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Anti-inflammatory and antiedematogenic activity of the *Ocimum basilicum* essential oil and its main compound estragole: In vivo mouse models

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ABSTRACT

The genus *Ocimum* are used in cooking, however, their essential oils are utilized in traditional medicine as aromatherapy. The present study was carried out to investigate the chemical composition and systemic anti-inflammatory activity of the *Ocimum basilicum* essential oil (EOOB) and its major component estragole, as well as its possible mechanisms of action. The *Ocimum basilicum* essential oil was obtained by hydrodistillation and analyzed by GC-MS. The anti-inflammatory action was verified using acute and chronic *in vivo* tests as paw edema, peritonitis, and vascular permeability and granulomatous inflammation model. The anti-inflammatory mechanism of action was analyzed by the participation of histamine and arachidonic acid pathways. The chemical profile analysis identified fourteen components present in the essential oil, within them: estragole (60.96%). The *in vivo* test results show that treatment with EOOB (100 and 50 mg/kg) and estragole (60 and 30 mg/kg) significantly reduced paw edema induced by carrageenan and dextran. The smallest doses of EOOB (50 mg/kg) and estragole (30 mg/kg) showed efficacy in the reduction of paw edema induced by histamine and arachidonic acid, vascular permeability inhibition and leukocyte emigration in the peritoneal fluid. Theses doses were capable of reducing the chronic inflammatory process. The results observed between the EOOB and estragole demonstrate efficacy in anti-inflammatory activity, however, the essential oil is more efficacious in the acute and chronic anti-inflammatory action. This study confirms the therapeutic potential of this plant and reinforces the validity of its use in popular medicine.

1. Introduction

Inflammation is the body's response to foreign stimuli. This is a defense mechanism whose function is to protect the body from assaults of an endogenous or exogenous etiologic agent. The inflammatory process comprises the vascular and cellular changes that aim to bring to the site of irritation the constant flux of blood

Abbreviations: NIH, National Institute of Health-USA; CONCEA, Conselho Nacional de Controle de Experimentação; Brasil, URCA; Regional University of Cariri, Brasil; HPLC-DAD, High-Performance Liquid Chromatography with Diode-Array Detection; sc, subcutaneous injection way; ip, intraperitoneal injection way; vo, oral way; EOOB, Essential Oil of *Ocimum basilicum*; LOX, Lipoxygenase enzyme; COX, Cyclooxygenase enzyme.

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and defense cells. The inflammatory response can occur in an acute and chronic form, and during this response chemical substances are produced that act as mediators inducing biochemical and molecular mechanisms that characterize clinical manifestations [1,2].

Chemical inflammatory mediators, such as histamine, arachidonic acid (AA) products, cytokines and chemokines, act through receptors in target cells of different tissues of the organism. These substances induce vascular and cellular events promoting the exudation and emigration of leukocytes [3]. Persistence of the inflammatory process, as well as the constant production of chemical mediators by cells and plasma proteins, can cause tissue damage and produce potentially harmful signals and symptoms. Some stimuli prolong inflammation making the defense process chronic with the presence of offending tissue cells like macrophages and lymphocytes [4]. These cells are capable of synthesizing lethal chemical substances to both the offending agent and the body itself [5–7].

Natural products derived from plants are still an important part of the traditional health care in developing countries. In recent years there has been a resurgence of interest in medicinal plants in all countries as alternative sources of drugs for intractable diseases, such as inflammatory diseases [8].The genus *Ocimum* belongs to the family Lamiacea originating in Southeast Asia and Central Africa, it possesses more than 3200 species widely spaced around the planet that are perfectly adapted to tropical and subtropical regions. The species of this genus are aromatic plants with diverse commercial purposes in popular medicine [9–11] and can be used as a new class of ecological products for controlling insect pests or Insect Pest management [12]. The species *Ocimum basilicum,* known popularly as basil and fragrant basil, is an aromatic underbrush, cultivated and commercialized in Brazil for food purposes, using fresh leaves for flavoring sauces and pastas [13,14]. In addition to its potential culinary use, the essential oil from basil is used as larvicide [15], repellents [16,17], antifungal [18,19] and antimicrobials [20].

Basil oils have marked differences in composition, and some chemotypes from different geographical origins have been chemically classified as “linalool,” “linalool and methylchavicol (estragole),” and “linalool and eugenol” [21]. Moreover, the aromatic and intraspecific variation in plant morphology, determined by genotype, greatly influences the essential oil composition of the leaves [22]. The major components of different parts of *Ocimum basilicum* were estragole, α-phellandrene, limonene, fenchone, however, linalool, eugenol and methylchavicol are the chemical fingerprint of this species [23].

Several biological activities have been shown for the essential oil of *O. basilicum* leaves as antifungal, insect-repelling activity [24], antigiardial [25], antioxidant capacity [26,27], anxiolytic, sedative [28] and others. For methylchavicol (estragole), insecticidal activity [29], anti-edematogenic effects [30], anti-Candida effects [31] and others, have been shown. There are few studies for this species in relation to anti-inflammatory effects and the possible mechanism of actions and, this work, portrays the first experimental report on acute and chronic *in vivo* experimental models. This paper showed an important and potential use of the essential oil of leaves from *Ocimum basilicum* and its major chemical component estragole as an anti-inflammatory and the influence on the histamine and eicosanoids pathways.

2. Materials and methods

2.1. Legal requirements: ethical aspects of the research

The research was conducted in strict compliance with the current rules and bioethical guidelines for trials involving living beings: animals (*Guide for the care and use of laboratory animals,* of NIH – National Institute of Health-USA, 1996; Federal Law No. 11,794/2008; Conselho Nacional de Controle de Experimentação – CONCEA); and flora and fauna integrity (Federal Law No. 9605/1998). This work was submitted to the Ethics Committee for Animal Research (CEUA) of the Regional University of Cariri – URCA for evaluation of experimental protocols, and was approved under No. 00040/2014.2.

2.2. Extraction of the essential oil

The leaves were collected from the garden of medicinal plants of the Regional University of Cariri-CE. A voucher of the plant specimen was deposited in the Carirense Dárdano de Andrade Lima Herbarium -HCDAL of the Regional University of Cariri–URCA, under registration number 10736. The plant was identified and classified by Profª Drª Maria Arlene Pessoa da Silva. The leaves (fresh) collected (3,950 g) were washed in running water and subjected to distillation drag with water vapor in Clevenger type device. The final oil was dried with the aid of anhydrous sodium sulfate, stored in 1 mL fractions in an amber bottle and maintained in a refrigerator for later analysis.

2.3. Chemical analysis of essential oil from leaves of Ocimum basilicum Linn

Analysis of the oil was performed using Shimadzu GC/MS – series QP2010 (GC/MS system) equipment, RTX – 5MS capillary column (30 m × 0.25 mm, 0.25 mm film thickness), helium gas as the carrier at 1.5 ml/min, gun temperature 250 °C; detector temperature 290 °C, column temperature 60 °C – 180 °C at 5 °C/min, followed by 180° – 280 °C at 10 °C/min (10 min). Scanning speed of 0.5 scan/sec of *m/z* 40 and 350, Split ratio (1:200). The injected volume was 1 ml of [25 μl (essential oil)/5 μl $CHCl_3$] (1:200), with solvent cutting time = 2.5min. The mass spectrometer was operated with 70 eV ionization energy and identification of individual components was based on their mass spectral fragmentation based on the NIST Mass 08 spectral library, retention indices, and comparison with published data.

2.4. In vivo experimental protocol

2.4.1. Drugs

All pure compounds were acquired from Sigma-Aldrich Corporation (St. Louis, MO, USA). The drugs were prepared on the day of the experiments and administered by subcutaneous injection (sc), intraperitoneal injection (ip) or orally (vo), in the volume of 0.1 ml/10 g of body mass. Control animals received the same volume of one of the sterile saline solutions, or vehicle.

2.4.2. Animals

For the realization of *in vivo* tests, randomly chosen mice were utilized (*Mus musculus*), albinos, Swiss strain for both sexes, with enclosed body mass of 20–30 g. These were kept packed in polypropylene and maintained in environment temperature between 22 ± 3 °C, dark/light cycles of 12 h and with free access to drinking water and specific rodent chow (Labina, Purina®).

2.4.3. Acute oral toxicity test in mice

The mice were randomly separated, weighed and divided in two groups (n = 5) and fasted for a period of 6 h with free access only to water, and the chow was allowed 3 h after administration. The EOOB was administered in the concentrations 2000 and 1000 mg/kg v.o (single dose), each animal received a different concentration with the volume of 0.1 ml/10 g. After administrations the animals were observed for the first 30, 60, 120, 240 and 360 min and at