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A medicinal plant compound, capnoidine, prevents the onset of inflammation in a mouse model of colitis

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ABSTRACT

Ethnopharmacological relevance: The traditional uses of *Corydalis dubia, Ajania nubigena* and *Pleurospermum amabile* in the Bhutanese traditional medicine for treating disorders related to inflammatory conditions and the *in vitro* anti-inflammatory activity of their crude extracts inspired the isolation and the investigation of anticolitic properties of four pure compounds.

Materials and methods: Three medicinal plants were collected from Himalayan Mountains of Bhutan. Capnoidine and scoulerine were isolated from *C. dubia,* linalool oxide acetate from *A. nubigena* and isomyristicin from *P. amabile* using natural product isolation protocols. Four compounds were investigated for their anti-inflammatory activities against IBD-colitis using chemically induced (TNBS) mice model of colitis. Capnoidine conferred the best preliminary protection against TNBS-induced colitis in mice and we have conducted in-depth pharmacological investigation of this compound including clinical symptoms, pathological signs, cytokine profiles, histological structure and inflammasomes using relevant bioassay protocols.

Results: Capnoidine-treated mice had significantly: a) improved clinical symptoms (body weight loss, mobility, piloerection and faecal consistency); b) reduced colon pathology (adhesion, oedema, ulceration, and colon length); c) altered inflammatory cytokines profiles within the colons; d) reduced levels of p-IκB-α (Ser32) and p-NF-κB p65 (Ser536) and e) reduced histological inflammation in the colon when compared with mice administered TNBS only.

Conclusion: Capnoidine presents as a potential new anti-inflammatory drug lead candidate for diseases where current standard-of-care often fails and is associated with major side effects. It also validates the traditional uses of *C. dubia* against inflammatory conditions and underlines the value of pursuing bioactive compounds derived from traditionally used ethnobotanical medicines.

1. Introduction

Natural products especially medicinal plants hold appeal for therapeutic drug discovery because they display superior chemical diversity and are considered evolutionarily optimised for biological function (Atanasov et al., 2015). Long used herbal medicines have the potential to contain compounds that are beneficial (Wangchuk et al., 2011). Drugs such as aspirin, taxol and vinblastine are all examples of currently used therapeutics derived from plants originating from traditional medicines (Mahdi et al., 2006; Noble, 1990; Wani et al., 1971). Traditional therapies, often known as complementary and alternative medicine (CAM), have the potential to be low cost and are well accepted by patients due to their "natural" origins. Patients with inflammatory bowel disease (IBD) rank among the highest users of CAM, with current or past use of CAM ranging from 21% to 60% (Cestari et al., 2011; Hilsden et al., 2011; Burgmann et al., 2004; Bensoussan et al., 2006). Despite their increasing use by IBD patients, a lack of understanding of the bioactive chemical components and mechanisms of anti-inflammatory action are obstacles to the incorporation of many CAM or dietary treatments into mainstream IBD medicine (Hong et al., 2012).

IBD represent a group of immune system-related disorders of the

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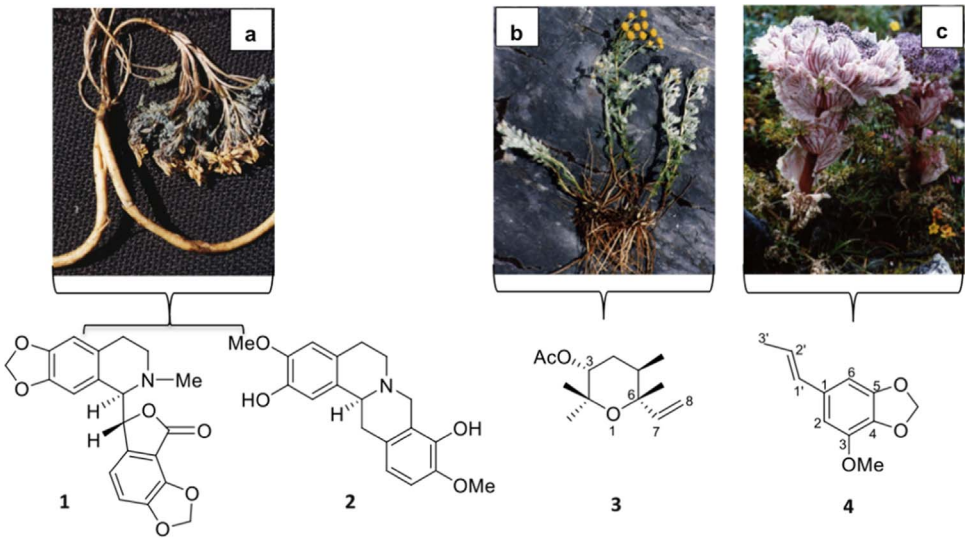

Fig. 1. The structures of the compounds isolated from three plant species and screened in the TNBS model of acute colitis. Capnoidine (**1**) and scoulerine (**2**) were isolated from *Corydalis dubia* (**a**) (Wangchuk et al., 2012), linalool oxide acetate (**3**) was isolated from *Ajania nubigena* (**b**) (Wangchuk et al., 2013b), and isomyristicin (**4**) was isolated from *Pleurospermum amabile* (**c**) (Wangchuk et al., 2014).

gastrointestinal tract, which is often difficult to treat due to its elusive nature and a complex interplay of genetic, microflora, dietary and environmental factors (Bouma and Strober, 2003; Strober and Fuss, 2011; Jostins et al., 2012; Ananthakrishnan, 2013; de Souza and Fiocchi, 2016). The term covers several diseases including ulcerative colitis (UC), Crohn's disease (CD) and indeterminate colitis that are grouped together on the basis of similar symptoms, including diarrhoea, pain, bloody stool, mal-absorption and, in some cases, are associated with cancer. The central pathological themes are the presence of high levels of pro-inflammatory cytokines such as tumour necrosis factor α (TNF-α), interferon-γ (IFN-γ), interleukin (IL)-1, IL-6, and IL-12 from both epithelial and immune cells resulting from exposure to pathogens, injury, microbe-associated molecular patterns or foreign substances (de Souza and Fiocchi, 2016). These pro-inflammatory cytokines induce the differentiation of T helper cell (Th) 1 and Th17 CD4+ T cells that are strongly associated with CD (Strober and Fuss, 2011). UC displays a more Th2-like pathological process with an increase in natural killer T cells and IL-13-producing T cells (Strober and Fuss, 2011). There are also a number of regulatory networks operating in IBD, such as increased IL-22 expression that protects the epithelial barrier (de Souza and Fiocchi, 2016; McLean et al., 2013) and regulatory T cell responses that attempt to control hyperactive T cell responses such as IL-17-producing neutrophils which promote further inflammation (Ueno et al., 2015) along with IL-22-mediated epithelial cell proliferation (de Souza and Fiocchi, 2016; McLean et al., 2013). This complex milieu of cytokines and factors presents a multitude of potential therapeutic targets for the treatment of these diseases.

Current treatment regimens for IBD include 5-aminosalicylic acid, corticosteroids and biologics, which shows only selective efficacies and can induce adverse effects (de Souza and Fiocchi, 2016; Braus and Elliott, 2009). Despite the availability of these drugs, the prevalence of IBD has been rising in developed countries, with North America and Europe experiencing the steepest increases in incidence (Ng et al., 2013; Heylen et al., 2014). Populations previously considered to be low risk (such as in Japan and India) are also experiencing an increase in the incidence of IBD, especially UC (Wangchuk et al., 2012). Until new arsenals of safe, effective and affordable drugs are made available, IBD will continue to be a significant health burden worldwide. Despite recent enthusiasm for the concept of computationally- and synthetically-derived drugs, there has been a decline in recent interest due to the relatively low number of drugs from these sources that have reached the marketplace (Haustedt et al., 2006). Computationally-optimised drugs have relatively low chemical diversity compared with those sourced from natural products (Atanasov et al., 2015) and medicinal plants used in CAM.

Recently, a systematic review of CAM treatments in IBD showed that 29 trials including phytotherapy, psychological therapy, acupuncture, and helminth therapy have been conducted to date (Langhorst et al., 2015). Even though many studies showed promising results - particularly those involving poly-ingredient phytotherapy - most trials required additional substantiation (Langhorst et al., 2015). Pre-clinical studies of medicinal plant extracts (Cestari et al., 2011; Brückner et al., 2012; de Almeida et al., 2013; Wang et al., 2014; El-Meligy et al., 2015; Castro et al., 2015) have also demonstrated anticolitic properties. Similarly, crude extracts of the medicinal plants, *Corydalis dubia*, *Ajania nubigena* and *Pleurospermum amabile*, which are used in Bhutanese traditional medicine (BTM) as febrifuge and for treating infections related to inflammatory conditions (Wangchuk et al., 2011), significantly reduced the production of TNF-α in LPS-activated human monocytic cells (THP-1) (Wangchuk et al., 2013a). Encouraged by the anti-inflammatory activities of their crude extracts, we set out to isolate and test the anticolitic properties of purified compounds from these three medicinal plants. Isolated bioactive compounds are simpler to study for their mechanism of drug action and are the ideal targets for drug discovery as compared to complex mixture of herbal medicines. Of 25 compounds that were isolated and whose structures were determined from these three plant species (Wangchuk et al., 2012, 2013b, 2014), we assessed four of the most abundant compounds for their anticolitic properties in a TNBS-mouse model of colitis. These four compounds (Fig. 1): capnoidine (**1**), scoulerine (**2**) linalool oxide acetate (**3**) and isomyristicin (**4**); have not been explored for anticolitic potential until now. Since capnoidine (**1**) imparted the best protection of mice against colitis-induced weight loss, we conducted a detailed assessment of this compound as a novel lead therapeutic agent.

2. Materials and methods

2.1. Plant materials

The selected plant materials – *Corydalis dubia* (Fumariaceae, herbarium voucher specimen number (HVSN) 78)), *Ajania nubigena* (Asteraceae, HVSN 73), *Pleurospermum amabile* (Umbelliferae, HVSN 29) – were collected from the Himalayan mountains of Lingzhi in Bhutan in July-August 2009 and the herbarium specimens were preserved at Menjong Sorig Pharmaceuticals (MSP), Ministry of Health, Thimphu, Bhutan. Although the whole plant of *C. dubia* is used in BTM, only the aerial parts of *A. nubigena* and *P. amabile* were used in this study. We collected those parts of plants from their natural habitats and dried them in the shade/sun in accordance with prescribed BTM traditional methods of drying.