REVIEW ARTICLE

Toxicol Rev 2005; 24 (1): 11-35
1176-2551/05/0001-0011/$34.95/0

Toxicological Aspects of the South American Herbs Cat's Claw (*Uncaria tomentosa*) and Maca (*Lepidium meyenii*)

A Critical Synopsis

Luis G. Valerio Jr[1] and *Gustavo F. Gonzales*[2]

1 Division of Biotechnology and GRAS Notice Review, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, Maryland, USA*

2 Department of Biological and Physiological Sciences, Faculty of Sciences and Philosophy, Universidad Peruana Cayetano Heredia, Instituto de Investigaciones de la Altura, Lima, Peru

Contents

* The opinions and conclusions expressed in this article are solely the views of the author and do not necessarily reflect those of the U.S. Food and Drug Administration.

Abstract

Recent exceptional growth in human exposure to natural products known to originate from traditional medicine has lead to a resurgence of scientific interest in their biological effects. As a strategy for improvement of the assessment of their pharmacological and toxicological profile, scientific evidence-based approaches are being employed to appropriately evaluate composition, quality, potential medicinal activity and safety of these natural products. Using this approach, we comprehensively reviewed existing scientific evidence for known composition, medicinal uses (past and present), and documented biological effects with emphasis on clinical pharmacology and toxicology of two commonly used medicinal plants from South America with substantial human exposure from historical and current global use: *Uncaria tomentosa* (common name: cat's claw, and Spanish: uña de gato), and *Lepidium meyenii* (common name: maca). Despite the geographic sourcing from remote regions of the tropical Amazon and high altitude Andean mountains, cat's claw and maca are widely available commercially in industrialised countries. Analytical characterisations of their active constituents have identified a variety of classes of compounds of toxicological, pharmacological and even nutritional interest including oxindole and indole alkaloids, flavonoids, glucosinolates, sterols, polyunsaturated fatty acids, carbolines and other compounds.

The oxindole alkaloids from the root bark of cat's claw are thought to invoke its most widely sought-after medicinal effects as a herbal remedy against inflammation. We find the scientific evidence supporting this claim is not conclusive and although there exists a base of information addressing this medicinal use, it is limited in scope with some evidence accumulated from *in vitro* studies towards understanding possible mechanisms of action by specific oxindole alkaloids through inhibition of nuclear factor (NF)-κB activation. Although controlled clinical studies have demonstrated reduction in pain associated with cat's claw intake in patients with various chronic inflammatory disorders, there is insufficient clinical data overall to draw a firm conclusion for its anti-inflammatory effects. An important observation was that experimental results were often dependent upon the nature of the preparation used. It appears that the presence of unknown substances has an important role in the overall effects of cat's claw extracts is an important factor for consideration. The available animal toxicological studies did not indicate severe toxicity from oral intake of cat's claw preparations but rather were

suggestive of a low potential for acute and subacute oral toxicity, and a lack of evidence to demonstrate genotoxic potential and mutagenic activity.

Maca is a clear example of a herb with substantial medicinal use in traditional herbal medicine by indigenous cultures in South America since the first recorded knowledge of it in the seventeenth century. The hypocotyls of maca are the edible part of the plant used for nutritional and proposed fertility-enhancing properties. Maca has been described to possess many other medicinal properties in traditional herbal medicine but only a few of them have been well studied scientifically. Published clinical studies of maca seem to be related to its property as a nutrient, for male fertility and for energy. There are inadequate data regarding the precise mechanism of action of maca. Some studies suggest that secondary metabolites found in maca extracts are important constituents responsible for its physiological effects. Maca has been reported in the scientific literature to have a low degree of acute oral toxicity in animals and low cellular toxicity *in vitro*.

An important finding unveiled by this review is the importance of standardisation in quality and additional basic and clinical research to scientifically validate and understand composition, biological activity, safety and risk. Development of a comprehensive pharmacological and toxicological profile through critical evaluation of existing and future experimental data, especially carefully conducted clinical studies would facilitate the scientific evidence-based approach to understanding potential biological effects of these major traditionally based herbals in current global use.

Over the last decade there has been increased interest in medicinal use of herbs and botanical products worldwide. Globally, the market for herbal ingredients in 2002 has been valued at $US20 billion with Europe, Asia and North America having the largest market shares, respectively.[1-3] Between 1990 and 1997, it is estimated that use of botanicals in the US increased by 380%.[4] The popularity of herbal medicines in the US is reflected by the estimated consumer expenditure of $US5.1 billion in 1997.[5] A recent survey report from the 2002 National Health Interview Survey (NHIS) conducted by the Centers for Disease Control and Prevention's National Center for Health Statistics found that 19% of American adults used some form of natural products including herbal medicine.[3] With this documented increase in popularity and widespread use of herbal medicines and botanical-based products, there emerges a reciprocal demand for the rate at which these materials are scientifically evaluated.

The Food and Nutrition Board of the Institute of Medicine of the National Academies recently published recommendations regarding scientific principals for evaluating and integrating the available data appropriately when different pieces of data exist (human, animal, *in vitro* etc.), which may not be conclusive or are inconsistent, as is frequently encountered with herbal ingredients.[6] The usefulness of this approach affords weighing the available evidence by emphasis on the plausibility and consistencies across different types of data in order to draw a conclusion regarding the toxicological safety and expected biological effects in humans.

In this article, we take a weight-of-evidence-based approach to address our current understanding of the known composition, medicinal uses (past and present), and documented biological effects with emphasis on pharmacology and toxicology for two selected South American medicinal plants: *Uncaria tomentosa* (common name: cat's claw, and Spanish: uña de gato) and *Lepidium meyenii* (common Spanish name: maca). It has been estimated there are 250 000 flowering plants on the earth and a large number of these species are endemic to South America, especially the Peruvian Amazon region since estimates place these flora at about 20 000 species or 8% of the total number of plants that exist on the earth.[7] Because of this exceptional diversity of plants in the Peruvian Amazon, this region is recognised as a significant resource for commercial sourcing and scientific study of natural products with potential for medicinal and pharmacological properties.[7] Despite South America's lower share of the global market for herbal ingredients, the region has evolved as an important geographical source for growing and exportation of specific plants intended for use as herbal ingredients designed for medicinal applications. For example, Euromed, a major US producer of botanical extracts, imported 85% of its St. John's wort from wild-harvested sources in Chile between 1995 and 1998.[8]

Further basis for selection to review *U. tomentosa* and *L. meyenii* is due to the increasing interest by investigators to characterise constituents of the herbs and discern potential biological effects, both beneficial and adverse. In addition, these plants maintain a well known historical background for traditional use in South America, and importantly have grown in popularity as herbal remedies outside of Peru and surrounding countries. This has resulted in an increased awareness of their ethno-traditional use and lead to the incorporation of cat's claw and maca as

ingredients in a wide variety of end-use consumer products (e.g. capsules, aqueous and powder extracts and teas). As such, the rapid product development and widespread usage of these popular herbals has led to an increased frequency and duration of exposure for which a scientific base for understanding the composition, medicinal uses, and potential biological and toxicological effects is needed.

Previous scientific reviews of *U. tomentosa* and *L. meyenii* have been useful in addressing our basic understanding but have been limited in scope.[9-11] Other reviews covering multiple South American medicinal plants have included some aspects related to toxicology, medicinal activity and pharmacological effects, but cover numerous herbals at once, are less comprehensive and do not focus on the most commonly used herbs with widespread human exposure.[12,13] In addition to the general lack of scientific understanding for many herbal medicines, there is also a recognised difference for the basis of herbal use between developing countries and industrialised nations.[14,15] For example, in developing countries, the use of herbals is a response to meeting basic or primary healthcare needs and also for historical or cultural reasons.[15] In contrast, developed countries have designated the use of herbals in medicine as complementary and alternative medicine (CAM), which normally co-exists with conventional medical practices. Consistent with this notion, the NHIS survey found that 54.9% of individuals who used CAM used it in conjunction with conventional medicine.[3] Although the basis for use of CAM and traditional herbal medicines is better understood, it is clear that anecdotal reporting is not sufficient as an acceptable approach to providing a complete scientific understanding between potential medicinal and biological effects and toxicology. This article, therefore, will address the available scientific evidence for strength or weakness of the relationship between potential medicinal and biological activity in order to provide a toxicological profile and a critical synopsis of the chemical, medicinal and biological aspects of cat's claw and maca, commonly used herbs from South America.

1. *Uncaria tomentosa:* Identity and Composition

U. tomentosa (Willd) is the best known plant from the Amazon River basin that has been used in traditional and cultural practices in South America for centuries, especially in Peru, for its potential medicinal effects on chronic inflammation, including arthritis and gastrointestinal illnesses. Its common name is cat's claw because of the curved hooked thorns on the vine which resemble claws of a cat. The Spanish translation is 'uña de gato', which is of particular importance since it is referred to as such and widely known to indigenous cultures in South America.[9,16] In addition to the Spanish-speaking populations, many Peruvian aborigine populations such as the Asháninka Natives have used and currently use the plant in traditional medicine for the treatment of several infectious and inflammatory diseases.[9] In the discussion that follows we will refer to *U. tomentosa* as 'cat's claw'.

Uncaria belongs to the family Rubiaceae.[17] Although the genus *Uncaria* is also found in the tropics of Southeast Asia, the species *U. tomentosa* and *U. guianensis* are of primary interest. In the Western world, *U. tomentosa* (yellowish-white flowers) is better known than *U. guianensis* (reddish-orange flowers), but both species share the same traditional ethnomedical applications and are used interchangeably as both are found in the Amazon rainforest and surrounding tropical areas outside Peru, including Colombia, Ecuador, Guyana, Trinidad, Venezuela, Suriname, Costa Rica, Guatemala and Panama.[18] Since the available published scientific literature on cat's claw has largely focused on *U. tomentosa* we will describe this species of the plant unless scientific studies using *U. guianensis* fall within the context of the review.

Cat's claw is described as a high climbing woody vine approximately 20m in length and is usually found in mature tropical forests at 500–600m altitude.[7] Common characteristics of the inner bark of the stem, which is one of edible parts of the plant used medicinally, are described as slightly bitter and odourless with an outer fibrous surface that is cinnamon in colour.[19] The bark of the root is the other part of the plant consumed for medicinal purposes[20] and is equally important in traditional healing to the Amazonian aborigine population in Peru.

1.1 Source

A common point of confusion in identifying 'active' cat's claw may be due to changes in alkaloid composition over seasons and plant life-cycles.[11,21] It has been established that the Asháninka natives identify medicinally active *U. tomentosa* as 'savéntaro',[9,11] and it is interesting that the healer-priests of this largest group of indigenous people of Peru can reportedly differentiate between medicinally active 'savéntaro' and a less potent variety of *U. tomentosa.*[9,11] As it turns out, there is a scientific basis for the preferred medicinally active variety of *U. tomentosa*. An Austrian research group discovered that in nature, *U. tomentosa* occurs in two chemotypes,[21] each varies greatly in its alkaloid content and as a consequence, in activity and potential medicinal use.[9] One chemotype contains tetracyclic (4-ring) indole and oxindole alkaloids in various parts of the plant and the other contains pentacyclic (5-ring) indole and oxindole alkaloids (table I). In demonstrating how the alkaloid content of cat's claw can potentially change over time, a study described samples of cat's claw root that were taken in 1983, 1985 and 1987 from the native Central

Table I. Some alkaloid constituents identified from cat's claw (*Uncaria tomentosa*)

Pentacyclic alkaloids	Tetracyclic alkaloids
Oxindoles	
Pteropodine	Rhynchophylline
Isopteropodine	Isorhynchophylline
Speciophylline	Corynoxeine
Uncarine F	Isocorynoxeine
Mitaphylline	
Isomitraphylline	
Indoles	
Akuammigine	Hirsutine
Tetrahydroalstonine	Dihydrocorynantheine
Isoajimalicine	Hirsuteine
	Corynantheine

Peruvian tropical forest near the Perené river and determined that the alkaloid composition changed significantly over this time period and plant generations.[11] Results showed that in cat's claw samples taken in 1983, the composition was principally of tetracyclic oxindole alkaloids, and then changed in 1985 to the pentacyclic chemical type, and back again in 1987 to the tetracyclic alkaloids. Investigators were unable to explain the change in alkaloid content over seasons but it is likely that the numerous life-cycles of the plant lead to the transformation in chemotype. These changes of patterns in the alkaloid content of cat's claw warrant further investigation.

Complicating the accurate identification of cat's claw (uña de gato) is the fact that this common name is shared with other plants with common features of curved thorns including *Acacia greggii* endemic to the Mexican-American border region; however, this variety derives from an entirely different plant genus and species than *U. tomentosa* and does not even belong to the Rubiaceae family. Moreover, there are 34 reported species from the *Uncaria* genus. Some, such as an Asian species (*U. gambir*) commonly known as 'pole catechu' have unrelated use applications including use as a tanning agent and also historical traditional usage as an antidiarrhoeal medicinal agent.[22]

Although large quantities of *U. guianensis* are harvested in South America for the European market, it is reported that in the US, the *U. tomentosa* plant is preferred for incorporation into consumer products.[23]

2. Chemistry

In 1996, there were 29 chemicals listed as constituents of cat's claw in James Duke's phytochemical and ethnobotanical database.[24] The number of compounds that have been identified thus far has increased to 50;[25] however, there are three classes of compounds that are thought to play an important role in the activity of cat's claw. These compounds are the alkaloids, quinovic acid glycosides and polyhydroxylated triterpenes.[26] The analytical measurement of the alkaloid class of compounds of cat's claw has been reported in numerous papers.[27-34] The presence of quinovic acid glycosides has also been well documented.[26,35-38] Recently, the first report of a naturally occurring pyroquinovic acid glycoside was derived from an extract of cat's claw obtained in Peru.[39] Other compounds derived from cat's claw with potential relevance to its medicinal activity include procyanidins,[40] polyoxygenated triterpenes,[41,42] catechins,[43] tannins and sterols such as β-sitosterol.[44]

As mentioned in section 1.1, investigators have pointed out that there are two chemotypes of *U. tomentosa* whose roots contain abundant amounts of either the tetracyclic oxindole alkaloids or the pentacyclic oxindole alkaloids (table I).[9,21,45] Plant chemotypes of the same species contain different chemical constituents, but are otherwise morphologically indistinguishable. Whether there also exist two chemotypes for *U. guianensis* is not clear; however, a major observation is that the bark of the cat's claw root and stem is thought to contain higher alkaloid content than the leaves.[26]

Much attention has focused on the chemistry of the oxindole alkaloids of cat's claw since scientific evidence has accumulated implicating these compounds for their therapeutic effects during traditional use of the herb. Oxindole alkaloids isomerise in aqueous solutions to give pH-dependent mixtures of isomers and it is thought a zwitter-ionic intermediate is formed through which the entire isomerisation process can be slowed by decreasing the polarity of the solvent.[9,46,47] Formation of isomers from the oxindole alkaloids is of significance to experimental efforts in studying the biochemical and pharmacological properties of the individual isomers since after only 2 hours at 37ºC the concentration reportedly decreases to 5% of the initial value and other isomers appear in the reagent solution.[9] Because of this instability, scientists are using equilibrated isomeric mixtures of oxindole alkaloids rather than the pure compounds.

According to one account on the distribution of pentacyclic alkaloids in cat's claw, there is a pattern to their presence in leaves, stem and root.[11] According to the description, akuammigine, a specific indole alkaloid, forms in leaves but not the root as a precursor to the formation of the oxindole alkaloid, uncarine F. Uncarine F isomerises to form additional oxindole alkaloids, speciophylline, pteropodine and isopteropodine. During transport of these compounds to the root, another oxindole alkaloid mitraphylline forms in the main stem. According to the account, the

isomer isomitraphylline is not found in the leaves or shoot but is found in the root where the deposition of all six pentacyclic oxindole alkaloids is characteristically present. However, a recent high-performance liquid chromatography-electron spectrometry/mass spectrometry (HPLC-ES/MS) quantitative analysis of alkaloids in crude wild stem bark, cultivated stem bark and leaves of cat's claw, found mitraphylline and isomitraphylline in detectible quantities in both leaves and bark. It was concluded that stem bark when grown in cultivation contains the same constituent metabolites as that from stem bark of the wild plant. However, the relative abundance of isopteropodine, a pentacyclic alkaloid constituent of pharmacological interest, was found at a higher concentration in wild bark compared with cultivated bark, and the lowest concentration of isopteropodine was detected in cultivated leaves.[26] A comparison of the chemical composition of wild leaves to cultivated leaves was not possible because of the unavailability of wild leaves.

3. Medicinal Uses

3.1 Traditional Use

Cat's claw has been reported to have broad medicinal use under the traditional practice of herbal medicine. In this sense, it has been historically relied upon by population groups in South America as an important primary remedy to help treat several disorders, including: chronic viral infections; bacterial infections; inflammatory and immunological disorders; asthma; dysentery; certain cancers including gastric; gastritis; gastric ulcers; parasites; haemorrhage; menstrual irregularity; and as a birth control agent.[10,16,48] The root and stem bark of cat's claw are the parts of the plant used in medicinal applications.[26] In traditional medicine, it has been documented that indigenous groups use the herb as a decoction by boiling 20g of cat's claw root bark in 1L of water for 45 minutes, then decanting the liquid and the water loss due to evaporisation restored to 1L.[9,11] It is reported this preparation represents an approximate 10-day supply.[9] However, these historical applications of use are based principally on use of traditional extracts that likely possessed different constituents in concentrated amounts (e.g. active compounds and contaminants) compared with the manufactured forms of cat's claw used today.

3.2 Current Use

In contrast to traditional practice, the use of cat's claw today by consumers in the US is as a complementary or alternative therapy rather than a primary medicine.[3] The perceived therapeutic benefit by consumers seeking complementary or alternative therapy is likely based on its use in traditional practice for inflammatory disorders,[18,49] a therapeutic application that emerged from the broad armamentarium of traditional medicinal uses for cat's claw.

Current consumer usage patterns seem to be broad and variable with uses in conditions such as: cancer; arthritis and other inflammatory disorders; problems with the prostate; asthma; ulcers; gastritis; viral infections; and other chronic diseases. Consistent with traditional practice, modern herbal product applications of cat's claw (capsules, tablets and teas) maintain the use of the root or stem bark of the herb as the source of the active constituents.

3.3 Dose and Dose Forms

The most effective oral dose for some of the aforementioned medicinal applications is not entirely clear. However, recent literature suggests an oral dose in a general range from 100 to 1000 mg/day.[50-52] It is thought this dose may provide a minimum intake of 10–30mg total alkaloids from *U. tomentosa*.[51] In traditional medicinal practice, however, the daily dosage for oral intake is approximately 60mL (2 fluid ounces) of a decoction as described in section 3.1 with an added equal amount of water to be taken before the first meal of the day.[11] Clearly, this form of administration is crude by today's modern healthcare standards since the purported active component of pentacyclic oxidinole alkaloids is not standardised and there is the likelihood for significant microbiological contamination.

There are numerous commercially available products of cat's claw in a diversity of forms including: 500mg capsules of dried and pulverised extract, with a recommended dosage of 1–2 capsules daily; a patented bark extract standardised to 8% carboxyl-alkyl-esters at a recommended dosage of 300 mg/day; a root extract containing a minimum of 1.3% pentacyclic oxindole alkaloids free of tetracyclic oxindole alkaloids, with a recommended dosage of one 20mg capsule three times daily for the first 10 days and then one capsule thereafter.[10] The variety of product forms of cat's claw available outside the US range from powders of whole dry inner bark of the vine, unprocessed or milled root bark, micropulverised bark, crude extracts of the whole plant or leaves, alcoholic tinctures, aqueous extracts, pressed tablets, capsules and tea. Depending on the source, many products are non-standardised and a reference standard would be important for quality control. In the US, cat's claw is found commercially in capsule form as a dried and pulverised preparation of the root bark. Some manufacturers employ thin layer chromatography as an analytical aid in species identification.

4. Clinical Features

4.1 Leukocytes

In thirteen volunteer subjects with HIV, oral intake of 20mg of a hydrochloric acid extract of cat's claw root daily for 5 months containing 12mg pentacyclic oxindole alkaloids/g and 300 mg/g sodium chloride as remnant from neutralisation, lead to increased levels in relative and absolute lymphocyte count in each subject.[9] However, the total number of leukocytes as a collective whole was not significantly changed at the end of testing from month 0 compared with month 5, and T4/T8 cell ratios remained unchanged. The number of patients evaluated was limited with no control group reported or other diagnostic parameters related to infection. No further medical history of the subjects was provided.

Two limited studies in which four and 12 healthy human volunteers were administered a commercially available aqueous extract of cat's claw at (approximately 5 mg/kg bodyweight [bw] daily) for 6 and 8 consecutive weeks, respectively, reported no clinically significant adverse effects or toxicity.[53,54] In the 6-week study, a washout period of 3 weeks was used to establish baseline blood cell counts after which cat's claw was administered orally, and bodyweights, symptoms and white blood cell counts (WBC) were taken. A slight but statistically significant increase in WBC was observed in one subject compared with the WBC baseline level for the individual before administration of cat's claw. No adverse effects (diarrhoea/constipation, headache, nausea, rash, pain) or other effects were reported in either study. In the 8-week randomised controlled trial, it was claimed an increase in DNA repair was observed after DNA damage was induced by a dose of hydrogen peroxide; however, further scientific evidence would be required in order to conclusively demonstrate such an effect is plausible and consistent with different types of cat's claw preparations and population groups.

4.2 Mutagenicity

In a double-blind, randomised study assessing the effects of a freeze-dried aqueous extract of cat's claw on the mutagenic activity of urine from 12 smokers and 12 nonsmokers, a decrease in mutagenic activity using the Ames assay was reported when urine from the smoker group was tested and compared with the nonsmoking control group.[55] The dose of cat's claw correlated linearly and significantly with the decrease of assayed mutagenic activity. In similar testing, two apparently healthy 35-year-old males, one of which was a smoker (20 cigarettes/day) the other a nonsmoker, were administered a decoction of 6.5g cat's claw bark/day for 15 days.[56] A urine donation from each individual was made before and during the 8 days after the last dose. Urine was concentrated by an Amberlite XAD-2 resin and 20, 50 and 100μL samples added to TA98 and TA100 strains of *Salmonella typhimurium* with and without β-glucuronidase activity. Results showed the urine from the nonsmoker did not possess mutagenic activity before, during or after treatment. The smoker's urine, however, had mutagenic activity before and a decreased level of activity at the end of the 15 days, which lasted another 8 days. Similar results using other strains were reported with or without the β-glucuronidase activation.

4.3 Osteoarthritis

In a 4-week double-blind, placebo-controlled study to test the effectiveness of *U. guianensis* for osteoarthritis of the knee, a freeze-dried aqueous cat's claw extract (100mg capsule daily) was administered to 45 osteoarthritis patients (30 in treatment group, 15 placebo control).[18] Criteria for inclusion included osteoarthritis of the knee that required NSAID therapy for at least 3 months prior to the study and evidence of knee pain on movement scored by the patient. A 7-day NSAID wash-out period was also required. Exclusion criteria included patients with serious pre-existing medical illness or secondary osteoarthritis. Haematological parameters (alanine aminotransferase/aspartate aminotransferase [ALT/AST], haematocrit, haemoglobin), pain, medical and subject assessment scores and adverse effects were collected at weeks 1, 2 and/or 4. Results demonstrated that this variety of cat's claw had no deleterious effects on blood or liver function or other adverse effects compared with placebo control. In a paradox, knee pain at rest or at night, and knee circumference were not significantly reduced by cat's claw treatment; however, pain associated with activity and patient assessment scores were significantly reduced, occurring within the first week of treatment. In this clinical study, *in vitro* testing was also conducted to complement the investigator's hypothesis for anti-inflammatory effects by testing for tumour necrosis factor-α (TNFα) and prostaglandin E_2 (PGE_2) production in a murine macrophage cell line following low concentrations (1–1000 ng/mL or 50 ng/mL) of pretreatment with cat's claw from both Amazonian species of the genus *Uncaria* (*tomentosa* and *guianensis*). A dose-dependent reduction in TNFα levels after stimulation with lipopolysaccharide (LPS) was observed in cells pretreated with both *U. tomentosa* and *guianensis* extracts. Inhibition of TNFα between each species were approximately equivalent. However, cat's claw extracts had no effect on unstimulated PGE_2 production but did significantly reduce PGE_2 production stimulated by LPS, suggesting inhibition of cyclo-oxygenase 2 (COX-2) expression. Taken together, these studies using scientific-based methodologies indicate the type of information being produced with regards to understanding the potential effects of the oral intake of therapeutic doses of cat's claw on the inflammatory

process. Further study will be required in order to fully ascertain the physiological mechanisms and responses in humans from chronic exposure to the prepared extract of the herb.

4.4 Rheumatoid Arthritis

The safety and clinical efficacy of a pentacyclic chemotype cat's claw extract was tested for 24 weeks in a double-blind, placebo-controlled study of 40 patients with active rheumatoid arthritis in combination with sulfasalazine or hydroxychloroquine.[49] Long-term treatment with 60mg of the extract resulted in a significant reduction in the number of painful joints compared with placebo control (53.2% vs 24.1% placebo control). Although the study reported minor side effects likely related to the cat's claw treatment, including diarrhoea and dyspepsia in three subjects, the extract was considered to be well tolerated.

4.5 Contraindications and Interactions

Overall, there are a limited number of published controlled clinical trials documenting clinically significant adverse health effects from acute or chronic ingestion of cat's claw.[18,49,53,54] One spontaneous case report, however, described a single patient with systemic lupus erythematosus who experienced acute renal failure associated with ingestion of 4 capsules/day of cat's claw.[57] Creatinine levels and proteinuria were increased in association with taking the herb compared with urinalysis before the use of cat's claw. The patient had taken cat's claw in combination with numerous oral medications (prednisone, atenolol, metolazone, furosemide and nifedipine) so it is unclear as to a cause-effect relationship, but upon discontinuation of cat's claw, biochemical parameters for renal function returned to normal.

Interactions with food or other herbs do not appear in the scientific literature for cat's claw. There is preliminary *in vitro* evidence that cat's claw may inhibit the major drug-metabolising enzyme cytochrome P450 3A4 (CYP3A4).[58] However, it remains a theoretical scenario that this effect might lead to modification in therapeutic efficacy of conventional drugs that are substrates for CYP3A4 (e.g. ketoconazole and numerous others) since this interaction has not been reported to occur in humans taking such medications and concomitant ingestion of the herb. Based on limited experimental evidence that cat's claw stimulates phagocytosis and may modulate immune system function,[9] it is also only theoretical that cat's claw could interfere with immunosuppressant drugs. In fact, there are no well documented reports of such adverse events in humans to substantiate this possibility. Still it has been recommended in herbal reference texts that, because of the potential immune-stimulating effects, individuals with autoimmune disorders or those who are on immunosuppressant drugs should avoid use of the herb to prevent rejection of implanted organs.[50,59,60] It has also been cited that cat's claw may reduce blood pressure and potentiate hypotension.[50,60]

Scientific evidence to support the safe use of cat's claw during pregnancy, lactation and in paediatrics is not available, and use under these conditions is not recommended.[50-52,60,61] Based on traditional herbal practice for its use in birth control, such an application would preclude its use during pregnancy.

5. Toxicological Studies

5.1 Animal Data

Acute and subacute oral toxicity testing in mice and rats has been conducted using aqueous extract preparations of *U. tomentosa*.[53,62-64] In a subacute repeated dose test, five male and five female specific pathogen-free Wistar rats were orally administered 1000 mg/kg of a cat's claw preparation containing 7.5mg total oxindole alkaloids/g each day for 28 days.[64] Control animals were administered the vehicle (distilled water). The health, behaviour, bodyweight and food consumption were recorded daily, and heart, spleen, kidneys, liver, adrenals, testes, and hematological parameters examined or analysed at the end of the test period in both treatment and control groups. Results showed that the aqueous extract preparation caused a significant increase in lymphocytes and decrease in percentage of neutrophil granulocytes in association with a relative increase in kidney weight in both sexes. Histological examination of tissue organs was found to be normal. Female Wistar/Furth (W/Fu) rats were gavaged daily for 8 weeks with a water-soluble extract of cat's claw at doses of 5–80 mg/kg bw.[65] WBCs were significantly elevated after treatment at the highest doses tested. No significant toxicity, histopathology of liver, kidney, spleen and heart, or changes in food consumption, body or organ weights were observed. Further testing of a separate group of animals included a pre-treatment with cat's claw extract for 8 weeks (40 and 80 mg/kg) after which whole body irradiation with 12Gy was performed. Following the irradiation, animals were allowed to repair for 3 hours and splenic single cell suspensions were harvested to evaluate DNA repair of single-strand breaks (SSB) and double-strand breaks (DSB) using the technique of alkaline and neutral elution. As expected, analysis of DNA showed that SSB and DSB were significantly higher in the irradiated treatment group compared with control non-irradiated animals. In the analysis of irradiated animals pretreated with the cat's claw extract, the investigators found an enhancement of DNA repair as the incidence of SSB and DSB was lower in the cat's claw-treated group compared with control irradiated animals, sug-

gesting a mechanism by which the herb may protect against chemical-induced tissue injury.

In a separate study, the acute oral dose that is lethal in rats to 50% of animals tested (LD_{50}) with an aqueous cat's claw extract could not be calculated because the maximum dose was not lethal; however, it was concluded that the LD_{50} was >8 g/kg, the maximum dose tested.[53] In the same rat study, a commercially available cat's claw powder and water/ethanol extract containing 4% alkaloids were also tested and the acute LD_{50} dose determined to be >2 and >5 g/kg, respectively. In mice, a freeze-dried aqueous root extract containing 35mg total pentacyclic oxindole alkaloids per gram was prepared as a 40% suspension in aqueous gum tragacanth and administered orally at a dosage volume of 40 mL/kg then observed for 14 days.[62] According to the study, two of ten mice died within 4 hours of treatment and examination of stomach, intestines, liver and spleen revealed evidence of haemorrhage. Normal bodyweight gains and autopsy findings were observed in the other animals compared with controls. The acute LD_{50} of mice in this study was established to be >16 g/kg bw, a high dose indicative of low toxicity. Consistent with these results, additional testing with mice using the cat's claw aqueous extract at a dose of 5 g/kg bw and intraperitoneal administration of a dose of 2 g/kg bw reported no apparent toxicity.[63] Overall, these short-term toxicity studies in rodents demonstrate a low level of toxicity from orally and intraperitoneal administered aqueous preparations of cat's claw.

Using the carrageenan-induced rat paw oedema as an animal model for inflammation, a quinovic acid glycoside was isolated as a potential active constituent of cat's claw and was tested. The compound reportedly reduced the inflammatory response by 33%.[27] More impressive, was the effective anti-inflammatory effect in the model by a chloroform/methanol (methyl alcohol) extract of cat's claw (50 mg/kg bw orally) showing a 69% reduction in oedema. However, the contribution of the quinovic acid glycoside constituent or any other constituent in the extracts tested towards the observed anti-inflammatory response by the chloroform/methanol extract was not determined, and some other cat's claw extracts tested did not exhibit similar or any anti-inflammatory effect in the model. Consistent with Aquino et al.,[27] a BALB/c mouse carrageenan-induced paw oedema model of inflammation, which tested a hydroalcoholic and aqueous freeze-dried extract of cat's claw for potential anti-inflammatory activity,[66] found that a hydroalcoholic extract showed a significantly higher level of anti-inflammatory activity in the model compared with the aqueous extract. Although both extracts produced dose-dependent effects at reducing paw inflammation, higher concentrations of the aqueous extract (200 mg/kg bw) were needed to observe the same effect produced by the hydroalcoholic extract (50 mg/kg bw). This suggests the contribution of non-polar constituents in producing anti-inflammatory effects. Interestingly, it was found that the hydroalcoholic extract contained a higher concentration of oxindole alkaloids compared with the aqueous extract, further suggesting suppression of inflammation by these chemical constituents.

Cat's claw root bark extract was administered to rats in drinking water and reported to be effective against chemically induced inflammation.[67] Liver metallothionein (MT) and histological sections of the midjejunum were used to index and evaluate inflammation in the model. Compared with positive controls, animals treated with cat's claw bark (5 mg/mL) in drinking water showed a significantly reduced level of MT and normal histological picture of mucosal architecture. The test material bark extract was analysed and found to contain the oxindole alkaloids listed in table I. Since the exposure period to cat's claw was only 7 days, it is not possible to conclude that anti-inflammatory effects would be observed under conditions of chronic exposure. Moreover, it is not clear that the animals were exposed to equal doses of the herb since it was administered *ad libitum* in drinking water.

In a mouse model of acute ozone (O_3)-induced inflammation, pre-treatment with an aqueous extract of a cat's claw bark (20 g/L administered at 50% and 100% dilution) in drinking water for 8 days significantly lowered the number of pulmonary inflammatory cells including infiltrating neutrophils and severity of pulmonary tissue damage measured as epithelial necrosis. Concomitant increases in the numbers of epithelial cells and bronchial epithelial height were observed in mice treated with the extract in a dose-dependent manner providing evidence for a protective effect by cat's claw on chemically induced inflammation and tissue damage of bronchiolar epithelial surfaces.[68]

Peruvian investigators found that phagocytosis in rats was increased when a cat's claw extract was administered at a dose of 400 mg/kg bw.[69] In a repeated-dose study, subacute administration via gavage of a cat's claw water extract depleted of indole alkaloids (table I) afforded recovery in rats with chemically induced leukopaenia.[65] All fractions of WBCs were proportionally and significantly increased in the cat's claw treatment group compared with controls; however, the mechanism of recovery is not understood.

5.2 *In Vitro* Studies

5.2.1 Cytotoxicity

The cytotoxicity of an aqueous extract of cat's claw bark was tested at various concentrations in cultured Chinese hamster ovary cells.[70] Using the neutral red assay, total protein content, te-

trazolium assay (MTT) and a Microtox® [1] bacterial test as endpoint systems of assay, the cat's claw extract tested did not exhibit cytotoxicity in each of the *in vitro* assays up to the highest concentration (100 mg/mL) tested. However, there was a significant dose response effect in each of the four tests, although the magnitude of the effect was not considered toxic.

In experiments to assess the capacity of cat's claw to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and prevent cytotoxicity in cell culture in response to UV irradiation and DPPH, RAW 264.7 cells were treated with freeze-dried (0.1–100 mg/L) and micropulverised (0.1–300 mg/L) cat's claw extracts collected from the Tulumayo agricultural station in Peru.[71] From the evaluation of free radical scavenging capacity, it was shown that both forms of extracts significantly inhibited DPPH free radical in a concentration-dependent manner. It was observed that the freeze-dried form was a more effective scavenger compared with the micropulverised form. The cytotoxicity (trypan blue exclusion) of the free radical DPPH in RAW 264.7 cells when simultaneously treated with freeze-dried cat's claw extract (10 mg/L) was significantly decreased compared with DPPH-treated cells without simultaneous addition of cat's claw. A 2-hour pretreatment of these cells with freeze-dried cat's claw followed by 1 hour UV light, resulted in a significant increase in cell viability compared with UV-treated cells not pretreated with cat's claw.

An aqueous extract of cat's claw bark was not cytotoxic as measured by trypan blue exclusion to epithelial (HT29) and macrophage (RAW 264.7) cells when treated at a concentration of 200mg extract/L overnight.[67] Viability of treated cells was >90% and not significantly different than control untreated cells. In addition, the cat's claw extract inhibited cellular toxicity induced by lipopolysaccharide (LPS), and LPS-mediated peroxynitrite formation providing experimental evidence for its potential to protect against oxidative stress *in vitro*.

Using an aqueous decoction, methanolic and dichloromethane extracts of cat's claw bark, the *in vitro* antioxidant activity of cat's claw was measured by the determination of thiobarbituric acid reactive substances content in rat liver homogenates after being induced by hydroperoxides and the presence of oxidative DNA sugar damage induced by iron salts.[72] These biochemical indexes of oxidative stress are well established indicators for lipid peroxidation and free radical damage to DNA. It was found that high concentrations (0.1–1 mg/mL) of the cat's claw decoction, which are unlikely to be achieved *in vivo,* were needed in order to inhibit oxidative stress induced by the toxicants. Lower concentrations (0.001–0.1 mg/mL) of a methanol extract were more effective in suppressing oxidative activity and DNA damage suggesting a contribution by non-polar constituents against oxidative stress.

In recent human and murine cell culture experiments evaluating cytoprotective activity of dietary antioxidants, cat's claw was tested as a commercially available freeze-dried concentrate (Vinicol™) from the *U. guianensis* species for potential to protect against the toxicity of the oxidants, 1,1-diphenyl-2-picrylhydrazyl (DPPH, 3 μmol/L), hydrogen peroxide (H_2O_2 50 μmol/L), and peroxynitrite (300 μmol/L) in cultured human gastric epithelial cells (AGS) and murine small intestinal epithelial cells (IEC-18).[73] After a 1-hour pretreatment with 10 μg/mL cat's claw extract, chemical oxidants were added to cells for 24 hours and cytotoxicity was measured by MTT assay and LDH release. Consistent with previous studies demonstrating reduced peroxynitrite-induced cytotoxicity in colonic (T84) cells by cat's claw,[74] the study with AGS gastric cells showed that the cat's claw extract protected against peroxynitrite- and H_2O_2-induced oxidative stress in the cell line equally as a green tea extract. However, whether cat's claw can exert such effects *in vivo* is still unclear.

5.2.2 Mutagenicity

The mutagenic potential of cat's claw was tested at 10, 50, 75, and 100 μg/plate in *Salmonella*/mammalian microsome TA 98, TA 100, TA 1535, TA 1537 and TA 1538 strains of *Salmonella typhimurium*, with and without metabolic activation from Aroclor 154-induced rat liver homogenate.[56] Cat's claw did not show evidence of mutagenicity with or without metabolic activation.

5.2.3 Antiproliferative Effects

Antiproliferative effects of cat's claw have been documented from *in vitro* testing using MCF-7 breast-cancer cells and bark and leaf extracts of the herb.[75] Depending on the extract used, the study reported concentrations that produce 50% inhibition (IC_{50}) from 10–20 mg/mL and an approximate 90% inhibition of cell growth at a concentration of 100 mg/mL.

Pentacyclic oxindole alkaloids from cat's claw have been documented to demonstrate antiproliferative effects on leukaemic HL60 and U-937 cells at concentrations ranging from 1×10^{-5} to 1×10^{-4} mol/L.[76] In another study, three human cell lines were tested for growth inhibitory activity after treatment with cat's claw water extracts.[77] Leukemic HL60s and EBV-transformed B lymphoma cell lines (Raji) were strongly suppressed in growth activity; however, the leukaemic K562 cell line was resistant to inhibition. The study concluded that the suppressive effect of the cat's claw water extract on tumour cell growth is possibly mediated through induction of apoptosis, which was demonstrated by nucleosomal DNA fragmentation after agarose gel electrophoresis and

1 The use of trade names is for product identification purposes only and does not imply endorsement.

DNA fragmentation quantification. It was further concluded from the *in vitro* study that cat's claw induced a delayed type of apoptosis in a dose-dependent manner.

In a similar study, an aqueous extract of cat's claw bark collected from the Amazonian area of Tingo Maria, Peru, containing oxindole alkaloids pteropodine, isopteropodine, mitraphylline and isomitraphylline was used in simultaneous treatment of HT29 epithelial and RAW 264.7 macrophage cells with peroxynitrite to induce apoptosis.[67] In both cell lines a significant reduction in apoptosis was observed in the cat's claw treatment group when compared with cells treated only with peroxynitrite. However, when macrophage RAW 264.7 cells were irradiated with UV light, cat's claw pretreatment did not significantly reduce apoptosis induced by UV irradiation.[71]

Further studies in cell culture with human and mouse epithelial lines have documented cat's claw to be more effective than ascorbate and comparable with green tea extract in reducing chemically induced apoptosis.[73]

5.2.4 Immunomodulation

Since in traditional herbal medicine, cat's claw is best known for its potential anti-inflammatory activity, many *in vitro* studies have focused on potential mechanisms by which this activity may be explained.[9,66,71,78-80] Although important observations have been documented, the precise mechanisms and effects of the herb on immunological systems still remain to be elucidated and established *in vivo*.

One studied pathway by which cat's claw and its active constituents is thought to have anti-inflammatory effects is by suppression of the activation of the NF-κB transcription factor.[66,67] NF-κB is an important regulator of the transcription of various pro-inflammatory mediators including cytokines such as TNFα.[81] When hydroalcoholic and aqueous freeze-dried extracts of cat's claw of known composition were compared for the ability to impair NF-κB DNA binding in an *in vitro* model of Jurkat T cells, activation of NF-κB was more significantly inhibited by the hydroalcoholic extract compared with the aqueous counterpart.[66] Interestingly, the hydroalcoholic extract contained 5.6% total oxindole alkaloids, whereas the aqueous extract contained only 0.26% oxindole alkaloids. This suggests that these constituents could potentially, as a group of compounds, contribute to the inhibition of transcription factors that regulate the production of proinflammatory mediators. However, inhibition of NF-κB activation occurred only at the highest concentration tested, suggesting impaired DNA binding due to cytotoxicity rather than an anti-inflammatory mechanism-based event. Since inhibition of COX-1 and COX-2 enzymes is associated with anti-inflammatory effects, the study also determined how the activity of these enzymes in Jurkat T cells was affected by treatment with both cat's claw extracts. The results indicate that each of the two different extracts weakly to moderately inhibited the activity of COX-1 and COX-2. Notably, the COX enzyme inhibition was not selective against one or the other of the enzymes. In a screening study of several plant extracts with putative anti-inflammatory properties, cat's claw extracts of bark and leaf were tested for their effect on the enzymatic activity of phospholipase A_2 (PLA), an enzyme that stimulates COX and 5-lipo-oxygenase pathways.[82] Results showed a 30% increase in PLA activity, which suggests that cat's claw may not possess anti-inflammatory properties or that PLA inhibition is not the mechanism for the putative anti-inflammatory effects of cat's claw.

Using an *in vitro* cellular model, a recent mechanistic study suggested that cat's claw has anti-inflammatory activity through suppression of TNFα production.[71] A freeze-dried cat's claw bark extract, significantly inhibited by >65% LPS, induced TNFα production in a murine macrophage cell line (RAW 264.7) using low concentrations and the effect was dose-dependent. The concentrations of cat's claw that inhibited TNFα (1.2–30 ng/mL) were well below the concentrations that afforded cytoprotection and antioxidant activity (3–10 mg/L) in the cellular model. A similar cytoprotective effect against LPS-induced TNFα production was also observed in cells pretreated with a micropulverised cat's claw preparation, although it was less effective than the freeze-dried material. In a subsequent study by Sandoval et al.,[83] the antioxidative and anti-inflammatory properties of *U. tomentosa* and *U. guianensis* were again determined by inhibition studies of TNFα and nitrite production in the RAW 264.7 cellular model of LPS exposure. However, two different species of cat's claw were compared for total oxindole and pentacyclic alkaloid content and it was found that *U. tomentosa* contained a 35-fold greater amount of these alkaloids than *U. guianensis*. Consistent with their previous work with the murine macrophage cell line, *U. tomentosa* was an effective inhibitor of TNFα production. However, the IC_{50} value for inhibition of TNFα production was significantly higher for the *U. tomentosa* species than *U. guianensis*, suggesting that the increased content of oxindole pentacyclic alkaloids present in *U. tomentosa* was not a factor in the influence of antioxidant and anti-inflammatory effects observed in the cellular model.

In other *in vitro* studies, investigators have suggested that cat's claw promotes phagocytosis, leading to a hypothesis that the herb is an immunostimulant.[9,11,79,80,84] This terminology may be misleading since anti-inflammatory agents are not by convention thought of as stimulators of the immune system. Nevertheless, there has been scientific interest in the pentacyclic oxindole alkaloid constituents of cat's claw (table I), as these compounds have been implicated in enhancing phagocytic activity of granulocytes

in cell culture.[79] This use has been patented.[85,86] In a complimentary *in vitro* study, two Peruvian commercial aqueous extracts of cat's claw bark with a characterised composition of oxindole alkaloids showed potent activity in stimulating interleukin-1 and interleukin-6 production by rat alveolar macrophages in a dose-dependent manner.[84] In a further *in vitro* study of immunostimulation, a mixture of 1 μmol/L pentacyclic oxindole alkaloids was incubated with the isolated cell supernatant of human endothelial cells (EA.hy926) and was able to stimulate and increase the proliferation of normal resting or weakly activated human B and T lymphocytes.[9,80] In contrast, the proliferation of B and T lymphoblasts and other cell lines under these conditions was significantly inhibited. The supernatant of untreated endothelial cells did not affect the proliferation of lymphocytes demonstrating the influence of the pentacyclic alkaloid mixture on endothelial cells to induce proliferation of lymphocytes. The investigators suggest that an unknown factor is released from the treated endothelial cells, which influences the proliferation of lymphocytes. In an interesting finding, a tetracyclic alkaloid mixture was antagonistic to the inducing effects on proliferation by the pentacyclic alkaloid mixture on Epstein-Barr virus-transformed human lymphoblastoid Raji and leukaemic Jurkat cells (up to 85% inhibition) without compromised cell viability. Further research seems reasonable in order to identify what factor(s), if any exist, might be responsible for these *in vitro* observations in the regulation of lymphocyte proliferation by pentacyclic oxindole alkaloid-stimulated endothelial cells.

5.2.5 Antiviral

Quinovic acid glycosides isolated from cat's claw have been shown to possess antiviral activity against vesicular stomatitis virus in HeLa cell cultures.[35] However, the concentration of quinovic acid glycoside required to exert antiviral activity approached the concentration eliciting cellular toxicity (80–150 mg/L). Moreover, the antiviral effect was not observed against rhinovirus type 1B human rhinovirus. Consistent with these results, a separate study also reported the antiviral activity of quinovic acid glycosides from cat's claw.[37]

5.2.6 Metabolism

There is an extremely limited amount of scientific information regarding metabolism and elimination of cat's claw. One well conducted *in vitro* study reported inhibitory effects of human CYP3A4 enzymatic activity using a fluorometric microtitre plate assay.[58] Comparatively, these investigators recently reported that common black tea and other herbal tea mixtures also demonstrated dose-dependent inhibitory activity towards human CYP3A4 and other human cytochrome P450 isoforms including CYP2C9, CYP2C19 and CYP2D6 under similar test conditions and methodology.[20] In a study by Budzinski et al.,[58] about 75% of the commercial products and 50% of the pure compounds tested showed significant inhibition of CYP3A4 mediated metabolism of 7-benzyloxyresorufin, suggesting this biochemical effect on a major biotransformation enzyme system may be prevalent across commonly used herbs. Cat's claw had the lowest IC_{50} values at <1% full strength, followed by *Echinacea angustifolia* roots, *Trifolium pratense* (wild cherry), *Matricaria chamomilla* (chamomile) and *Glycyrrhiza glabra* (licorice), which had IC_{50} values ranging from 1% to 2% of full strength. This suggests the need for *in vivo* studies on the pharmacokinetics and tissue distribution of a potential botanical-drug interaction, identification of the mechanism of the interaction and active constituent(s) responsible for the effect.

6. *Lepidium meyenii*: Identity and Composition

Maca is a Peruvian plant of unique character because it grows exclusively between 4000 and 4500m altitude in a habitat of intense cold, extremely intense sunlight and strong winds where no other cultivated plant may survive. This plant has been reported to have been cultivated for many centuries in the central highlands of the Peruvian Andes, in the former Chinchaycocha (Plateau of Bombón) present day Carhuamayo in the Junin Plateau close to Cerro de Pasco.[87,88]

L. meyenii (maca) is a cultivated plant that belongs to the family of the Brassicaceae (Cruciferae), the Tribe of Lepidieae, Section Monoploca and Genus *Lepidium*.[89] The natural habitat in which maca is cultivated and grows has an average minimum temperature of −1.5°C and a maximum of 12°C.[90] In this environment, maca is a biennial plant in which a vegetative phase is followed by a reproductive phase.[90] In favourable conditions, the plant is annual.[89] Maca is a mat-like plant, small and flat in appearance.[91]

L. meyenii is the only species cultivated as a starch plant.[89] *L. meyenii* was first described by botanist Wilhelm Gerhard Walpers in 1843.[89,92] This botanist described *L. meyenii* in Pisacoma, Puno (Southern Andes of Peru) far away from the known distribution of maca in the Central Andes. Morphology of the specimen described by Walpers does not show the enlarged tuberous hypocotyl observed in the plant from the Central Andes. For this reason it has been suggested that the plant from the Central Andes belongs to another species.[93] This will require further research.

The Andean species of *Lepidium* include several wild species besides the cultivated maca.[93] At least seven wild species of *Lepidium* have been reported in Peru.[94] In 1982, it was declared by the International Board for the Protection of Genetic Resources to be in danger of extinction as a domesticated plant.[91]

The edible part of the plant is a radish-like tuber that constitutes the hypocotyl and the root of the plant. The hypocotyl has about a 2–8cm diameter. Maca is an octoploid with 64 chromosomes.[95] It has been described with different ecotypes according to the colour of the hypocotyls as shown in figure 1. In a study performed in the city of Carhuamayo, Junin, Peru, there were 13 ecotypes reported ranging from white to black.[90] The most frequent presentation (47.8%) was the yellow colour, and this is commercially the most preferred.[90] Analysis of three ecotypes (yellow, black and red) in Carhuamayo, Junin, showed some differences in nutritional components. The differences were observed in the amount of pure protein, soluble sugars direct reducing, riboflavin, potassium and iron. The order of difference was red-yellow-black maca.[96]

Maca has been widely distributed in Peru; however, most of the studies related to medicinal and nutritional properties have been performed with maca derived from the Central Andes of Peru mainly from Carhuamayo, Junin.

There is much confusion as to the origin and distribution of maca in ancient Peru. Some authors say that it was the birth of the Cusco and the Inca civilisation that fully exploited maca.[97] In another paper it is stated that "during the Inca Empire maca consumption was limited to the nobility, the clergy, and the privileged classes; it was also given as a prize to warriors".[98] These statements are inaccurate. In 1553[87] and in 1653[88] chronicles of the Spaniard Conquest of Peru reported that maca was exclusively cultivated in Chinchaycocha (Plateau of Bombon) in the central Andes of Peru. This population was conquered by the Incas in 1470,[99] and there is no evidence that Incas in Cusco cultivated or used maca before 1470 or before the Spanish conquest in the sixteenth century.

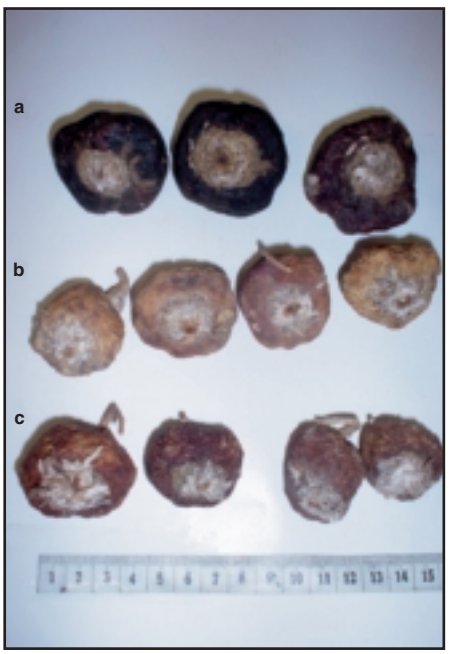

Fig. 1. Dry hypocotyls of maca (*Lepidium meyenii*) with three distinct ecotypes: (**a**) black; (**b**) yellow; and (**c**) red.

6.1 Source

The hypocotyls of maca are dried naturally. The fresh hypocotyls are exposed to the sunlight for 4–6 days until they are dry. Thereafter, they are stored in a cool, dark place until they are used.[100] Natives from the Central Andes do not use fresh maca. The fresh hypocotyls are considered harmful. Dried, the hypocotyls can be stored for several years. For eating purposes, the dried hypocotyls are hydrated and boiled in water until they are soft.[99]

Although maca has a strong peculiar flavour that is not acceptable to many people, it is an important dietary staple, rich in sugars, protein, starches and essential minerals. In most cases, its peculiar flavour can be disguised by other components used in preparation of juices and other concoctions.[89]

Maca hypocotyls are consumed baked, boiled into porridge, or fermented to make a beverage called 'maca chicha'.[100] They are mostly used because of their nutritional value[101] and medicinal properties.[102] Sources of maca are broad. It is possible to find the following products for consumption: whole maca; maca flour for baking; maca baked goods; maca blender drinks; a fresh, fermented drink called in Spanish 'maca chicha'; distilled maca liquors; and in pharmaceutical preparations. Commercial products include pills, capsules, flour, liquor, tonic and mayonnaise.[102]

7. Chemistry

In relation to the chemical composition, we divide those compounds related to primary metabolites, which correspond to the nutritional component of the hypocotyls, and the secondary metabolites. The chemical composition of maca has been reported to support the reputed medicinal benefits of maca as a food supplement.[101,102]

7.1 Primary Metabolites

The nutritional value of the hypocotyls of maca is as high as that of maize, rice and wheat.[102] The nutritional profile from maca is reported to be better than that of potato.[101] The dried hypocotyls of maca are approximately 13–16% protein, and are rich in essential amino acids.[91] Fresh hypocotyls contain 80% water[102] and have high amounts of iron and calcium.[91]

The first description of the chemical composition of dry maca (yellow ecotype) obtained from the plateau of Junin in 1967 reported 35.51% of moisture, and 10.30g of protein in 100g of dry maca calculated as concentration of total nitrogen multiplied by 6.25. The maca provided 384.0 kcal per 100g.[103]

A more complete description of the composition of dry maca was published by Dini et al.:[101] 10.2% proteins; 59% carbohydrates; 2.2% lipids; and 8.5% of fibre.[101] Free fatty acids are also present in maca, the most abundant being linoleic, palmitic and oleic acids.[101] Saturated fatty acids represent 40.1%, whereas unsaturated fatty acids are present at 52.7%. Several of the unsaturated fatty acids are known as macaenes. Maca contains amino acids (mg/g protein) like leucine (91.0mg), arginine (99.4mg), phenylalanine (55.3mg), lysine (54.3mg), glycin (68.30mg), alanine (63.1mg), valine (79.3mg), isoleucine (47.4mg), glutamic acid (156.5mg), serine (50.4mg) and aspartic acid (91.7mg). Other amino acids present but in less proportion are histidine (21.9g), threonine (33.1mg), tyrosine (30.6mg), methionine (28.0mg), hydroxyproline (26mg), proline (0.5mg) and sarcosine (0.70mg). Minerals reportedly found in maca were iron (16.6mg/100g dry matter), calcium (150mg/100g dry matter), copper (5.9mg/100g dry matter), zinc (3.8mg/100g dry matter) and potassium (2050mg/100g dry matter) among others.[101]

Table II shows a comparison of the concentration of some amino acids in maca between two studies performed in 1990[101] and 1995.[104] Table III shows data related to differences in nutritional components between three ecotypes of maca (yellow, red and black).[96] The differences observed between these three ecotypes may also result in different biological responses.

Table II. Some amino acids (g/100g protein) present in hypocotyls of *Lepidium meyenii* (maca) from Junin, Peru[101,104]

Amino acid	Maca from 1990[101]	Maca from 1995[104]
Isoleucine	4.7	4.3
Leucine	9.1	6.8
Valine	7.9	6.3
Lysine	5.4	5.8
Phenylalanine + tyrosine	8.6	4.8
Threonine	3.3	4.5
Methionine	2.8	3.3

Table III. Nutritional differences between three ecotypes of *Lepidium meyenii* (maca)[96]

Analysis[a]	Red maca	Yellow maca	Black maca
Fibre (g%)	5.45	5.30	4.95
Carbohydrates (g%)	62.60	62.69	63.82
Pure protein (g%)[b]	9.97	8.25	7.7
Starch (g%)	37.52	37.86	38.18
Soluble sugars (g%)[c]	6.03	6.17	7.02
Riboflavin (mg%)	0.50	0.61	0.76
Potassium (mg%)	1160	1130	1000
Iron (ppm)	62	80	86

a The unit data are related to g/100g maca and mg/100g maca.

b Calculated from protein nitrogen by 6.25.

c Indirect reducing ppm.

ppm = parts per million.

7.2 Secondary Metabolites

The first attempt to determine the secondary metabolites in maca was reported in 1961.[105] The study reported the presence of alkaloids (up to four fractions), glucosides, tannins and scarce saponins.[105]

7.2.1 Glucosinolates

Maca contains glucosinolates as its major secondary metabolite.[106] The enzyme myrosinase, activated in damaged plant tissue and also present in the microflora of the human digestive tract, converts these glucosinolates to a number of compounds including isothiocyanates.[107] Thus, it is possible to find these metabolites in maca extracts.[108] The most abundant glucosinolates detected in fresh and dry hypocotyls and leaves of maca were the aromatic glucosinolates, benzylglucosinolate (glucotropaeolin),[101,102,109] *p*-methoxybenzylglucosinolate[102,106] and *m*-methoxybenzylglucosinolate.[98,109] The presence of *p*-methoxybenzylglucosinolate[102,106] has not been confirmed in other studies.[98,109]

The main component of maca seems to be benzylglucosinolate.[109] Among commercial products, no glucosinolates were detected in the liquor or tonics of maca, while mayonnaise of maca had only trace amounts of those glucosinolates, including allyl glucosinolate (sinigrin) an aliphatic glucosinolate.[102] The pills, capsules and flour of maca had the same glucosinolates as those observed in hypocotyls, but in variable amounts.[102] The absolute content of glucosinolates in fresh maca hypocotyls is relatively higher than that reported in other cruciferous crops.[102] The richest sources of glucosinolates were seeds, fresh hypocotyls and sprouts, in that order.[102] Phenylacetonitrile, a degradable product of benzylglucosinolate, has also been found in the aerial part of the *L. meyenii*.[110] It is important to note that glucosinolate content in cruciferous vegetables grown at the same site for 2 years under different climatic conditions is highly variable.[111]

7.2.2 Sterols

Sterols observed to be present in maca were: β-sitosterol, campesterol and stigmasterol.[108]

7.2.3 Polyunsaturated Fatty Acids

Maca also contains novel polyunsaturated fatty acids called macaenes and macamides (benzylated alkamides).[108] These include three new compounds: (i) *N*-benzyl octanamide; (ii) *N*-benzyl-16-hydroxy-9-oxo-10E, 12E, 14E-octadecatrieneamide;

and (iii) *N*-benzyl-9,16-dioxo-10E, 12E, 14E-octadecatrieneamide.[108] Other macamides have also been described as: (i) *N*-benzyl-5-oxo-6E,8E-octadecadienamide; (ii) *N*-benzylhexadecanamide; and (iii) 5-oxo-6E,8E-octadecadienoic acid.[112] A benzylated derivative of 1,2-dihydro-*N*-hydroxypyridine, named macaridine has been reported to be present in the plant.[112]

The analysis of several commercially available maca products showed a similar qualitative pattern but significant differences in the quantitative composition. The percentage of total macaenes and macamides in the preparations varied from 0.15% to 0.84%, resulting in daily intakes for the consumers from 1.52 to 14.88mg, respectively.[113]

7.2.4 Carbolines

The methanol extract of maca also contained (1R,3S)-1-methyltetrahydro-β-carboline-3-carboxylic acid.[98]

7.2.5 Other Compounds

Uridine and malic acid are other compounds present in maca hypocotyls.[98] Prostaglandins have also been described in maca.[101] Flavonoids[96] and anthocyanines[114] have also been reported. Among the flavonoids that have been found is the flavonol, quercetin.[115] Anthocyanines are responsible for the external colour of the hypocotyls. Two new imidazole alkaloids (lepidiline A and lepidiline B) have been isolated from hypocotyls-root extract of *L. meyenii* and identified as: (i) 1,3-dibenzyl-4,5-dimethylimidazolium chloride; and (ii) 1,3-dibenzyl-2,4,5-trimethylimidazolium chloride.[116] Another alkaloid was isopteropodin.[115]

7.2.6 Components of the Aerial Parts of *Lepidium meyenii*

The essential oil profile of maca identified 53 components. Phenyl acetonitrile (85.9%), benzaldehyde (3.1%) and 3-methoxyphenylacetonitrile (2.1%) were the major components of the steam distilled oil.[110]

8. Medicinal Uses

Maca is cultivated principally for nutritional and medicinal purposes. With respect to these uses, the hypocotyl root axis is the valuable portion of the plant. Original descriptions of the use of the hypocotyls are for its potential nutritional[87,88] and fertility-enhancing properties.[88,117]

8.1 Historical Use

Maca was probably domesticated in San Blas, Junin (present day, Ondores, Junin, Peru), between 1300 to 2000 years ago. Up until the twentieth century, maca was an Andean crop cultivated in the Peruvian Departments of Junin and Pasco.

Cieza published in 1553 '*The Chronicle of Peru'* first volume. In the chapter related to the province of Bombon (Chinchaycocha), Cieza describes a place where maize, the common food in ancient Peru, is not cultivated. He refers to the place as a colder area where the natives used certain roots for maintenance.[87] Father Cobo,[88] a priest who visited Peru between 1603–29, was the first person to describe maca and its properties. He stated as translated from his book published in 1653 that this plant grows in the harshest and coldest areas of the sierra, where no other plant for man's sustenance can be grown. Cobo also referred to the use of maca for fertility, and observed that the population in these places was higher than others (not using maca) under similar environmental conditions.

The Spaniard Juan Tello de Sotomayor reported after a visit to the area of Huanuco (vicinity of Cerro de Pasco) in 1572 that the Chichaycochas had used maca for bartering since the time of the Incas, as there was no other crop on their lands[100] This means that the Chynchaycochas used maca as a food since no other crops could be grown on their lands.[87,88]

The distribution or the knowledge about maca in the ancient Peru remains obscure. According to Cobo, maca is cultivated only in the Peruvian province of Chinchaycocha (currently, Carhuamayo). Spaniard, Hipólito Ruiz, who visited Peru between 1777–78 had also reported that areas of production and consumption of maca were Carhuamayo, Pampa de los Reyes, Ninacaca and its vicinity. These places are known as districts of Carhuamayo and Ondores in the department of Junin. Ruiz also referred to the fertility-enhancing properties of maca.[117] He said: "many people believe that they make men and women fertile".[117]

In the nineteenth century, very little was written about maca except the taxonomic description by the botanist, Wilhelm Gerhard Walpers of a plant found in Pisacoma, Puno as *L. meyenii* Walpers.[89,92]

In the twentieth century, the first scientific evidence of an effect of maca came from Chacón, a Peruvian biologist.[105] She reported that maca administered for 6 months produced an increase in the number of offspring. In the same study,[105] it was claimed that maca improved rat spermatogenesis; however, the study only included two male rats treated with maca and one male rat as a control. An alkaloid extract was intraperitoneally injected and the effect was assessed 72 hours later.[105] A major limitation of the study is the number of rats tested, which makes it difficult to draw a conclusion on this effect. On the other hand, the spermatogenic cycle in the rat lasts 12.5 days.[118] Therefore, it is impossible to detect after 72 hours the reported effect of increased spermatogenesis. In addition, the study only showed a picture of histological sections of seminiferous tubules, without any quantification of different germ cells. Further studies have demonstrated that the first effect of *L. meyenii* is on spermiation stage (VIII) rather than on the mitosis of spermatogonia. An increase of spermiation and

lengths of stages VII–VIII were observed at day 7 of treatment, whereas an increase of mitosis (stages IX–XI) was observed at day 21 of treatment.[119]

The experimental design used by Chacón[105] to determine the effect of maca in rats has been rated as far from satisfactory by other researchers.[108]

Fertility was also reported to increase in guinea-pigs treated with 22.5, 45 or 90 g/day of maca for 100 days. Survival of offspring was also higher with 90 g/day of maca.[120]

8.2 Current Use

Many reports have claimed different effects for orally consumed maca. These include effects on male impotence, increased sex hormones, menstrual and menopausal symptoms in women,[121] increased energy, stamina and endurance in athletes,[98,121,122] promoting mental clarity and improving chronic fatigue syndrome,[121] that maca fosters vitality,[98,121] and significantly helps restore hormonal balance in women.[122] Other referenced medicinal properties attributed to maca including stress reduction, regulation of hormonal secretion, stimulation of metabolism, memory improvement, antidepressant activity and effectiveness in combating anaemia, leukaemia, AIDS and cancer.[102] However, almost all of these effects have not been demonstrated scientifically.

8.3 Dose and Dose Forms

There are no established scientific criteria for dosing levels of maca for medicinal use. Using macamides and macaenes as markers, it has been recently demonstrated that several commercially available products of maca have different quantitative composition.[113]

There is still controversy related to the dose used in rats and humans to produce desired effects. In fact, the administration in male rats of 2 g/kg bw published in several studies[113] represents about 140g in an individual with a bodyweight of 70kg, whereas the administration of 3.0g of maca to a 70kg individual, as published previously,[123] represents 0.04 g/kg in the rat.

Most of the natives in the central Andes of Peru use maca in daily amounts >100g a day, equivalent to 1.4 g/kg. This is an important issue to be considered in further clinical studies.

Pharmaceutical preparations in South America usually contain 500mg of *L. meyenii* in a tablet or capsule, and it is dispensed by pharmacists in dosages of 1500 or 3000 mg/day. Maca is a nutraceutical product and does not require a medical prescription.

9. Clinical Features

9.1 Nutrition

There are few studies related to the nutritional effects of maca. In one study, mice fed cooked maca showed better growth than those fed raw maca or control. No signs of malnutrition or increased weight were observed in any of the study groups (raw maca, cooked maca, control). Serum values of total protein and albumin were statistically higher in the groups of mice fed with cooked maca compared with raw maca and commercially balanced food groups.[124]

Supplementation of maca in diets improved growth rates and survival of rainbow trout *Oncorhynchus mykiss* (walbaum) alevins and juveniles.[115]

9.2 Sexual Function

A 10% ethanol suspension of two extracts of maca (M-01 and M-02) administered orally for 22 days, enhanced sexual function of mice and rats, as evidenced by an increase in the number of complete intromissions and the number of sperm-positive females in normal mice, and a decrease in the latent period of erection in male rats with erectile dysfunction.[108] While control mice averaged only 16 intromissions in 3 hours, the mice receiving maca extract M-01 completed 46 intromissions during the 3-hour period. In the third group receiving the second maca extract, M-02, the frequency jumped to a stunning 67 intromissions in just 3 hours. Another finding of the experiment was that in mice with erectile dysfunction, the latent period of erection (the interval between erections) decreased following administration of maca extracts.[108] The oral administration of M-01 (containing high polysaccharide levels) at a dose of 4 g/Kg increased the number of sperm-positive females.[108]

In a recent study, rats were treated for 15 days with pulverised extract of maca at doses of 0.015 or 0.075 g/kg. After 1 day of treatment, both doses acutely decreased mount latency (ML), intromission latency (IL) and intercopulatory interval, while 0.075 g/kg decreased post-ejaculatory latencies (PEL). After 15 days, decreased ML, IL, PEL and ejaculatory latency (EL) were observed in a dose-dependent fashion.[125] These effects seem to be independent of spontaneous locomotor activity. Further study showed that a hexane extract of maca improved the majority of the sexual parameters measured in male rats.[126] Sub-acute oral administration of the hexane maca extract improved sexual performance most effectively in sexually inexperienced male rats.

Men treated with maca (1.5 or 3.0g) reported an increase in sexual desire after 8 weeks of treatment compared with a placebo group. This effect was maintained after 12 weeks of treatment.[126]

However, the prevalence of men manifesting increase of sexual desire was 40.0% and 42.2% at 8 and 12 weeks of treatment, respectively.[127] The low prevalence is probably related to the dosage used as being too low to produce an effect in the remaining 60% of the population studied.

9.3 Fertility

Studies in rats[105] and guinea-pigs[120] have reported an increase in fertility. There is no reference in any peer-reviewed journal on the effect of maca on fertility in these species. A study in mice treated with maca for 30 days did not demonstrate a difference in the rate of embryos.[128] This study failed to control the amount of maca consumed since 5.0g of the powder was dissolved in 100mL of water, and mice had free access to drinking water. Therefore, it is difficult to determine if each animal received the same amount of maca.

9.4 Spermatogenesis

An aqueous extract of maca in doses of 2 g/kg bw improved spermiation, mitosis and epididymal sperm count after 21 days of treatment in adult male rats.[119,129] Spermiation occurs first, and it is observed at day 7 of treatment. This is accompanied by an increase in lengths of stages VII and VIII. At day 14 and 21 an increase in lengths of onset of mitosis was observed (stages IX–XI). At all times, epididymal sperm count was increased.

The length of stage VIII of the seminiferous tubule epithelium cycle at day 7 of treatment was higher with 1 g/kg of maca than with 0.1 g/kg or 0.01 g/kg.[119] However, 0.01 g/kg of maca also resulted in a significantly higher value of stage VIII than control.[119]

Treatment of rats with maca at high altitude prevented the high altitude-induced spermatogenic disruption.[119] In humans, there is some historical account of discovery of maca use to prevent low fertility dating back from the Spaniards when they first occupied Peru.[100] Because of reported fertility problems at high altitude, the Spaniards discovered maca from the native Andes people and was suggested that later the natives were required to give maca as a tax because of its beneficial value. In modern times, treatment of healthy men with 1.5 or 3.0g of maca obtained from a pharmaceutical company and administered orally for 4 months resulted in increased seminal volume, sperm count per ejaculum, motile sperm count, and sperm motility. In four of the nine subjects who had low basal serum follicle stimulating hormone levels, the sperm count was not increased after maca treatment.[129]

9.5 Hormone Levels

In men treated with 1.5 or 3.0g of maca, the serum levels of luteinising hormone, follicle stimulating hormone, prolactin, testosterone, 17-hydroxyprogesterone and estradiol levels were not different at 1, 2, 3[130] or 4[129] months of treatment.

In rats treated with 2 g/kg of an aqueous extract of maca, it was observed that serum testosterone increased at day 14 but not at day 7 or 21.[119] Treatment of an ethanolic extract of maca did not produce differences in serum testosterone levels after 21 days of treatment.[131]

In male mice, an increase was observed in serum testosterone levels after 30 days of treatment with maca.[128]

In female mice, high serum progesterone levels were observed after 30 days of treatment with maca. However, when maca was administered with another plant, *Jatropha macrantha,* no difference in progesterone levels were observed compared with control. Serum progesterone levels in female mice treated with *J. macrantha* was similar to controls. This looks more of a random finding rather than a real effect of maca on progesterone levels. Serum estradiol levels were not different in female mice treated with maca.[128] The other limitation of this study is that it is not easy to determine the amount of maca consumed by each mouse.

It will be necessary to perform a dose-response study to determine if different doses of maca affect serum testosterone levels, and also to determine the effect after prolonged exposure. A comparison of testosterone levels between different species while controlling the amount of maca consumed should also be performed.

In fish, the period of sex differentiation is most sensitive to the possible effect action of phytochemicals with steroid activity. However, there was no significant difference in sex ratios between the control and maca-treated groups of rainbow trout.[115]

9.6 Energy

Different doses of an aqueous extract of maca (4,10, 20 and 40 g/kg) have been evaluated to test its effect on endurance and energy capabilities by measuring the swimming activity of mice. The results demonstrated that increased swim time was related to the increased content of polysaccharides in the aqueous extract of maca. Recovery from muscle fatigue measured by lactic acid and malonic acid production after strenuous physical activity was observed to be better in the samples with a higher content of polysaccharides.[132]

9.7 Haematology and Immunity

In a study in which rainbow trout were fed maca for 15 weeks, no difference in haemoglobin levels were observed.[115] In men treated with 1.5 or 3.0g of maca for 12 weeks, no difference in haematocrit was observed when compared with a placebo group (unpublished observation).

Increased total leukocyte counts were observed in fish fed diets supplemented with maca meal compared with control, suggesting that immunity is improved by maca.[115] It is necessary to test this observation in another species including humans.

9.8 Stress

Mice treated with maca for 15 weeks were submitted to different tests after non-lethal electric discharges. Mice treated with maca showed a lower score of neuroticism than control and they had a more rapid recovery to normal after neuroticism.[133]

9.9 Antioxidant Activity

An aqueous extract of maca (0.3–3mg) was demonstrated to have the capacity to scavenge free radicals and protect cells against oxidative stress.[134]

9.10 Contraindications and Drug Interactions

The popular literature, producers and distributors (without scientific evidence shown) of commercially available maca, indicate that their product in different applications is safe, as they frequently make this assertion on the basis of centuries of traditional use in natives as well as in foreign consumers. The National Medicines Comprehensive Database lists maca as 'possibly safe' when used orally and appropriately, over a short-term period.[59] The plant appears to be safe in healthy adult men in amounts up to 3 g/day for 12 weeks;[130] however, there is a lack of clinical safety studies and reliable information to clearly demonstrate safety under intended conditions of use. Furthermore, there is insufficient information in the scientific literature to establish safety in sensitive and susceptible population groups such as in children and use during pregnancy and lactation.

To our knowledge, there are no well known reports of adverse reactions after consuming *L. meyenii* in food. However, it is recommended that maca be consumed after boiling because consumption of fresh maca may induce adverse health effects. One of our co-workers in our laboratory in Peru consumed fresh maca and she reported pain in the stomach (personal communication).

A recent clinical study on the effect of maca on men did not reveal any side effects after 12 or 16 weeks of treatment with 1.5 or 3.0g of a pharmaceutical preparation of maca.[127,130]

There is no information in the literature about contraindications, although some commercial producers recommend not using maca in the presence of hypertension, or conditions of prostate disease. The latter may be due to the belief that maca increases serum testosterone levels. It was demonstrated by Gonzales et. al.[123,130] that maca at the administered dosage of up to 3.0 g/day in men did not increase serum testosterone levels. A recent clinical study conducted by this author's (Gonzales) laboratory demonstrated that maca administered for 12 weeks in healthy men reduced blood pressure (unpublished observations).

It is unknown if any drug interactions exist between maca consumption and administration of prescribed or over-the-counter drugs.

10. Toxicological Studies

There are no articles in peer-reviewed journals that list the toxic properties of *L. meyenii*. However, there are local publications in Peru regarding toxicological effects of maca in mice and cultured cells.

10.1 Animal Data

Studies were performed in 30-day-old mice receiving acute doses of micro-pulverised maca ranging from 11 to 15 g/kg. The mice were evaluated from 8 hours after administration up to 7 days post-administration. Results suggest that ingestion of doses <15 g/kg are innocuous to mice. The Williams criteria was used for the scoring of the LD_{50} results.[135] Another study administered increasing doses of maca to Swiss mice and they were observed for a period of 3 days. The maximal doses used (16.129 g/kg) did not produce any deaths, suggesting that the LD_{50} was >16.129 g/kg according the Williams criteria.[136]

10.2 *In Vitro* Studies

Using bioassays of *Artemia salina,* Leanch observed that micro-pulverised maca has an LD_{50} of 822.68 g/mL.[137] Similarly, viability of a cell line of macrophages in culture (RAW264.7) showed that extract of maca was innocuous.[136] The oil from aerial parts of *L. meyenii* was selectively toxic towards the cyanobacterium *Oscillatoria perornata* compared with the green alga *Selenastrum capricornutum*, with complete growth inhibition at 100 mg/L.[110] These studies suggest the absence of toxicity of maca as assessed under *in vitro* and *in vivo* models.

10.3 Other Studies

The oil from aerial parts of *L. meyenii* has been tested for phytotoxic, cyanobactericidal and anti-termite activity. The oil

showed no phytotoxic activity under the conditions used.[110] Anti-termite activity was observed with some of the minor components of the essential oil of *L. meyenii*, namely benzylthiocyanate, 3-methoxy-phenylacetonitrile and β-ionone.[110]

11. Conclusions

11.1 *Uncaria tomentosa*

Cat's claw was ranked as the seventh most popular herb in US sales in 1997[138] and, therefore, merits attention for review of documented scientific evidence from *in vitro*, animal and human studies regarding its biological and toxicological effects in relation to potential medicinal activity.

Several basic questions remain regarding the observed biological effects of cat's claw that have been documented in the scientific literature. One is whether documented observations using isolated pure compounds derived from the plant (e.g. individual pentacyclic alkaloids) have the same or similar property and effect as the whole plant extract preparation. Clearly, in studies that employ controlled amounts of purified cat's claw material that has been well characterised for composition, the relationship of ingredient to observed effects is relatively more certain than in studies that utilised uncharacterised cat's claw as the test material. Another important question in considering the experimental evidence for potential cat's claw medicinal activity is the fact that different extraction systems influence the composition of the extract and thus may affect in a relatively significant way, the biological activity responsible for the medicinal effects and the potential toxicity. For example, an aqueous alcoholic extraction system may produce a mixture that contains secondary metabolites that may have relative significance to biological activity and toxicity compared with an extraction system that uses only water. When water is used as the extracting solvent, it can be expected that more polar compounds are extracted compared with an alcoholic extraction system. The study by Aguilar et al.,[66] is a case in point by which observed differences in biological effects were produced from different extraction systems, as suppression of NF-κB DNA binding was more evident when a hydroalcoholic extract of cat's claw was used compared with a water extract of the herb. Whether the difference in observed biological effects is due to extraction procedure alone is not entirely clear when comparing separate studies because although the test material may have been obtained by the same extraction system (e.g. alcohol), it is still not clear that the outcome is driven by the extraction procedure alone. This is because the extracts from separate studies have not been obtained from the same specific plant material. Furthermore, the plausibility that biological activity observed from *in vitro* experimentation will occur *in vivo*, regardless of the extraction procedure employed, is clearly not established. Detailed pharmacokinetic studies in the presence or absence of various cat's claw preparations must be completed in order to determine if *in vitro* results translate to *in vivo* effects.

A problematic aspect in understanding potential biochemical and toxicological events relevant to the medicinal use of cat's claw and many other herbs is that the exact amounts of active chemical constituents are frequently unknown. Whenever new preparations are created, it can be expected that the presence of these constituents of interest will vary from preparation to preparation.[14,24,26-34] It is generally accepted by herbalists that variation in composition and bioavailability can derive from inconsistent harvesting, storage and processing, or differences in the plant genotype, chemotype or growing conditions.[14] Another logical assumption is that variation in bioavailability may also derive directly from the exposure level as it relates to the route of exposure of the formulation. As a result, bioequivalence across different product formulas and test materials may be inconsistent and lead to observed differences in reported outcome from animal and human studies. Lastly, differences between animal and human metabolism can also account for variation in bioavailability resulting in inconsistent reporting of biochemical, pharmacological and toxicological effects.[6]

An important aspect regarding potential medicinal activity of cat's claw seems to be the presence or absence of oxindole alkaloid constituents. The extent of evidence implicating the contribution of this chemical class of constituents towards eliciting potential medicinal effects is limited; however, it appears across different types of data (*in vitro*, animal, clinical) and endpoints (e.g. anti-proliferative and anti-inflammatory effects). Further work is needed to develop analytical standards for known compounds in this class, which could help establish standardisation of commercial extracts among different preparations, and additional research into potential pharmacological effects would be essential information for assessing the utility of the herb.

There have been few published controlled clinical trials in humans with cat's claw to corroborate both traditional and current medicinal use applications. The clinical studies that have been conducted are limited in scope for addressing potential medicinal activity in relation to effects on the immune system.[9,18,49] Consistent with traditional herbal practice for use of cat's claw as a remedy for inflammatory disorders, one clinical study using a pentacyclic chemotype cat's claw extract treatment over 24 weeks reported an effectiveness of the herb in the suppression of pain from rheumatoid arthritis.[49] Another study, using a freeze-dried aqueous extract conducted over 4 weeks, did not find significant reduction of inflammation as measured by knee circumference in

patients with osteoarthritis; however, it did report a significant reduction in pain.[18] As the whole plant extract is reported to be nonspecific in modulating purported immunological effects, co-administration with antioxidants has been suggested to produce 'synergistic' effects.[10] This combination of therapeutic regimens is clinically unproven and further clinical evidence for the activity of cat's claw to suppress chronic inflammation is required before a recommendation could be made regarding potential synergism.

The clinical studies we evaluated found that the herb is well tolerated with the highest dose administered being 6.5 g/person of a decoction (boiled down concentrate of water-soluble components of the herb).[56] Other dosing in clinical studies from capsules and dried extract forms ranged from 5–100 mg/day for 8 weeks to 5 months.[9,54] The only reported side effect associated with the ingestion of cat's claw was diarrhoea in three out of 40 subjects after 24 weeks of treatment with 60mg extract of the pentacyclic chemotype.[49] Outside of these studies addressing inflammation, there is a lack of clinical data to support other medicinal uses widely known from traditional herbal practice, including antiviral effects, gastric cancer and asthma. In two double-blinded, randomised clinical studies assessing the mutagenic activity of urine from smokers and nonsmokers, cat's claw extract did significantly decrease the mutagenic activity of urine from the tested smoker group suggesting the potential to suppress mutagenicity *in vivo*; however, this is far from demonstrating anti-carcinogenic effects.[55,56] Clearly, more well designed and well conducted clinical studies are needed to assess the other potential medicinal properties known from traditional practice and to determine the most relevant for further investigation, especially if the herb is going to be used in combination with anticancer drugs.

It has been recently estimated that approximately one in five Americans (or approximately 16%) ingest at least one herbal product while concurrently taking prescription medications.[5,139] Cat's claw is a commonly used herb, and as such, its concomitant use with prescription medications could be an important aspect to consider in its safety profile. In *in vitro* studies by Budzinski et al.,[58] the inhibition of CYP3A4 activity by cat's claw suggests the possibility for the occurrence of herbal-drug interactions. Although this notion is not supported by sporadic case reports suggesting the interaction occurs in humans, the preliminary *in vitro* work by Budzinski et al.[58] does raise the question of the ability of cat's claw to modulate important enzyme systems involved in the biotransformation of xenobiotics.

A better studied example of the mechanism of action of a commonly used herb to modulate drug metabolising enzyme activity is the case of *Hypericum perforatum* (St. John's wort). St. John's wort has been well documented to induce intestinal and hepatic CYP3A4 and P-glycoprotein.[140-143] A recent advancement in this regard is that extracts of St. John's wort preparations and individual constituents were found to be potent inducers of CYP3A4 and P-glycoprotein through activation of the nuclear factor pregnane X receptor (PXR).[144] Choudhuri and Valerio[145] reviewed St. John's wort and recent knowledge regarding its molecular mechanism of action through activation of the PXR, and concluded that such information can be useful as a clinical risk management tool. The induction of CYP3A4 by St. John's wort through activation of the PXR has been implicated in the loss of efficacy of various prescribed drugs and verified in documented case reports of drug interactions.[146-149] Likewise, other commonly used herbs have been documented to modulate the activity of major human biotransformation enzyme systems and have also been implicated in interactions with prescription drugs emphasising the prevalence of this effect across herbals.[139,150,151] For example, echinacea, the most commonly used natural product according to the most recent National Health Interview Survey,[3] is capable of producing significant inhibition of intestinal CYP3A activity and induction of hepatic CYP3A.[152] This observation suggests consideration of the relative contribution of metabolism by both hepatic and extra hepatic tissues to understanding the overall clearance pathway for a particular drug substrate affected and the individual modulating effects on CYP enzymes as essential in predicting the clinical significance of human herb-drug interactions. However, in the case of cat's claw and many herbals, the lack of clinical case reports, specific mechanistic and *in vivo* pharmacokinetic information characterising the interaction with and metabolism by major biotransformation enzymes precludes conclusiveness for the likelihood of induction of herbal-drug interactions. Once the various active components in cat's claw and similar herbs are characterised and understood, and the mechanisms driving such interactions are elucidated, potential interactions can be better predicted for better risk management by physicians regarding recommendations for their avoidance or concomitant use.

Overall, the animal studies conducted using aqueous (polar) and hydroalcoholic (relatively non-polar) extract preparations of cat's claw indicate a low degree of acute oral toxicity, and show preliminary evidence for anti-inflammatory activity in the carrageenan-induced paw oedema model. The mechanistic basis for the effects against inflammation have been studied and may be at least partially explained by *in vitro* cellular models that suggest such effects may be produced via combined anti-oxidant effects, suppression of TNFα production[71,83] and the activation of NF-κB, an important regulator of the transcription of various pro-inflammatory mediators.[65,66] Although the nature of this evidence is merely an association between the mechanistic studies conducted *in vitro* and effects observed *in vivo,* it is clear that further

studies on cytokine production and the key enzymes in the formation of proinflammatory eicosanoids from arachidonic acid such as COX-2 could be undertaken as they would be helpful to biochemically characterise the ability of cat's claw to produce anti-inflammatory effects. Moreover, the evaluation of scientific studies in this review found consistent reports that aqueous, methanolic and commercially available freeze-dried extracts of cat's claw tested were not mutagenic or cytotoxic at high concentrations (100 mg/mL) in a variety of *in vitro* cellular models.[56,67,70,73,74] Albeit the primary drawback to *in vitro* cytotoxicity testing is the lack of multiple organ interactions as observed in a whole organism, the absence of significant toxicity and histopathology in acute and subchronically treated animals, lack of significant clinical toxicity from ingestion of cat's claw extract preparations in controlled clinical studies ranging from 15 days to 24 weeks duration, and lack of multiple spontaneous human case reports of significant human adverse effects and toxicity suggest the herb is relatively safe and well tolerated under normal dose and use conditions. However, additional basic research and chronic human clinical studies would be the most suitable means for further evaluating the overall potential medicinal activity and toxicological safety of the herb. Because of the exceptional interest in their use, such information as well as post-marketing surveillance is needed in an evidence-based approach to understanding the chemical, medicinal and toxicological aspects of cat's claw and that of many other herbs.

11.2 *Lepidium meyenii*

It is possible that the beneficial effects of maca on sexual function and fertility may be explained by its concentration of proteins and vital nutrients. However, most data related to effects of maca on these parameters were not related to higher bodyweight in men or rats.[119] In addition, a recent study with different ecotypes of maca showed that treatment of male rats for 42 days with black, yellow and red maca resulted in higher epididymal sperm count only in rats treated with black or yellow maca but not in those treated with red maca (unpublished observation). These data suggest that secondary metabolites are likely responsible for most of the effects of maca.

There are few studies evaluating the mechanisms of action for the beneficial effects of maca. Some investigators have made an association between the presence of secondary metabolites with potential medicinal effects. For example, one paper suggests that maca alkaloids, steroids, glucosinolates, isothiocyanates and macamides are probably responsible for its potential properties as a fertility enhancer, aphrodisiac, adaptogen, immunostimulant, anabolic agent and for its influence on hormonal balance.[153] The available scientific evidence described above do not support all claimed properties for maca. Further studies should be conducted to study all of these properties.[153]

Macamides have been proposed as the compounds responsible for the improvement of sexual behaviour in rats and mice.[108]

The first attempt to demonstrate an effect of any particular secondary metabolite with biological activity was published in 1981.[106] Johns identified benzyl isothiocyanate in maca by TLC and HPLC, and suggested that this compound is responsible for the influence of the plant on the reproductive system and fertility.[106] However, mitosis is required during spermatogenesis and glucosinolates and one of its derivates, benzyl isothiocyanates, have been identified as anti-mitotic agents, which is consistent with their role in the hypothesis that these naturally occurring compounds, that are found in cruciferous vegetables at high concentrations, are proposed to have anti-carcinogenic properties.[107]

Maca also contains sterols, such as campesterol, stigmasterol and β-sitosterol;[107] however, β-sitosterol has been found to be an anti-fertility agent in male rats rather than a compound that enhances fertility.[154] (1R,3S)-1-methyltetrahydro-β-carboline-3 carboxylic acid was identified as a component of maca.[98] β-carbolines inhibit apoptosis[154] and this may be a mechanism to enhance spermatogenesis.

Studies of the effect of aqueous extract of maca on spermatogenesis in male rats were performed.[119,129] All phenolic compounds present in maca are not extracted in the aqueous preparation.[134] This suggests that some active principles are present in the aqueous extract. We have demonstrated that the initial effect of maca on spermatogenesis is improving spermiation and length of stage of spermiation (stage VIII).[129] This was not observed when ethanolic extracts of maca were used instead of aqueous extract.[131] However, sperm count was increased in rats treated with aqueous[129] or ethanolic[131] extract suggesting that there is more than one active principle acting on spermatogenesis.

Most advertisements for the effects of maca suggest its potential on sexual desire, copulatory behaviour and increased fertilising ability of sperm as a result of changes in sex hormone levels. However, most of the studies demonstrate that sex hormone levels were not affected.[119,129-131] Only one study in mice demonstrated that serum testosterone increases after 30 days of treatment.[128] The fact that the sex ratio and gonad development were not affected in rainbow trout that were fed diets containing maca meal (up to 15%) suggest that the activity of sex hormones are not affected by treatment with maca. In addition, treatment during 14 days with an aqueous extract of maca did not modify seminal vesicle weight in androgen-dependent organs in male rats.[129]

Maca contains water-soluble scavengers that may contribute to the decomposition of peroxyl radicals produced during inflam-

matory states.[134] The observed cytoprotective effects of maca may be due in part to its capacity to diminish the deleterious effect of cell death induced by peroxynitrite.[134] Maca may help to maintain a balance between oxidants and antioxidants.[134] The antioxidant activity of maca may be also due to its isothiocyanate content. Isothiocyanates have been shown to have antioxidant activities and anticarcinogenic properties.[107]

Much work remains to be done regarding the mechanism of action of maca. The Gonzales laboratory has identified that studying the effects of different secondary metabolites found in maca extracts and the physiological pathways they affect for their actions as important areas for future research.

Maca is a clear example of a plant with potential medicinal properties and substantial historical use, namely in traditional practice by indigenous cultures in Peru as a herbal medicine since the first recorded knowledge of it in the seventeenth century. Presently, the use of this plant is growing and has been the subject of advertisements with names such as 'Peruvian Ginseng' or 'Andean Viagra', which can be very misleading to consumers. Clearly, further research is needed to address the safety, pharmacology and toxicology of this medicinal plant. It is essential to establish a scientific evidence-based approach to further understanding its potential biological effects in humans and address its safe use.

Acknowledgements

The authors thank Dr Ronald F. Chanderbhan for his helpful comments of this manuscript. No sources of funding were used to assist in the preparation of this manuscript. The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

References

1. Dev S. Ethnotherapeutics and modern drug development: the potential of Ayurveda. Curr Sci 1997; 73: 909-28
2. Dev S. Ancient-modern concordance in Ayurvedic plants: some examples. Environ Health Perspect 1999; 107: 783-9
3. Barnes P, Powell-Griner E, McFann K, et al. Complementary and alternative medicine use among adults: United States, 2002. Hyattsville (MD): US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics; 2004 May. Advance data report no. 343
4. Mahady, Gail B, Fong HS, et al., editors. Botanical dietary supplements: quality, safety and efficacy. Lisse: Swets & Zeitlinger, 2001
5. Eisenberg DM, Davis RB, Ettner SL, et al. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. JAMA 1998; 280: 1569-75
6. Committee on the Framework for Evaluating Safety of Dietary Supplements Food and Nutrition Board on Life Sciences, Institute of Medicine and National Research Council of the National Academies. Scientific principals for integrating and evaluating the available data. In: Dietary supplements: a framework for evaluating safety. Washington, DC: The National Academies Press, 2004: 1-13
7. Desmarchelier C, Schaus FW. Sixty medicinal plants from the Peruvian amazon: ecology, ethnomedicine and bioactivity. 1st ed. Lima: Bio2000, 2000
8. Mulry M. Review of the First International Conference on St John's wort. HerbalGram 1999; 45: 61-5
9. Keplinger K, Laus G, Wurm M, et al. *Uncaria tomentosa* (Willd.) DC-ethnomedicinal use and new pharmacological, toxicological and botanical results. J Ethnopharmacol 1999; 64: 23-4
10. Williams JE. Review of antiviral and immunomodulating properties of plants of the Peruvian rainforest with a particular emphasis on uña de gato and sangre de grado. Altern Med Rev 2001; 6: 567-79
11. Reinhard KH. *Uncaria tomentosa* (Willd.) D.C.: cat's claw, uña de gato, or savéntaro. J Altern Complement Med 1999; 2: 143-51
12. Hammond G, Fernández ID, Villegas LF, et al. A survey of traditional medicinal plants from the Callejón de Huaylas, Department of Ancash, Peru. J Ethnopharmacol 1998; 61: 17-30
13. Villegas LF, Fernández ID, Maldonado H, et al. Evaluation of the wound-healing activity of selected traditional medicinal plants from Peru. J Ethnopharmacol 1997; 55: 193-200
14. Fong HHS. Integration of herbal medicine into modern medical practices: issues and prospects. Integr Cancer Ther 2002; 1: 287-93
15. World Health Organization. IARC monographs on the evaluation of carcinogenic risks to humans: some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Vol. 82. Lyon: IARC Press, 2002
16. Taylor L. Herbal secrets of the rainforest: technical data report for cat's claw 'uña de gato' (*Uncaria tomentosa*). 2nd ed. Austin (TX): Sage Press Inc., 2002
17. Duke J, Vasquez R. Amazonian ethobotanical dictionary. Boca Raton (FL): CRC Press, 1994
18. Piscoya J, Rodriguez Z, Bustamante SA, et al. Efficacy and safety of freeze-dried cat's claw in osteoarthritis of the knee: mechanisms of action of the species *Uncaria guianensis*. Inflamm Res 2001; 50: 442-8
19. Gattuso M, Di Sapio O, Gattuso S, et al. Morphoanatomical studies of *Uncaria tomentosa* and *Uncaria guianensis* bark and leaves. Phytomedicine 2004; 11: 213-23
20. Foster BC, Vandenhoek S, Hana J, et al. In vitro inhibition of human cytochrome P450-mediated metabolism of marker substrates by natural products. Phytomedicine 2003; 10: 334-42
21. Laus G, Keplinger K. Radix *Uncarie tomentosae* (Wild.) DC. Z Phytother 1997; 18: 122-6
22. Jones K. The herb report: uña de gato, life-giving vine of Peru. Am Herb Assoc 1994; 10: 4
23. The review of natural products by facts and comparisons. St Louis (MO): Wolters Kluwer Co., 1999
24. Beckstrom-Sternberg S, Duke J, Wain K. Chemicals in *Uncaria tomentosa* DC (Pedaliaceae): the ethnobotany database. Fulton (MD): Agricultural Research Service, 1996
25. Heitzman ME, Neto CC, Winiarz E, et al. Ethnobotany, phytochemistry and pharmacology of *Uncaria* (Rubiaceae). Phytochem 2005; 66: 5-29
26. Montoro P, Carbone V, Quiroz Jde D, et al. Identification and quantification of components in extracts of *Uncaria tomentosa* by HPLC-ES/MS. Phytochem Anal 2004; 15: 55-64
27. Aquino R, De Feo V, De Simone F, et al. Plant metabolites: new compounds and anti-inflammatory activity of *Uncaria tomentosa*. J Nat Prod 1991; 54: 433-59
28. Laus G, Brössner D, Keplinger K. Alkaloids of Peruvian *Uncaria tomentosa*. Phytochemistry 1997; 45: 855-60
29. Hemingway SR, Phillipson JD. Alkaloids from S. American species of *Uncaria* (Rubiaceae) [abstract]. J Pharm Pharmacol 1974; 26 Suppl.: 113P
30. Stuppner H, Sturm S. Capillary electrophoretic analysis of oxindole alkaloids from *Uncaria tomentosa*. J Chromatogr 1992; 609: 375-80
31. Ganzera M, Muhammad I, Khan RA, et al. Improved method for the determination of oxindole alkaloids in *Uncaria tomentosa* by high performance liquid chromatography. Planta Med 2001; 67: 447-50
32. Stuppner H, Sturm S, Konwalinka G. HPLC analysis of the main oxindole alkaloids from *Uncaria tomentosa*. Chromatographia 1992; 34: 597-00
33. Kitajima M, Hashimoto K, Yokoya M, et al. A new gluco indole alkaloid, 3, 4-dehydro-5-carboxystrictosidine, from Peruvian una de gato (*Uncaria tomentosa*). Chem Pharm Bull (Tokyo) 2000; 48: 1410-2
34. Muhammad I, Dunbar D, Khan R, et al. Investigation on uña de gato I. 7-deoxyloganic acid and 15N NMR spectroscopic studies on pentacyclic oxindole alkaloids from *Uncaria tomentosa*. Phytochemistry 2001; 57: 781-5

35. Aquino R, Conti C, Stein ML. Plant metabolites: structure and in vitro antiviral activity of quinovic acids glycosides from *Uncaria tomentosa* and *Guettarda platypoda*. J Nat Prod 1989; 52: 679-85
36. Aquino R, De Tommasi N, De Simone F, et al. Triterpenes and quinovic acid glycosides from *Uncaria tomentosa*. Phytochemistry 1997; 45: 1035-40
37. Cerri R, Aquino R, De Simone F, et al. New quinovic acid glycosides from *Uncaria tomentosa*. J Nat Prod 1988; 51: 257-61
38. Yepez M, Lock de Ugaz O, Alvarez ACM, et al. Quinovic acid glycosides from *Uncaria guianensis*. Phytochemistry 1991; 30: 1635-7
39. Kitajima M, Yokoya M, Takayama H, et al. Two new nor-triterpene glycosides from Peruvian 'uña de gato' (*Uncaria tomentosa*). J Nat Prod 2003; 66: 320-3
40. Montenegro De Matta S, Delle Monache F, Ferrari F, et al. Alkaloid and procyanidins of an *Uncaria* sp. from Peru. II. Fármaco 1976; 31: 527-35
41. Aquino R, De Simone F, Vincieri F, et al. New polyhydroxylated triterpenes from *Uncaria tomentosa*. J Nat Prod 1990; 53: 559-64
42. Kitajima M, Hashimoto K, Yokoya M, et al. Two new 19-hydroxyursolic acid-type triterpenes from Peruvian 'uña de gato' (*Uncaria tomentosa*). Tetrahedron 2000; 56: 547-52
43. Wirth C, Wagner H. Pharmacologically active procyanidines from the bark of *Uncaria tomentose*. Phytomedicine 1997; 4: 265-7
44. Senatore A, Cataldo A, Laccarino FP, et al. Ricereche fitochjmjehe e biologiche sull *Uncaria tomentosa*. Boll Soc Ital Biol Sper 1989; 656: 517-20
45. Laus G, Keplinger K. Separation of steroisomeric oxindole alkaloids from *Uncaria tomentosa* by high performance liquid chromatography. J Chromatogr A 1997; 662: 243-9
46. Laus G, Brössner D, Senn G, et al. Analysis of the kinetics of isomerization of spiro oxindole alkaloids. J Chem Soc Perkin Trans 1996; 2: 1931-6
47. Laus G. Kinetics of isomerization of tetracyclic spiro oxindole alkaloids. J Chem Soc Perkin Trans 1998; 2: 315-7
48. Obregón Vilches L. Uña de gato. Género Uncaria, estudios botánicos, químicos y farmacológicos de *Uncaria tomentosa* y *Uncaria guianensis*. Lima (Perú): Instituto de Fitoterapia Americano ed., 1997: 53-7
49. Mur E, Hartig F, Eibl G, et al. Randomized double blind trial of an extract from the pentacyclic alkaloid-chemo-type of *Uncaria tomentosa* for the treatment of rheumatoid arthritis. J Rheumatol 2002; 29: 678-81
50. Wolters Kluwer Health Inc. eFacts: review of natural products. Facts and comparisions. Cat's claw [online]. Available from URL: http://www.efactsweb.com/RNP/RNPMonoDetail.asp?.itemID = 61063&optIndexID = 61064 [Accessed 2004 Sep 1]
51. Schauss AG. Cat's claw (*Uncaria tomentosa*). Nat Med J 1998; 1: 16-9
52. Gruenwalk J, Brendler T, Jaenicke C, editors. PDR for herbal medicines. Montvale (NJ): Medical Economics Company Inc., 2000
53. Sheng Y, Bryngelsson C, Pero RW. Enhanced DNA repair, immune function and reduced toxicity of C-MED100™, a novel aqueous extract from *Uncaria tomentosa*. J Ethnopharmacol 1999; 69: 115-26
54. Sheng Y, Li L, Holmgren K, et al. DNA repair enhancement of aqueous extracts of *Uncaria tomentosa* in a human volunteer study. Phytomedicine 2001; 8: 275-82
55. Leon FR, Ortiz N, Antunez de Mayolo A, et al. Antimutagenic activity of a freeze-dried aqueous extract of *Uncaria tomentosa* in smokers and non-smokers. Third European Colloquium on Ethnopharmacology; 1996 May 29-Jun 2; Genova (Italy): European colloquim on Ethnopharmacology, 1996: 255
56. Rizzi R, Re F, Bianchi A, et al. Mutagenic and antimutagenic activities of *Uncaria tomentosa* and its extracts. J Ethnopharmacol 1993; 38: 63-7
57. Hilepo JN, Bellucci AG, Mossey RT. Acute rental failure caused by 'cat's claw' herbal remedy in a patient with systemic lupus erythematosus [letter]. Nephron 1997; 77: 361-9
58. Budzinski JW, Foster BC, Vandenhoek S, et al. An in vitro evaluation of human cytochrome P450 3A4 inhibition by selected commercial herbal extracts and tinctures. Phytomedicine 2000; 7: 273-82
59. Hammerly M, Murray MT, Spoerke DG. Micromedex systems. AltMedex. Cat's claw [online]. Available from URL: http://csi.micromedex.com/DKS/DATA/AL/AL0069.HTM?.Top = Yes [Accessed 2005 Jan 13]
60. Natural Medicines Comprehensive Database. 2004. 'Maca' [online]. Available from URL: http://www.naturaldatabase.com/monograph.asp?.mono_id = 555&brand_id = [Accessed 2004 Apr 20]
61. McGuffin M, Hobbs C, Upton R, et al. editors. American Herbal Products Association's Botanical Safety Handbook. Boca Raton (FL): CRC Press, 1997
62. Kynoch SR, Lloyd GK. Acute oral toxicity to mice of substance E-2919. Huntington (England): Huntington Research Centre, 1975. (Data on file)
63. Kreutzkamp B. Niedermolekulare Inhaltsstoffe mit immunstimulierenden Eigenschaften aus *Uncaria tomentosa* and Okoubaka aubrevillei und anderen Drogen [dissertation]. Munich: University of Munich, 1984
64. Svendsen O, Skydsgaard K. Test report (Extraction radicis *Uncariae tomentosae*). Denmark: Scantox Biological Laboratory Ltd, 1986. (Data on file)
65. Sheng Y, Pero RW, Wagner H. Treatment of chemotherapy-induced leukopenia in a rat model with aqueous extract from *Uncaria tomentosa*. Phytomedicine 2000; 7: 137-43
66. Aguilar J, Rojas P, Marcelo A, et al. Anti-inflammatory activity of two different extracts of *Uncaria tomentosa* (Rubiacea). J Ethnopharmacol 2002; 81: 271-6
67. Sandoval-Chacon M, Thompson JH, Zhang XJ, et al. Antiinflammatory actions of cat's claw: the role of NF-kappaB. Alimentary Pharmacol Ther 1998; 12: 1279-89
68. Cisneros FJ, Jayo M, Niedziela L. An *Uncaria tomentosa* (cat's claw) extract protects mice against ozone-induced lung inflammation. J Ethnopharmacol 2005; 96: 355-64
69. Mestanza D. Evalución de la actividad immunoestimulante de *Uncaria tomentosa* (Willd.) DC. uña de gato en ratones albinos. Bioversidad Salud 1999; 1: 16-9
70. Santa Maria A, Lopez A, Diaz MM, et al. Evaluation of the toxicity of *Uncaria tomentosa* by bioassays in vitro. J Ethnopharmacol 1997; 57: 183-7
71. Sandoval M, Charbonnet RM, Okuhama NN, et al. Cat's claw inhibits TNFα production and scavenges free radicals: role in cytoprotection. Free Radic Biol Med 2000; 29: 71-8
72. Desmarchelier C, Mongelli E, Coussio J, et al. Evaluation of the in vitro antioxidant activity in extracts of *Uncaria tomentosa* (Willd.) DC. Phytother Res 1997; 11: 254-6
73. Miller MJ, Angeles FM, Reuter BK, et al. Dietary antioxidants protect gut epithelial cells from oxidant-induced apoptosis. BMC Complement Altern Med 2001; 1: 1-10
74. Sandoval M, Ronzio RA, Mwanza DN, et al. Protection from peroxynitrite-induced apoptosis by plant-derived antioxidants [abstract]. FASEB J 1997; 11: A648
75. Riva L, Cordini D, Di Frunzo G, et al. The antiproliferative effects of *Uncaria tomentosa* extracts and fractions on the growth of breast cancer cell line. Anticancer Res 2001; 21: 2457-61
76. Stuppner H, Sturm S, Geisen G, et al. A differential sensitivity of oxindole alkaloids to normal and leukemic cell lines. Planta Med 1993; 59 Suppl. A: 583
77. Sheng Y, Pero RW, Amiri A. Induction of apoptosis and inhibition of proliferation in human tumor cells treated with extracts of *Uncaria tomentosa*. Anticancer Res 1998; 18: 3363-8
78. Akesson CH, Pero RW, Ivars F. C-Med 100, a hot water extract of *Uncaria tomentosa*, prolongs lymphocyte survival in vivo. Phytomedicine 2003; 10: 23-33
79. Wagner H, Kreutzkamp B, Jurcic K. Die Alkaloide von *Uncaria tomentosa* und ihre Phagozytose-steigernde Wirkung. Planta Med 1985; 5: 419-23
80. Wurm M, Kacani L, Laus G, et al. Pentacyclic oxindole alkaloids from *Uncaria tomentosa* induce human endothelial cells to release a lymphocyte-proliferation-regulating factor. Planta Med 1998; 64: 701-4
81. Baeuerle PA, Henkel T. Function and activation of NF-κB in the immune system. Annu Rev Immunol 1994; 12: 141-79
82. Bernard P, Scior T, Didier B, et al. Ethnopharmacology and bioinformatics combination for leads discovery: application to phospholipase A_2 inhibitors. Phytochemistry 2001; 58: 865-74
83. Sandoval M, Okuhama NN, Zhang XJ, et al. Anti-inflammatory and antioxidant activities of cat's claw (*Uncaria tomentosa* and *Uncaria guianensis*) are independent of their alkaloid content. Phytomedicine 2002; 9: 325-37
84. Lemaire I, Assinewe V, Cano P, et al. Stimulation of interleukin-1 and -6 production in alveolar macrophages by the neotropical liana, *Uncaria tomentosa* (una de gato). J Ethnopharmacol 1999; 64: 109-15
85. Keplinger K, Wagner H, Kreutzkamp B. Oxindole alkaloids having properties stimulating the immunological system. US Patents 4,244,901, 1989
86. Keplinger K, Wagner H, Kreutzkamp B. Oxindole alkaloids having properties stimulating the immunological system. US patents 4,940,425, 1990
87. Cieza de Leon P. Chronicle of Peru. First part 1553; 240: 354
88. Cobo B. History of the new world. Madrid: Biblioteca de autores españoles, 1956: 1-430

89. Quiroz C, Aliaga R. Maca (*Lepidium meyenii* Walp.) Andean roots and tubers: ahipa, arracacha, maca and yacon. In: Hermann M, Hellers J, editors. Promoting the conservation and use of underutilized neglected crops. Rome: International Plant Genetic Resources Institute, 1997; 21: 173-97
90. Tello J, Hermann M, Calderon A. La maca (*Lepidium meyenii* Walp.) cultivo alimenticio potencial para las zonas andinas. Boletín Lima 1992; 14: 59-66
91. National Research Council. Lost crops of the Incas: little-known plants of the Andes with promise for worldwide cultivation. Washington, DC: The National Academies Press, 1989: 56-65
92. Balick MJ, Lee R. Maca: from traditional food crop to energy and libido stimulant. Altern Ther Health Med 2002; 8: 96-8
93. Toledo J, Dehal P, Jarrin F, et al. Genetic variability of *Lepidium meyenii* and other *Lepidium* species (Brassicaceae) assessed by molecular markers. Ann Bot (Lond) 1998; 82: 523-30
94. Bracko L, Zarucchi J. Catalogue of the flowering plants and gymnosperms of Peru. In: monographs in systematic botany. New York: Missouri Botanical Garden 1993; 45: 1-1286
95. Quiroz CF, Epperson A, Hu J, et al. Physiological and cytological characterization of maca, *Lepidium meyenii* Walp. Econ Bot 1996; 50: 216-23
96. Yllescas M. Chemical and physicochemical study of three ecotypes of *Lepidium meyenii* from Carhuamayo [dissertation]. Lima: Faculty of Pharmacy and Biochemistry. Universidad Nacional Mayor de San Marcos, 1994
97. Bianchi A. Maca *Lepidium meyenii*. Boletín Latino americano y del caribe de plantas medicinales y aromáticas 2003; 2: 30-6
98. Piacente S, Carbone V, Plaza A, et al. Investigation of the tuber constituents of maca (*Lepidium meyenii* Walp). J Agric Food Chem 2002; 50: 5621-5
99. Carrillo F, Poma de Ayala G, editors. Cronistas Indios y Mestizos. Lima: Enciclopedia Histórica de la Literatura Peruana 7. Editorial Horizonte, 1992: 343
100. Rea J. Andean roots. In: Bermejo JEH, Leon J, editors. Neglected crops: 1492 from a different perspective. Plant production and protection series no. 26. Rome: Food and Agriculture Organization (FAO), 1994: 165-79
101. Dini A, Migliuolo G, Ratrelli L, et al. Chemical composition of *Lepidium meyenii*. Food Chem 1994; 49: 347-9
102. Li G, Ammermann U, Quiros CF. Glucosinolate contents in maca (*Lepidium peruvianum* Chacon) seeds, sprouts, mature plants and several derived commercial products. Econ Bot 2001; 55: 255-62
103. Baquerizo-Vasquez GL. Estudio químico-bromatológico del *Lepidium meyenii* Walp. (maca) y el Aiphanes var. Deltoidea burret (Shica-shica) [dissertation]. Lima: Faculty of Medicine, Universidad Nacional Mayor de San Marcos, 1968
104. Espinoza CL, Poma IP. Determinación de aminoácidos esenciales de la maca (*Lepidium meyenii* Walp.) y elaboración de una mezcla proteina a base de alimentos andinos [dissertation]. Huancayo (Peru): Faculty of Engineering in Alimentary Industries, Universidad Nacional del Centro del Peru, 1995
105. Chacón G. Phytochemical Studies of *Lepidium meyenii* Walp [dissertation in Spanish]. Lima: Universidad Nacional Mayor de San Marcos, 1961
106. Johns T. The anu and the maca. J Ethnobiol 1981; 1: 208-12
107. Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 2001; 56: 5-51
108. Zheng BL, He K, Kim CH, et al. Effect of a lipidic extract from *Lepidium meyenii* on sexual behavior in mice and rats. Urology 2000; 55: 598-602
109. Dini I, Tenore GC, Dini A. Glucosinolates from maca (*Lepidium meyenii*). Biochem Syst Ecol 2002; 30: 1087-90
110. Tellez MR, Khan IA, Kobaisy M, et al. Composition of the essential oil of *Lepidium meyenii* (Walp.). Phytochemistry 2002; 61: 149-55
111. Ciska E, Martyniak-Przybyszwska B, Kozlowska H. Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. J Agric Food Chem 2000; 48: 2862-7
112. Muhammad I, Zhao J, Dunbar DC. Constituents of *Lepidium meyenii* 'maca'. Phytochemistry 2002; 59: 105-10
113. Ganzera M, Zhao J, Muhammad I, et al. Chemical profiling and standardization of *Lepidium meyenii* (maca) by reversed phase high performance liquid chromatography. Chem Pharm Bull (Tokyo) 2002; 50: 988-91
114. Jeri H. Estudio fitoquímico de la maca [dissertation]. Pasco (Peru): Engineering Faculty. Universidad Nacional Daniel Alcides Carrion, 1990
115. Lee K-J, Dabrowski K, Rinchard J, et al. Supplementation of maca (*Lepidium meyenii*) tuber meal in diets improves growth rate and survival of rainbow trout *Oncorhynchus mykiss* (Walbaum) alevins and juveniles. Aquac Res 2004; 35: 215-23
116. Cui B, Zheng BL, He K, et al. Imidazole alkaloids from *Lepidium meyenii*. J Nat Prod 2003; 66: 1101-3
117. Ruiz H. Relación histórica del viaje a los reinos del Peru y Chile, 1777-1778. Madrid: Madrid Acad De Ciencias Exactas, Fis y Nat, 1952: 1-526
118. Aslam H, Rosiepen G, Krishnamurthy H, et al. The cycle duration of the seminiferous epithelium remains unaltered during GnRH antagonist-induced testicular involution in rats and monkeys. J Endocrinol 1999; 161: 281-8
119. Gonzales GF, Gasco M, Córdova A, et al. Effect of *Lepidium meyenii* (maca) on spermatogenesis in male rats acutely exposed to high altitude (4340 m). J Endocrinol 2004; 180: 87-95
120. Alvarez CJ. Utilización de diferentes niveles de maca en la fertilidad de cobayos [dissertation]. Pasco (Peru): Faculty of Agriculture and Cattle Sciences, Universidad Nacional Daniel Alcides Carrión, 1993
121. Willard T. Sowing your wild oats and reaping love´s benefits. Total Health 2000; 22: 62-3
122. Ahmed AJ. Maca-stimulin libido Redux. Total Health 2003; Suppl. 25: 15-6
123. Gonzales GF, Córdova A, Gonzales C, et al. Improved sperm count after administration of *Lepidium meyenii* (maca) in adult men. Asian J Androl 2001; 3: 301-4
124. Canales M, Aguilar J, Prada A, et al. Nutritional evaluation of *Lepidium meyenii* (maca) in albino mice and their descendants [in Spanish]. Arch Latinoam Nutr 2000; 50: 126-33
125. Cicero AF, Bandieri E, Arletti R. *Lepidium meyenii* Walp. improves sexual behaviour in male rats independently from its action on spontaneous locomotor activity. J Ethnopharmacol 2001; 75: 225-9
126. Cicero AF, Piacente S, Plaza A, et al. Hexanic maca extract improves rat sexual performance more effectively than methanolic and chloroformic maca extracts. Andrologia 2002; 34: 177-9
127. Gonzales GF, Córdova A, Vega K, et al. Effect of *Lepidium meyenii* (maca) on sexual desire and its absent relationship with serum testosterone levels in adult healthy men. Andrologia 2002; 34: 367-72
128. Oshima M, Gu Y, Tsukada S. Effects of *Lepidium meyenii* Walp and Jatropha macrantha on blood levels of estradiol-17β, progesterone, testosterone and the rate of embryo implantation in mice. J Vet Med Sci 2003; 65: 1145-6
129. Gonzales GF, Ruiz A, Gonzales C. Effect of *Lepidium meyenii* (maca) roots on spermatogenesis of male rats. Asian J Androl 2001; 3: 231-3
130. Gonzales GF, Córdova A, Vega K, et al. Effect of *Lepidium meyenii* (maca), a root with aphrodisiac and fertility-enhancing properties, on serum reproductive hormone levels in adult healthy men. J Endocrinol 2003; 176: 163-8
131. Gonzales GF, Rubio J, Chung A, et al. Effect of alcoholic extract of *Lepidium meyenii* (maca) on testicular function in male rats. Asian J Androl 2003; 5: 349-52
132. Zheng BH, He K, Hwang ZY, et al. Effect of aqueous extract from *Lepidium meyenii* on mouse behavior in forced swimming test. In: Quality management of nutraceuticals. ACS symposium series 803; 2002; 259-68; Washington, DC: American Chemical Society, 2002
133. Tapia A, López C, Marcelo A, et al. The maca (*Lepidium meyenii*) and their effect anti-stress in an animal model in mice [in Spanish]. Acta Andina 1999- 2000; 8: 45-56
134. Sandoval M, Okuhama NN, Angeles FM, et al. Antioxidant activity of the cruciferous vegetable maca (*Lepidium meyenii*). Food Chem 2002; 79: 207-13
135. Park TH, Kwon OS, Park SY, et al. N-methylated beta-carbolines protect PC12 cells from cytotoxic effect of MTP+ by attenuation of mitochondrial membrane permeability change. Neurosci Res 2003; 46: 349-58
136. Marcelo A, Canales M, Aguilar J. Toxicity testing of maca. Lima: Instituto de Medicina Tropical Alexander von Humboldt, 2004. (Data on file)
137. Obregon L. Maca. Planta medicinal y nutritiva del Peru. 1st ed. Lima: Instituto de Fitoterapia Americana, 1998: 143-44
138. Blumenthal M, editor. The Complete German Commission E monographs: therapeutic guide to herbal medicines. Boston (MA): American Botanical Council, 1998: 12
139. Sparreboom A, Cox MC, Acharya MR, et al. Herbal remedies in the United States: potential adverse interactions with anticancer agents. J Clin Oncol 2004; 22: 2489-503

140. Wang Z, Gorski JC, Hamman MA, et al. The effects of St John's wort (*Hypericum perforatum*) on human cytochrome P450 activity. Clin Pharmacol Ther 2001; 70: 317-26
141. Wang Z, Hamman MA, Huang S-M, et al. Effect of St John's wort on the pharmacokinetics of fexofenadine. Clin Pharmacol Ther 2002; 71: 414-20
142. Hennessy M, Kelleher D, Spiers JP, et al. St Johns wort increases expression of P-glycoprotein: implications for drug interactions. Br J Clin Pharmacol 2002; 53: 75-82
143. Dürr D, Stieger B, Kullak-Ublick GA, et al. St John's wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. Clin Pharmacol Ther 2000; 68: 598-4
144. Moore LB, Goodwin B, Jones SA, et al. St John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. Proc Natl Acad Sci U S A 2000; 97: 7500-2
145. Choudhuri S, Valerio LG. Usefulness of studies on the molecular mechanism of action of herbals/botanicals: the case of St John's wort. J Biochem Mol Toxicol 2005; 19: 1-11
146. Piscitelli SC, Burstein AH, Chaitt D, et al. Indinavir concentrations and St John's wort. Lancet 2000; 355: 547-8
147. Ruschitzka F, Meier PJ, Turina M, et al. Acute heart transplant rejection due to Saint John's wort [letter]. Lancet 2000; 355: 548-9
148. Markowitz JS, Donovan JL, DeVane CL, et al. Effect of St John's wort on drug metabolism by induction of cytochrome P450 3A4 enzyme. JAMA 2003; 290: 1500-4
149. Barone GW, Gurley BJ, Ketel BL, et al. Drug interaction between St. John's wort and cyclosporine. Ann Pharmacother 2000; 34: 1013-6
150. Huang S-M, Lesko LJ. Drug-drug, drug-dietary supplement, and drug-citrus fruit and other food interactions: what have we learned? J Clin Pharmacol 2004; 44: 559-69
151. Raucy JL. Regulation of CYP3A4 expression in human hepatocytes by pharmaceuticals and natural products. Drug Metab Dispos 2003; 31: 533-9
152. Gorski JC, Huang S-M, Zaheer N, et al. The effect of echinacea on CYP3A activity in vivo. Clin Pharmacol Ther 2004; 75: 89-100
153. Valentova K, Ulrichova J. *Smallanthus sonchifolius* and *Lepidium meyenii*: prospective Andean crops for the prevention of chronic diseases. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2003; 147: 119-30
154. Malini T, Vanithakumari G. Antifertility effects of beta-sitosterol in male albino rats. J Ethnopharmacol 1991; 35: 149-53

Correspondence and offprints: Dr *Luis G. Valerio*, Jr, Division of Biotechnology and GRAS Notice Review, Office of Food Additive Safety (HFS-255), Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 5100 Paint Branch Parkway, College Park, MD 20740, USA.
E-mail: Luis.Valerio@cfsan.fda.gov