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Research paper

Anti-inflammatory, analgesic activities and gastro-protective effects of the phenolic contents of the red alga, *Laurencia obtusa*

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ABSTRACT

Introduction: The present study was conducted to evaluate anti-inflammatory, analgesic and gastro-protective activities of methanol/dichloromethane extract and its semi-purified fractions from the red alga *Laurencia obtusa*.
Method: Anti-inflammatory activity was evaluated *in vitro* using cytometric bead array technology to follow up the secretion of TNF-α in lipopolysaccharide activated THP-1 cells and *in vivo* using carrageenan-induced rat paw edema test. Edema was induced with 1% carrageenan after 30 min of intra-peritoneal treatments. Paw thickness was checked at $t = 1, 2, 3, 4$ and 5 h. Analgesic activity was carried out using acetic acid-induced writhing test in mice. 1% of acetic acid was administrated intraperitoneally at the dose of 10 mL/kg, 30 min after treatment and the number of writhing was recorded during 30 min. Gastro-protective effect was determined using HCl/EtOH induced gastric ulcers in rats. 10 mL/kg of HCl/ethanol (60% ethanol in 150 mM HCl) was orally administrated to all groups after treatment. 1 h later, stomachs were examined for the presence of lesions.
Results: Crude extract and ethanol fraction showed an excellent inhibition of TNF-α secretion and a significant decrease in paw thickness ($P < 0.05$), 3 h after carrageenan injection. Ethanol fraction also showed a significant ($P < 0.05$) analgesic activity and an excellent decrease of gastric damages.
Conclusion: This study indicates that the red alga *L. obtusa* may be useful for developing potential therapeutic substances with anti-inflammatory, analgesic and gastro-protective activities. These activities, observed especially with the crude extract and the ethanol fraction may be related to their phytochemical composition.

1. Introduction

Medicinal plants, used in complementary and alternative medicine are being widely investigated, in several parts of the world, to analyze their potential as preventive and therapeutic agents [1]. In the last decades, the marine environment has been recognized to be a rich natural source of new promising compounds which exhibit interesting biological and pharmacological activities, including anti-inflammatory effects [2]. Approximately 15,000 pharmacologically active compounds have been isolated from marine species with unique compounds that are not present in terrestrial organisms [3]. A large number of marine bioactive compounds are under investigation and/or are being used in the treatment of various diseases [4].

Red algae elaborate a wide variety of structurally unusual secondary metabolites that may inhibit inflammatory reactions by suppressing the production of inflammatory mediators [5] and prevent or treat gastric ulcers and cancers caused by oxidative stress [6]. Natural compounds derived from the edible algae could be used as anti-inflammatory, analgesic and anti-ulcerogenic therapeutics as they have been taken in alimentation and used in traditional medicines in the last centuries [7].

Among all marine red algal flora, many species of the genus *Laurencia* (Family Rhodomelaceae) are used as human food in

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many countries like China, Japan, Korea, Malaysia, Thailand, Indonesia, Philippines and other South East Asian countries in the form of soup, salad, curry, etc. They are a cheap source of minerals, trace elements, dietary fibers, proteins and vitamins [8]. Species of the genus *Laurencia* are also known to produce a wide range of natural products like sulphated polysaccharides, polyphenols, sesquiterpenes, diterpenes and halogenated C15 acetogenins [9–11]. These compounds have been shown to possess antioxidant, anticancer, antibacterial, anti-asthmatic, and anti-inflammatory activities [12–16]. *Laurencia obtusa* (*L. obtusa*) (Rounded Brittle Fern Weed), is a widespread red algae in the Mediterranean Sea [17,18] Atlantic Ocean and Indian Ocean in South Africa and Tanzania [19]. It is used in traditional medicine in South India [20] and has been shown to be a rich source of novel bioactive secondary metabolites possessing antioxidant activity [21], antimalarial activity [22], cytotoxic activity [23] and antimicrobial activity [24].

Taken together, these results, especially the antioxidant activity of *L. obtusa*, lead to the hypothesis that *L. obtusa* might have anti-inflammatory potential.

Several anti-inflammatory agents are used to treat different pain types associated with inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications in the world because of their demonstrated efficacy in reducing pain and inflammation [25]; their basic mode of action is inhibition of the pro-inflammatory enzyme cyclooxygenase 2 (COX-2). Although effective at relieving pain and inflammation, NSAIDs are associated with a significant risk of serious gastrointestinal adverse events [26]. In chronic use, some representative NSAIDs (e.g. phenylbutasone, aspirin and sulindac) were proven to induce the production of mighty pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α) [27,28].

Overproduction of TNF-α believed to cause a wide range of human diseases [29]. It is a crucial mediator of NSAIDs-induced gastric mucosal injury [30]. In spite of enormous efforts, the only available drugs to inhibit TNF-α activity, in clinics, are protein-based drugs (Etanercept, Infliximab, Adalimumab and Anakinra) with many adverse effects such as aplastic anaemia, pancytopenia, vasculitis, demyelination and congestive heart failure [31]. Hence, the search for natural anti-inflammatory and analgesic substances that can block TNF-α signaling without side effects remains necessary. The present study was carried out to investigate the anti-inflammatory and analgesic activities with gastroprotective effect of the red alga *L. obtusa.*

2. Materials and methods

2.1. Reagents and chemicals

All solvents were commercially obtained (Sigma–Aldrich) at the highest commercial quality and used without further purification except where noted. Folin–Ciocalteu phenol reagent was obtained from BDH laboratory (Poole, England). Aluminum chloride ($AlCl_3$) was procured from Sigma Aldrich (Steinheim, Germany). Carrageenan from BDH Chemicals Ltd (Poole England), Acetylsalicylate of Lysine (ASL) and Diclofenac were purchased from Sigma Chemical (Berlin, Germany). Ranitidine and Omeprazol were obtained from Medis (Tunis, Tunisia). RPMI-1640 medium with GlutaMax™ and penicillin-streptomycin were obtained from Life Technologies (Saint Aubin, France), fetal bovine serum from BioWhittaker (Verviers, Belgium) and Celastrol from SurroMed (USA). Lipopolysaccharide (LPS) from *Salmonella abortus equi* was obtained from Sigma Aldrich (L'Isle d'Abeau Chesnes, France). Human Th1/Th2Cytokine Cytometric Bead Array (CBA) kit was obtained from BD Bioscience (Le Pont de Claix, France).

2.2. Sample collection and extract preparation

The red alga *L. obtusa* was collected from the coastal region of Bizerte (Tunisia) in the Mediterranean Sea, in June 2010, at a depth of 1–2 m. The identification of specimen was carried out in the National Institute of Marine Sciences and Technologies, Salambo, Tunisia. A voucher specimen was deposited in the Department of Pharmacology, Faculty of pharmacy of Monastir under the reference (Lo 2865). The collected samples were cleaned by rinsing with sea water and distilled water, to remove associated debris and epiphytes. Seaweeds were then air dried in the shade at $30 \pm 2\,^{\circ}C$ for 1–2 weeks and powdered using electric mixer grinder. About 1000 g of weighed dried powder of the whole algal biomass were finely packed in small bags (5×10 cm) of Whatman filter paper No. 1 (15 g of powder in each bag), all bags were sealed and soaked in 2 L of methanol-dichloromethane (1:1, v/v) for 48 h with frequent stirring, then, filtered. A ratio of 1:2 (w/v) solvent to the dry mass of the seaweed material was used for the extraction. This process was repeated three times on the residue. The crude extract was concentrated to solvent free by evaporation in a rotating evaporator (Buchi, B-480) at low temperature ($<40\,^{\circ}C$).

2.2.1. Purification and fractionation of the crude extract

Methanol/dichloromethane extract of *L. obtusa* was fractionated and purified, using C18 cartridges (Sep-pack, Supelco), by gradient elution with 500 mL of different organic solvents in the order of decreased polarity: ethanol, acetone and methanol/dichloromethane (1:1) to give three semi-purified fractions: ethanol (F-EtOH), acetone (F-Ac) and methanol/dichloromethane (F-MeOH/CH_2Cl_2) fractions [32,33]. Organic solvents were removed from recuperated fractions using rotating evaporator at $40\,^{\circ}C$.

2.3. Total phenolic content

Total phenolic content (TPC) of the crude extract and its semi-purified fractions from *Laurencia obtusa* was estimated by the method of Taga et al.]34]. The Folin–Ciocalteu reagent determines total phenols, producing blue color by reducing yellow hetero polyphosphate molybdate tungstate anions. The absorbance of all sample solutions was measured at 720 nm using spectrophotometer (Jenway 6505 UV/Vis). A calibration curve of gallic acid (GA) (0.03–1 mg/mL) was prepared and TPC was determined.

2.4. Total flavonoids content

The total flavonoid contents (TFC) of the crude extract and its semi-purified fractions were determined by aluminum chloride ($AlCl_3$) colorimetric method [35]. The absorbance of all sample solutions was measured at 415 nm using UV–VIS spectrophotometer. The TFC was determined on a standard curve using rutine as standard. The mean of three readings was used and expressed as mg of rutine equivalents per 1 g of sample (mg RE/1 g sample).

2.5. Cell culture

Acute monocytic leukemia cells (THP-1, ATCC® TIB-202), purchased from American Type Culture Collection (ATCC), were maintained in RPMI-1640 medium with GlutaMax™, supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin–streptomycin (10,000 units/mL and 10,000 μg/mL). Cells were grown in humidified atmosphere with 5% CO_2 at $37\,^{\circ}C$ in 25 cm^2 and 75 cm^2 flasks up to 70–80% confluency prior to treatment. Cells were replicated every 2–3 days and the medium changed.