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Journal of Ethnopharmacology 96 (2005) 577–584
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

An active ingredient of Cat's Claw water extracts Identification and efficacy of quinic acid

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Received 8 September 2004; received in revised form 1 October 2004; accepted 7 October 2004

Abstract

Historic medicinal practice has defined Cat's Claw, also known as Una de Gato or *Uncaria tomentosa*, as an effective treatment for several health disorders including chronic inflammation, gastrointestinal dysfunction such as ulcers, tumors and infections. The efficacy of Cat's Claw was originally believed, as early as the 1960s, to be due to the presence of oxindole alkaloids. However, more recently water-soluble Cat's Claw extracts were shown not to contain significant amounts of alkaloids (<0.05%), and yet still were shown to be very efficacious. Here we characterize the active ingredients of a water-soluble Cat's Claw extract called C-Med-100 as inhibiting cell growth without cell death thus providing enhanced opportunities for DNA repair, and the consequences thereof, such as immune stimulation, anti-inflammation and cancer prevention. The active ingredients were chemically defined as quinic acid esters and could also be shown to be bioactive in vivo as quinic acid.

Keywords: Cat's Claw; *Uncaria tomentosa*; Quinic acid; Carboxy alkyl esters (CAEs); In vitro/in vivo efficacy

1. Introduction

Uncaria tomentosa commonly known as Una de Gato or Cat's Claw has been widely used historically as a natural remedy, and it is currently present in a number of nutritional formulations to treat a large variety of health disorders (Blumenthal, 2003). So far to our knowledge most of the commercial preparations of Cat's Claw were based on oxindole alkaloid content (Keplinger et al., 1999), and not on their water solubility, bioavailability, clinical efficacy or lack of alkaloid content such has been the case with Cat's Claw water extracts such as C-Med-100® or Activar AC-11® (Sheng et al., 2000B; Sandoval et al., 2002).

The precise chemical identification of the active ingredients of C-MED-100® and Activar AC-11® have not been achieved as yet, but their chemical and biological characterization has been completed enough to standardize their commercial manufacture (Pero, 2000). They were formulated to mimic historical medicinal use of which the most important step is exhaustive hot water extraction for 18 h at 95 °C, and were spray dried to contain 8–10% or 16–20% carboxy alkyl esters (CAEs), respectively, as the only active ingredients found to be present. Daily oral doses of C-Med-100® in humans between 250 and 700 mg have been shown consistently to be efficacious (Lamm et al., 2001; Sheng et al., 2000A, 2000B, 2001).

The CAEs in C-Med-100 gave profound nutritional support as a dietary supplement because they enhance both DNA repair and immune cell responses, which in turn are critical physiological processes that regulate aging (Sheng et al., 2000B and as cited above). Both of these processes involved regulating the nuclear transcription factor kappa beta (NF-κβ). NF-κβ is well known to control both the nuclear events that salvage cells from apoptotic cell death as well as from pro-inflammatory cytokine production (Beg and Baltimore, 1996; Wang et al., 1996). Hence, this mechanism directly

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doi:10.1016/j.jep.2004.10.002

connects induction of apoptosis to programmed cell toxicity with inhibition of pro-inflammatory cytokine production and inflammation.

Apoptosis is another essential biochemical process in the body that regulates cells from division (replication) into differentiation and toward an increased functional capacity. Cells entering apoptosis will not only be stimulated to differentiate and take on function but they will eventually die from this 'programmed cell death'. Thus, increased apoptosis resulting from NF-κβ inhibition by C-Med-100® would both (i) effectively kill tumor cells because they would be forced out of replication by apoptosis and into eventual death, and (ii) at the same time increase immune cell responsiveness because more immune competent cells would be forced to differentiate and live longer by the paralleled enhancement of DNA repair. NF-κβ also sends signals to inflammatory cells to initiate them to produce cytokines (e.g. TNF alpha and the interleukins) that in turn stimulate phagocytic cells to kill more invading infectious agents, which at least in part is accomplished by producing pro-oxidant-generating inflammatory cytokines. Thus inhibiting NF-κβ has anti-inflammatory properties because it prevents over-reaction of the inflammatory process that can be very harmful to normal tissues of the body.

In addition, because pro-inflammatory cytokines are a major source of endogenous free radical production in humans, NF-κβ inhibition is expected to be anti-mutagenic by reducing genetic damage that can accumulate over time. The consequences would be that aging is curtailed because fewer radicals are produced to damage the DNA and to inhibit its natural repair. As a result, C-Med-100® should be considered an ultimate nutritional supplement for anti-aging remedies because it prevented free radical damage via NF-κβ inhibition, induced differentiation and immune cell responsiveness via the apoptotic pathway, enhanced DNA repair, and killed tumor cells, all of which in turn are major factors of why we age (Sandoval-Chacon et al., 1998; Sheng et al., 1998; Sandoval et al., 2000; Åkesson et al., 2003B). Here it is further disclosed that the CAEs characterized as the active ingredients of C-Med-100 have been identified and structurally elucidated as quinic acid analogs.

2. Materials and methods

2.1. Source of extracts of Uncaria tomentosa (Cat's Claw)

The primary historic medicinal preparation of *Uncaria tomentosa* involves heating bark to near boiling temperatures covered in water overnight, decanting the partially evaporated water extract and drinking it as a tea. Whereas such ethanopharmacological preparations have been shown repeatedly to be efficacious, high concentrations of tannins have contributed significantly to their toxic side effects by oral administration. However, this problem has been circumvented in water extracts of *Uncaria tomentosa* (Cat's Claw) destined for human consumption by utilizing C-Med-100® and Activar AC-11® supplied by Optigenex, Inc. (New York, NY) for these studies. C-Med-100® and Activar AC-11® were manufactured by a proprietary ultrafiltration process (Pero, 2000) that contained 8–10% CAEs or 16–20% CAEs, respectively, having no components >10,000 MW and that were essentially free of indole alkaloids (0.05%) (Sheng et al., 2000B; Sandoval et al., 2002, and this study). In order to chemically stabilize the manufacturing process the water extracts were spray dried on maltodextrin and finally processed into tablets or capsules. Whereas these extracts were 100% water soluble and thus directly bioavailable, they were also mimetic of ethnopaharmacological preparations only depleted of potential toxic side effect components.

2.2. Chemicals

Quinic acid (QA) was from Sigma and quinic acid lactone (QAL) was synthesized to 99% purity by Professer Robert Kane at Baylor University in Waco, Texas. Ammonia-treated QA (QAA) was made by neutralizing QA with 1% ammonia and then lypholyzing to dryness.

2.3. Isolation and purification of the active ingredient of water-soluble extracts of Cat's Claw, e.g. C-Med-100®

There are two commercial formulations of water-soluble *Uncaria tomentosa* extracts called C-Med-100® and Activar AC-11® standardized to 8–10% CAEs and 16–20% CAEs, respectively. Briefly, they are produced from heating 150 g of bark in 5 l of tap water for 12 h at 95 °C, decanting the soluble fraction, ultrafiltrating the resulting water extract to remove all components >10,000 MW and larger, and finally drying the <10,000 MW fraction according to U.S. Patent 6,039,949. For chemical analysis of the active ingredients in water extracts of Cat's Claw, they were first dissolved in water and then the spray drying agent (maltodextrin) removed by precipitation with 90% aqueous ethanol. The resultant solution was spotted on thin layer chromatographic (TLC) silica gel 60 F_{254} plates, and then chromatographed in a system of 1% ammonia in 95.5% ethanol. The TLC plate was scraped in 1-cm sections from baseline to solvent front, followed by elution of each section with 1% aqueous ammonia. Elution from silica gel 60 F_{254}TLC plates with aqueous ammonia proved to be necessary because of very tight binding of the active ingredient to silica. Although the $R_f = 0.3$ spot was essentially free from other Cat's Claw components, it contained relative large amounts of dissolved inorganic silica. In order to remove the inorganic component(s) introduced from the purification scheme off from silica TLC plates, the 1% aqueous ammonia solution was freeze dried and then the eluant redissolved in methanol leaving behind the solubilized silica. Distilled water was added to the methanol eluant, then again freeze-dried, before redissolving in water again for bioassay of tumor cell growth inhibition using HL-60 cells as already