Tris-HCl buffered saline pH 7.4; the extract was centrifuged at 10000*rpm* for 30 min. The clear supernatant was then used for lectin activity evaluation, protein content determination and lectin purification. OBSL was purified on a fetuin-agarose affinity column (1cm x 10cm), and the bound lectin was eluted using 3% acetic acid prepared in 150mM NaCl. The eluted lectin was dialyzed exhaustively against distilled water, lyophilized to dryness and preserved at -20 °C until further use. Just before use, the purified lectin was dissolved in a minimal amount of distilled water. Protein concentration was determined by Bicinchoninic Acid (BCA) method (Walker 2009) using bovine serum albumin (BSA) as the standard.

2.4 *In-vivo* antinociceptive activity of OBSL

2.4.1 Hotplate test

Mice were submitted to a plate heated to 50±5 °C to induce pain according to the methodology of Eddy (Eddy and Leimbach 1953). Mice were segregated into five groups; each had five animals. The first three groups received intraperitoneally 3, 6 and 12mg/kg of purified OBSL; respectively, the 4th group acted as a negative control where animals only received saline. The fifth group had diclofenac sodium, which acted as a reference drug. The hotplate cut-off time was 45s to avoid animal paw lesions.

2.4.2 Acetic acid-induced writhing

Mice were distributed into five groups exactly as shown in section 2.4.1. 0.7% acetic acid (10 mL/kg body weight) was used as the primary pain inducer agent. The writhing signs were reported after 10 min from the duration of 20 min. Thirty minutes before the acetic acid administration, the animals were pretreated with intraperitoneal injection with the OBSL in doses of 1.5, 3 and 6mg/kg. The negative control animals were injected with 0.7% acetic acid. Diclofenac Na (10 mg/kg/*ip*.) was used as a reference drug.

2.5 Statistical analysis

The results are reported as the Means ± S.E.M for the

antinociceptive studies; the statistical analysis was performed using one-way ANOVA followed by Bonferroni's test. A *p*-value of less than 0.05 was taken as the significance level. Analysis was performed using SPSS 26.0 package (Armonk, NY: IBM Corp., USA)

3. Results and Discussion

Thermal and chemical pain induction models are well-known methods for evaluating pain perception mechanisms and the effectiveness of new analgesics (Bahamonde et al. 2013; Khan et al. 2010; Khatun et al. 2015; Somchit et al. 2004). In the current study, the previously purified *Ocimum basilicum* seed lectin (OBSL) (Dafalla et al. 2022) was tested for its ability to act as an antinociceptive agent.

3.1 Hotplate pain induction assay model

Intraperitoneal administration of OBSL to experimental animals at varying doses of 3, 6 and 12mg/Kg resulted in remarkable pain perception intervention; however, significant results were obtained with 6mg/Kg at 30 minutes of administration (*p* 0.012). On the other hand, at 60 minutes of administration, both 3 and 6 mg/Kg doses of OBSL had also shown significant results (*p* 0.027 and 0.027, respectively). Interestingly, after 60 minutes of OBSL administration, 3 and 6 mg/Kg OBSL doses exhibited a better analgesic effect than the standard drug diclofenac sodium, indicating the effectiveness of this protein in pain perception intervention. Of interest, when we used OBSL at a dose of 12mg/Kg, the results were insignificant ($p > 0.05$) (Figure 1). However, these results were not of a surprise to us, as many drugs, when used at different dosing, were shown to exhibit a different mode of action. For example, aspirin at low doses inhibits thromboxane A2 formation, whereas, at a dose greater than 300mg, it acts as an analgesic (Ugurlucan et al. 2012), so we cannot exclude a similar mechanism in the case of OBSL. As hotplate pain sensation is a central specific nociceptive test in which sedative (opioid) agents exert their mechanisms through supra-spinal and spinal receptors, the obtained analgesic effect of OBSL could logically be related to the central mechanism *via* supra-spinal and spinal receptors (Silva et al. 2010).

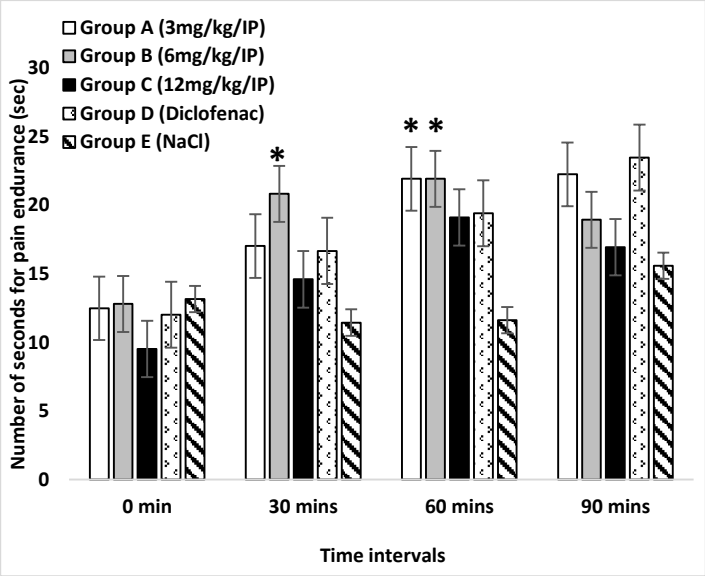

Fig. 1: The pain endurance measured after 30 min intervals from administering different doses of OBSL compared to the negative control, asterisk signs represent the significant mean difference at $P_{0.05}$, 95% CI

3.2 Acetic Acid Assay Model

Intraperitoneal injection of 0.7% acetic acid into mice created substantial pain exhibited by the mice writhing signs. The pain could significantly (*p* 0.02, 0.019 and 0.014, respectively) be relieved upon administration of different doses of lectin (OBSL) (1.5, 3, and 6mg/Kg); in which the best result was obtained with 6mg/Kg OBSL (Figure 2). Writhing assessment is a chemical method used to trigger the pain of peripheral or central origin by injecting irritant principles like acetic acid and phenyl-p-benzoquinone in mice. In particular, many publications have reported the acetic acid–prompted abdominal writhing model as a classic non-selective animal pain model (Sharma et al. 2019) for evaluating analgesic agents (Gawade 2012; Pavao-de-Souza et al. 2012). Lectins purified from different parts of plants have been shown to possess varying degrees of antinociception (Araújo et al. 2011; Campos et al. 2016; Cavalcante da Silva et al. 2021; de Freitas Pires et al. 2013). Since acetic acid triggers writhing tests through both central and peripheral analgesia, it will be difficult to relate the obtained analgesic effect by OBSL to either of the two mechanisms.

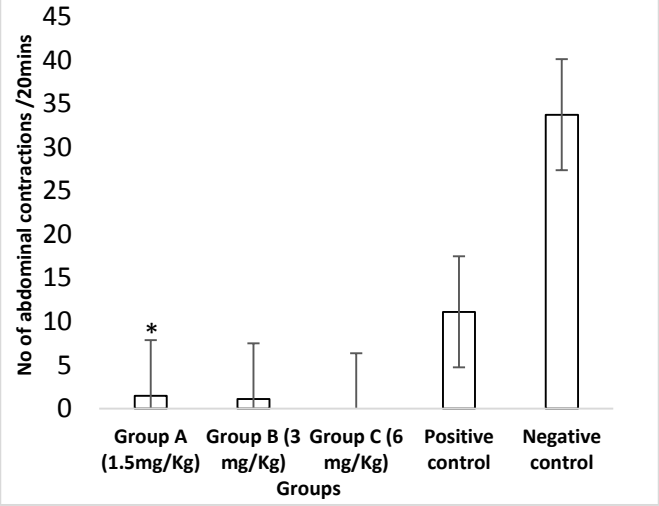

Fig. 2. Effect of OBSL (different doses) in the number of writhes in the nociception model induced by 0.7% acetic acid. Values are expressed as the mean for each group. While the asterisk sign represents the significant mean difference at $p_{0.05}$, 95% CI.

4. Conclusions

From our findings, the investigated plant OBSL possesses a potent antinociceptive property and can be used as a natural painkiller product for pharmacological purposes. Although these results may put forward the basis for the possible future applications of these interesting proteins, extensive toxicological and immunological studies may seem imperative.

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