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Original article

Isolation, identification and molecular docking as cyclooxygenase (COX) inhibitors of the main constituents of *Matricaria chamomilla* L. extract and its synergistic interaction with diclofenac on nociception and gastric damage in rats

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ABSTRACT

Chamomile (*Matricaria chamomilla* L., *Asteraceae*) is a medicinal plant widely used as remedy for pain and gastric disorders. The association of non-steroidal anti-inflammatory drugs (NSAIDs) with medicinal plant extracts may increase its antinociceptive activity, permit the use of lower doses and limit side effects. The aim was to isolate and identify the main chemical constituents of *Matricaria chamomilla* ethanolic extract (MCE) as well as to explore their activity as cyclooxygenase (COX) inhibitors *in silico*; besides, to examine the interaction between MCE and diclofenac on nociception in the formalin test by isobolographic analysis, and to determine the level of gastric injury in rats. Three terpenoids, α-bisabolol, bisabolol oxide A, and guaiazulene, were isolated and identified by ^{1}H NMR. Docking simulation predicted COX inhibitory activity for those terpenoids. Diclofenac, MCE, or their combinations produced an antinociceptive effect. The sole administration of diclofenac and the highest combined dose diclofenac-MCE produced significant a gastric damage, but that effect was not seen with MCE alone. An isobologram was constructed and the derived theoretical ED_{35} for the antinociceptive effect was significantly different from the experimental ED_{35}; hence, the interaction between diclofenac and MCE that mediates the antinociceptive effect is synergist. The MCE contains three major terpenoids with plausible COX inhibitory activity *in silico*, but α-bisabolol showed the highest affinity. Data suggest that the diclofenac-MCE combination can interact at the systemic level in a synergic manner and may have therapeutic advantages for the clinical treatment of inflammatory pain.

1. Introduction

Chamomile (*Matricaria chamomilla* L., *Matricaria recutita* L., *Matricaria suaveolens* L.) is a widely recognized medicinal plant from the Asteraceae family; it is native to southern and eastern Europe and cultivated also in countries of America and Asia. Chamomile flower heads contains an essential oil (0.4–1.5%) which has an intense blue color, being chamazulene and α-bisabolol some of its main constituents [1,2]. Chamomile is used in traditional medicine for the treatment of gastrointestinal problems such as dyspepsia, epigastric swelling, colic, ulcers, impaired digestion, diarrhea, and flatulence. Chamomile has also been used in the treatment of restlessness and in mild cases of insomnia due to nervous disorders. Topical use of chamomile has been beneficial for the treatment of inflammation and irritations of the skin, mucosa of mouth and gums, as well as hemorrhoids [1,2].

Chamomile extract and/or its constituents have demonstrated antinociceptive and anti-inflammatory activities in animal models [1–4]. In clinical studies, the fluid extract from chamomile has

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shown an analgesic effect in patients with aphthous stomatitis and other painful ulcers of the oral mucous membrane as well as its administration was very well tolerated [5]; besides, twice-a-day chamomile compresses application on peristomal skin lesions in colostomy patients revealed to be more effective in decreasing the pain, the inflammation and the itching than a treatment with once-a-day hydrocortisone 1% ointment [6]. Schneider et al. [7] assessed the non-inferiority of therapy based on a homeopathic ointment preparation (*Chamomilla recutita, Arnica Montana, Calendula officinalis, Hananelis virginica, Echinacea angustifolia,* and *Echinacea purpurea* as the main constituents) compared with a treatment based on diclofenac 1% gel in patients with tendinopathies of diverse etiology; then, the results exhibited that the homeopathic therapy was non-inferior to the diclofenac therapy on all efficacy variables, for example, motility-related variables trended toward the superiority of the homeopathic ointment. Indeed, it has been reported that the association of non-steroidal anti-inflammatory drugs (NSAIDs) with plant extracts can increase its analgesic activity, what permits the use of lower doses and thus limiting side effects. In the study of Schneider et al. [7]. it would have been very useful to assess the efficacy and security of the homeopathic ointment-diclofenac combination; perhaps, the combination had reached a better analgesic effect in those patients with tendinopathies. Moreover, it is suggested that the new phytopharmaceutical products or herbal preparations should be evaluated in the first instance in experimental animal models, and later in humans.

Additionally, the participation of COX in nociception and inflammation is undeniable as well as the discovery of COX-2 provided the rationale for the development of a new class of NSAIDs, the selective COX-2 inhibitors, with the aim of reducing the gastrointestinal toxicity associated with the administration of NSAIDs by virtue of COX-1 sparing [8]. In this regard, the inhibitory activity of chamomile or its constituents on COX has been suggested as a mechanism of action for their analgesic or antiinflammatory properties [3,9]. Furthermore, molecular docking is a powerful tool to simulate *in silico* the interaction and complex formation between a ligand and its target protein; hence, molecules with high binding affinity (low docking energy) for a targeted protein exhibit therapeutic efficacy, as the case of a docking study of α-bisabolol which revealed its strong binding affinity to pro-inflammatory cytokines [10].

Thus, the aim of this work was to isolate and identify the main chemical constituents of *Matricaria chamomilla* ethanolic extract (MCE) as well as to explore their activity as cyclooxygenase (COX) inhibitors *in silico*; besides, to examine the effects from the interaction between MCE and diclofenac on nociception and gastric injury in rats.

2. Materials and methods

2.1. Chemicals and drugs

Matricaria chamomilla ethanolic extract (MCE) was supplied by Gehrlicher Pharmazeutische Extrakte GmbH (Germany) and it was obtained according to the European Pharmacopoeia, 7th Edition 2011; the certified essential oil content was 1.11% (m/m) and α-bisabolol content was 0.56% (m/m). Diclofenac and formaldehyde were purchased from Sigma (St. Louis, MO, USA). Solvents were purchased from Tecsiquim S.A (Mexico) and further purified by fractional distillation.

2.2. Chemical compounds isolation and identification

MCE was concentrated to dryness using a rotary evaporator in vacuo at 40 °C. The oily brown viscous residue was then separated by column chromatography carried out through glass columns (2 × 50 cm) packed with silica gel 60 μm (mesh 0.063–0.2 mm, Merck, Germany), and eluted with mixtures of hexane, ethyl acetate and ethanol, followed by an 10% increase in the polarity. Fractions were collected, concentrated and controlled by thin layer chromatography (TLC) on silica $60F_{254}$ aluminum plates (Merck, Germany) as well as were visualized by ultraviolet irradiation in a chromato-vue apparatus; then, similar fractions were combined according to their purities. Compounds identification was achieved through ^{1}H-nuclear magnetic resonance (NMR) spectra that were measured on Varian Mercury spectrometer operating at 400 MHz. Chemical shifts (δ) are given in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, as well as deuterated chloroform ($CDCl_3$) for NMR from Sigma (St. Louis, MO, USA) was used as solvent for the compounds isolated from MCE.

2.3. Molecular docking study

Ligands were constructed by ChemBioOffice 2010 and the energy minimization performed with Molecular Mechanic by ChemBioDraw ultra 12 software. An approach to the real molecular spatial array was achieved by Gaussian 03W and GaussView 3.0, using a Hartree-Fock calculation for the molecule in neutral basal state (without electrical charge). Once finished the calculation, the final molecule structure and the information were saved in the mol2 format for posterior manipulation. The 3D structure of COX-1 and COX-2 in PDB format were obtained from the Swiss Institute of Bioinformatics (SIB) [11]; the enzyme identification for rat COX-1 (PTGS1) from National Center for Biotechnology Information (NCBI) is: 10116, and from Universal Proteine Resource (UniProt): Q63921, as well as for human COX-2 (PTGS2), NCBI: 9606 and UniProt: P35354. Finally, after obtainment of ligands and target proteins, the docking procedure was performed and optimized by the SIB web site service.

2.4. Animals

Male Wistar rats aged 7–9 weeks (weight range: 180–220 g) from our own breeding facilities were used in this study; animals were housed in regular plastic cages at 22–24 °C temperature, under a 12 h light–dark cycle, as well as they had free access to food (standard Purina chow diet, USA) and purified water ad libitum. Efforts were made to minimize animal suffering and to reduce the number of animals used. Each rat was used in only one experiment and at the end of the experiments they were sacrificed in a CO_2 chamber. All experiments followed the Guidelines on Ethical Standards for Investigation in Animals [12], and the criteria outlined in the Guide for the Care and Use of Laboratory animals [National Institutes of Health Publ. 86–23, rev. 1985]; also, the protocol was approved by the Institutional Animal Care and Use Committee (CINVESTAV-IPN, Mexico).

2.5. Measurement of antinociceptive activity

Nociception and antinociception were assessed using the formalin test, as previously described [13]. Briefly, 15 μL of diluted formalin (1%) were injected subcutaneously (s.c.) into the plantar surface of the right hind paw ($n = 8$ each group), and the resulting flinching behavior was considered to be an expression of nociception. Graphs of the number of flinches against time were constructed, and the resulting curves were biphasic. After the initial acute phase (0–10 min) there was a short quiescent period, and this was followed by a prolonged tonic response (15–60 min). The area under the curve for both phases was estimated, and a significant reduction in the area was interpreted as an anti-nociceptive effect.