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Antiinflammatory and immunomodulatory activity of an ethanolic extract from the stem bark of *Terminalia catappa* L. (Combretaceae): *In vitro* and *in vivo* evidences

Oyindamola O. Abiodun [a], Alba Rodríguez-Nogales [b], Francesca Algieri [b], Ana Maria Gomez-Caravaca [c,d], Antonio Segura-Carretero [c,d], M. Pilar Utrilla [b], M. Elena Rodriguez-Cabezas [b,1], Julio Galvez [b,*,1]

[a] Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria
[b] CIBER-EHD, Department of Pharmacology, ibs.GRANADA, Centre for Biomedical Research (CIBM), University of Granada, Granada, Spain
[c] Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avenida Fuentenueva s/n, 18071 Granada, Spain
[d] Research and Development Centre for Functional Food (CIDAF), Health-Science Technological Park, Avenida del Conocimiento 37, 18016 Granada, Spain

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ABSTRACT

Ethnopharmacological relevance: *Terminalia catappa* Linn (Combretaceae) is a medicinal plant with anti-inflammatory, anti-diarrhoeal and antioxidant properties, frequently found in tropical regions. Considering its characteristics, it could be useful for the treatment of inflammatory bowel disease, which is associated with inflammation, oxidative stress and an immune dysfunction. Thus this study evaluates the immunomodulatory properties and the intestinal anti-inflammatory effect of an ethanolic extract of the stem bark of *T. catappa* (ETCB) both *in vitro* (in RAW 264.7 macrophages) and *in vivo*, in the trinitrobenzenesulfonic acid (TNBS) model of rat colitis.

Materials and methods: The phenolic compounds in ETCB were identified and quantified using HPLC-DAD-qTOF-MS. The immunomodulatory activity ETCB was tested *in vitro* by determining the macrophage production of IL-1β and nitrites. *In vivo* studies were performed in the TNBS model of rat colitis. ETCB was given (25, 50 and 100 mg/kg/day) orally for two days prior to colitis induction and thereafter for 7 days. Response to treatment was assessed by scoring the gross appearance of the colon, and determining myeloperoxidase activity, gene expression of pro-inflammatory cytokines like TNF-α, IL-23 and IL-6, chemokines, inducible nitric oxide synthase and proteins crucial in the maintenance of the intestinal mucosal barrier integrity like mucins (MUC-2, MUC-3) and villin.

Results: ETCB was able to inhibit IL-1β and nitrite production *in vitro* in RAW 264.7 macrophages. Moreover, treatment of TNBS colitic rats with ETCB resulted in a decreased colonic damage score and weight/length ratio. It also reduced the colonic neutrophil infiltration indicated by a lower myeloperoxidase activity and prevented the depletion of colonic glutathione levels in colitic rats. In addition, treatment with ETCB down-regulated the gene expression of pro-inflammatory mediators (TNF-α, IL-23, IL-6 and CINC-1) and iNOS in colitic rats. Moreover, the gene expression of mucosal barrier proteins like MUC-2, MUC-3 and villin were up-regulated in colitic rats treated with ETCB. The dose of ETCB that produced the most significant beneficial effect was 100 mg/kg. Regarding the chemical composition of ETCB, 31 phenolic compounds were identified, including ellagic acid, catalagin and gallic acid.

Conclusion: The beneficial effect of ETCB in the TNBS induced colitis in rats could be related to its antioxidant, immunomodulatory and anti-inflammatory activities, which could be attributed to the phenolic compounds identified.

Abbreviations: COX-2, Cyclooxygenase-2; CINC-1, cytokine-induced neutrophil chemoattractant-1; ETCB, ethanolic extract of the stem bark of *T. catappa*, GSH, glutathione; IBD, inflammatory bowel disease; iNOS, inducible nitric oxide synthase; IL, interleukin; LOQ, limit of quantification; LPS, lipopolysaccharide; MPO, myeloperoxidase; TNBS, trinitrobenzene sulfonic acid; TNF-α, tumour necrosis factor-α

* Correspondence to: CIBER-EHD, Department of Pharmacology, Centre for Biomedical Research (CIBM), University of Granada, Avenida del Conocimiento s/n18016, Armilla, Granada, Spain.
E-mail address: jgalvez@ugr.es (J. Galvez).

[1] Both authors contributed equally to the supervision of the study.

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1. Introduction

Inflammatory bowel disease (IBD) is a chronic idiopathic inflammation of the intestine that consists of two main forms, ulcerative colitis and Crohn's disease. Both are characterized by periods of exacerbation of the symptoms followed by others in which there is a remission of the disease (Singh et al., 2012). Typically, these intestinal conditions are characterized by chronic diarrhoea, abdominal pain and bleeding due to ulceration of the inner lining of the colon and/or rectum. Although the aetiology of IBD is largely unknown, it involves a complex interaction between genetic, intestinal microbiota and environmental factors that triggers an inappropriate mucosal immune response (Khor et al., 2011). The main goals of the pharmacological treatments of IBD include remission of symptoms during the acute flare and control of chronic inflammation to avoid or delay the occurrence of new flares. Nowadays, various drugs are used, including aminosalicylates, corticosteroids, immunosuppressants or biologicals, like anti-tumour necrosis factor (TNF)-α agents. Most of these drugs have shown efficacy, but also adverse effects, together with inconvenient dosing schedule and some of them a prohibitive price, which limit their prescription and/or long-term use (Siegel, 2011). This situation demands the need for the search for new therapies that combine efficacy, convenient dosing and fewer side effects.

There is an increasing interest in the traditional remedies as a source of alternative or complementary therapies for the treatment of IBD. In the present study, we have focused our interest in *Terminalia catappa* Linn (Combretaceae), popularly known as "igi furutu" in Yoruba ethnomedicine. Different parts of the plant (leaves, fruits and barks) are used in folk medicine as antipyretic, for hemostatic purposes and prevention of hepatoma, hepatitis and gastritis (Chen et al., 2000; Lin et al., 1997). This plant is also used for the treatment of dermatitis (Ajaiyeoba et al., 2003) as well as for its anti-inflammatory, antifungal and antibacterial properties (Abiodun et al., 2011). The stem bark, in particular, is mainly used to treat gastrointestinal disorders (Nunes et al., 2012) and for its wound healing and antibacterial properties (Khan et al., 2014).

Compounds such as gallic acid, corilagin, ellagic acid, rutin, 3-β-D-glucuronopyranoside, kaempferol, which have been reported to be present in the leaf of *T. catappa*, possess varying degree of antioxidant, protective and intestinal anti-inflammatory effect in rat models of colitis (Marin et al., 2013; Xiao et al., 2013). Moreover, tannin and flavonoid glycosides, also present in the plant, show free radical scavenging ability (Lin et al., 2001).

Therefore, the aim of this study is to investigate both the anti-inflammatory and the immuno-modulatory activities of *T. catappa in vitro* and *in vivo*, focusing on the effects of the extract on some of the mediators involved in the intestinal inflammatory response, which would support the traditional use in gastrointestinal complaints.

2. Materials and methods

2.1. Chemical and reagents

All the drugs and chemicals used were purchased from Sigma–Aldrich Chemical (Madrid, Spain), unless otherwise stated. Absolute ethanol, acetonitrile, and glacial acetic acid were purchased from Fisher Chemicals (ThermoFisher, Waltham, MA, USA). Solvents used for extraction and analysis were of analytical and HPLC-MS grades, respectively. Double-deionized water with conductivity lower than 18.2 MΩ was obtained with a Milli-Q system from Millipore (Bedford, MA, USA). The standard compounds, gallic acid, ellagic acid and quercetin were acquired from Sigma–Aldrich Chemical (Madrid, Spain).

2.2. Ethical consideration

Experimental procedures and protocols used in this study conform to the "Guide to the care and use of laboratory animals in research and teaching" (NIH publications volume 25 no. 28 revised in 1996). The experimental protocol (Protocol CEEA 2010-286) was approved by the Commission of Ethics in Animal Experimentation of the University of Granada (Spain).

2.3. Plant collection

The stem bark of *T. catappa* was collected in January 2013 in Ibadan, Oyo state, Nigeria (7°23′47″N 3°55′0″E). *T. catappa* was identified and authenticated by Mr Osiyemi Oluwaseun at the Forest Herbarium, Ibadan, where a voucher specimen was deposited (FHI 107812).

2.4. Extraction of plant material

The stem bark (1600 g) of *T. catappa* was subjected to exhaustive extraction. Briefly, powdered plant material was percolated in 95% ethanol for the first 24 h. The ethanolic extract was filtered and concentrated under vacuum at 40 °C using a rotary evaporator. Thereafter, the marc was further percolated in 95% ethanol for 24 h and processed as described above. The extraction process was repeated 4 times for the plant material. The yield of ethanol extract was determined and the extract stored at −20 °C until needed for study.

2.5. Extraction of phenolic compounds

A stock solution of ETCB (5.5 mg/mL) was prepared in aqueous solution of ethanol at 80% (v/v). The extract solution was sonicated and filtered (0.2 μm pore size, 13 mm, polytetrafluoroethylene).

2.6. Determination of phenolic compounds by HPLC-DAD-qTOF-MS

An Agilent 1200-LC system (Agilent Technologies, Santa Clara, California, USA) equipped with a vacuum degasser, autosampler, binary pump, and DAD was used for the chromatographic determination. Briefly, a Poroshell 120 EC-C18 (4.6 mm × 100 mm, particle size 2.7 μm) (Agilent Technologies, Palo Alto, California, USA) was used to separate the compounds. The analytical method was based on previous studies (López-Cobo et al., 2015), with some modifications. The separation was done at a flow rate of 1 mL/min throughout the gradient. The mobile phases used were water with acetic acid (0.5%) (Phase A) and acetonitrile (Phase B), and the solvent gradient changed according to the following conditions: 0 min, 2.5% B; 5 min, 10% B; 9 min, 15% B; 10 min, 16% B; 12 min, 18% B; 17 min, 20% B, 21 min, 50% B, 23 min, 75% B, 28 min, 100% B, 30 min, 100% B, 32 min, 2.5% B, 34 min, 2.5% B. The injection volume was 5 μL and UV spectra were recorded from 200 to 600 nm, whereas the chromatograms were registered at 240, 280, 330 and 520 nm.

The system was coupled to a 6540 Agilent Ultra-High-Definition (UHD) Accurate-Mass quadrupole-time-of-flight (QTOF), which was equipped with an Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface (Agilent Technologies, Santa Clara, California, USA). The MS operating conditions were as follows: gas temperature, 360 °C; drying gas, nitrogen at 12 L/min; nebulizer pressure, 50 psig; sheath gas temperature, 370 °C; sheath gas flow, nitrogen at 12 L/min. The voltages were optimized and set with the appropriate polarity for working in the negative ionization mode. MS spectra were acquired over a mass range from m/z 50–1500. Automatic MS/MS experiments were carried out using the followings collision energy values: m/z 100, 30 eV;