Curcuma longa L. ameliorates asthma control in children and adolescents: A randomized, double-blind, controlled trial

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ABSTRACT

Ethnopharmacological relevance: Roots of Curcuma longa L. are used as medicine for millennia. They possess several pharmacological properties, including anti-inflammatory action, and can be suitable for asthma treatment.

Aim of the study: We aimed to test the hypothesis that, in children and adolescents with persistent asthma, the administration of powdered roots of C. longa for 6 months, in addition to standard treatment, compared to placebo, will result in better disease control.

Patients and methods: We conducted a randomized, double-blind, placebo-controlled, phase II clinical trial. Patients were randomly assigned to receive 30 mg/kg/day of C. longa for 6 months, or placebo. Data were collected prospectively. All patients were categorized for asthma severity and control according to GINA-2016 and underwent pulmonary function tests.

Results: Overall, both groups experienced amelioration of their frequency of symptoms and interference with normal activity, but no differences were found between the two treatment groups. However, patients receiving C. longa experienced less frequent nighttime awakenings, less frequent use of short-acting β-adrenergic agonists, and better disease control after 3 and 6 months.

Conclusion: The powdered roots of C. longa led to less frequent nighttime awakenings, less frequent use of short-acting β-adrenergic agonists, and better disease control after 3 and 6 months, when compared to placebo.

1. Introduction

Asthma is a highly prevalent chronic inflammatory disease whose main characteristics are bronchial hyper-responsiveness, variable limitation of air flow, and airway remodeling. Symptoms include episodes of wheezing, dyspnea, and cough (Global Initiative for Asthma, 2016). The disease leads to significant morbidity worldwide, and its prevalence is increasing in the last 20 years.

The treatment for asthma aims at controlling inflammation in the long term, while keeping symptoms at minimum and ameliorating the lung function. The main drugs used for long-term control are: inhaled corticosteroids and inhaled long-acting β-adrenergic agonists (LABA). Oral corticosteroids and short-acting β-adrenergic agonists (SABA) are used for asthma attacks. Although most patients experience good disease control, many patients do not (Global Initiative for Asthma, 2016). In addition, chronic exposure to corticosteroids can cause serious adverse effects, especially in children. Therefore, new, safe and effective drugs for asthma are needed.

Curcuma longa L. (turmeric, Zingiberaceae) is a perennial plant, extensively grown in Asia and tropical countries, like Brazil. Roots of C. longa have been used for millennia as medicine for many purposes, including respiratory diseases (Jagetia and Aggarwal, 2007) and, more specifically, asthma (WHO, 1999). However, its traditional use for asthma lacks scientific validation.

The characteristic yellow, golden color is due to the presence of curcuminoids [curcumin (75–81%), demethoxycurcumin (15–19%), and bisdemethoxycurcumin (2.2–6.6%)] (Patil et al., 2009). There is evidence that the mixture of curcuminoids is more effective than each one alone (Balaji and Chempakam, 2010). Curcumin possesses several biological activities, including: anti-inflammatory, antibacterial, antiviral, antifungal, antioxidant, wound healing, among many others (Jagetia and Aggarwal, 2007). Curcumin modulates the inflammatory response and release of cytokines by suppressing nuclear factor kappa-B (NF-κB) activation, through inhibition of phosphorylation and...
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powdered plant material. The powdered plant material of
Medicinal Plants at UNAERP (voucher HPMU-3215). The roots were
a.m., and a voucher specimen was deposited in the Herbarium of
methodology (Jayaprakasha et al., 2002) and validated by ultra-per-

dards were obtained from the rhizomes following previously described
encapsulated (250 mg, corresponding to 11 mg of curcumin and 2 mg of
37 kHz). The supernatant was
MS/MS). Phytocomplexes (5 mg) were mixed to methanol (1 mL) and
for herbal interventions (Gagnier et al., 2006). Children and adolescents
Brazilian Registry of Clinical Trials (http://www.ensaiosclinicos.gov.
ent was required. This study conforms to the standards of the Helsinki
board (protocol #17207213.4.0000.5440), and signed informed con-
clinical trial. The study was approved by a local institutional review
1.1. Patients and Methods

This was a phase 2, double-blind, randomized, placebo-controlled
clinical trial. The study was approved by a local institutional review
board (protocol #17207213.4.0000.5440), and signed informed con-
cept was required. This study conforms to the standards of the Helsinki
1983. The study was registered in the Brazilian Registry of Clinical Trials (http://www.ensaiosclinicos.gov.
protocol of drug and placebo
C. longa was grown at the rural area of Jardinopolis, Sao Paulo,
Brazil (latitude 21°4′33″ S, longitude 47°44′48″ W). The plant was
Identified by Dr. Lin Chau Ming (State University of Sao Paulo,
B. root material of C. longa was not included. Exclusion criteria were:
judgment by the researchers.
1.2. Preparation of drug and placebo

C. longa was grown at the rural area of Jardinopolis, Sao Paulo,
Brazil (latitude 21°4′33″ S, longitude 47°44′48″ W). The plant was
identified by Dr. Lin Chau Ming (State University of Sao Paulo,
Botucatu, SP, Brazil). C. longa is acknowledged as medicine in Brazil by
the National Sanitary Surveillance Agency (Agência Nacional de Vigilância Sanitaria, ANVISA). Rhizomes of C. longa, cultivated in the
region of Jardinopolis (Sao Paulo, Brazil, latitude: 21°01′04″, longitude:
47°45′50″, altitude: 590 m). The roots were harvested in 2012, at 9
a.m., and a voucher specimen was deposited in the Herbarium of
Medicinal Plants at UNAERP (voucher HPMU-3215). The roots were
washed in water, sliced, dried in a circulating-air oven at 45 °C for 36 h,
and sieved up to particle size of 40 mesh. The resulting powder is called
powdered plant material. The powdered plant material of C. longa
was encapsulated (250 mg, corresponding to 11 mg of curcumin and 2 mg of
demethoxycurcumin). Placebo was maltodextrin, encapsulated identi-
cally.

1.3. Quality control

The content of curcuminoids were determined in the powdered
roots by high-performance liquid chromatography (HPLC), at the
Laboratory of Medicinal Plants, Department of Plant Biotechnology,
University of Ribeirao Preto. Curcumin and demethoxycurcumin standards were obtained from the rhizomes following previously described
methodology (Jayaprakasha et al., 2002) and validated by ultra-per-
formance liquid chromatography-tandem mass spectrometry (UPLC-
MS/MS). Phytocomplexes (5 mg) were mixed to methanol (1 mL) and
sonicated for 15 min in ultrasound (Eco-sonics Q - 5.9/37, W 154 and
37 kHz). The supernatant was filtered through Millipore filter (0.22 μm
pore size) and injected in HPLC.

The HPLC used was a Shimadzu™ (Kyoto, Japan) model LC-10-AVP
equipped with a SIL-10AF autoinjector, a SPD-M20A photodiode array
detector and a Phenomenex™ (Torrance, CA, USA) Luna C18 column
(250 × 4.6 mm i.d.; 5 μm particle size). The injection system (Shimadzu™) was used a 20 μL sample loop. A Shimadzu SPD-M10A VP
series variable-wavelength detector set at 420 nm was used for detec-
tion. A Millipore™ (Bangalore, India) membrane filter (0.22 μm pore
size) was used for filtration.

1.3.1. Chromatographic conditions

The mobile phase comprised of (A) water/methanol/acetic acid
(93:5:2; v/v/v) and (B) acetonitrile/methanol (95:5; v/v) and gradient
elution was performed for 30 min at a flow rate of 1 mL min−1.
Quantitative levels of curcuminoids were determined using the above
solvents programmed linearly from 48 to 68% in B for 0–15 min. The
gradient went then from 68 to 48% in B for 15.01–30 min. The injection
volume was 20 μL and the detection wavelength at 420 nm, as previ-
ously described (Jayaprakasha et al., 2002).

1.3.2. Preparation of stock solutions for HPLC

Methanolic stock solutions of curcumin and demethoxycurcumin
were prepared separately at a concentration of 250–15.625 μg mL−1.

1.3.3. Determination of curcumin and demethoxycurcumin in samples

Samples (25 μL) were injected in the HPLC. Curcuminoids concen-
tration was determined using linear equation considering dilution
dilution. Curcumin and demethoxycurcumin contents were expressed as
mg g−1 dry weight (DW).

1.3.4. Ultra-performance liquid chromatography-mass spectrometry
(UPLC-MS) analysis of the methanolic extract of C. longa, curcumin and
demethoxycurcumin

The identities of curcumin and demethoxycurcumin present in the
methanolic extract of C. longa roots were confirmed by UPLC-MS-MS
analysis using a Waters (Milford, MA, USA) Acquity UPLC-Class
system equipped with a PAD and a Waters Xevo TQ-S tandem quad-
rupe mass spectrophotometer and fitted with a Supelco Ascentis Express C18 column (100 × 4.6 mm i.d.; 2.7 μm particle size) (Fig. 1).

The mobile phase used for gradient elution consisted of 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid (solvent
B) at a flow rate of 0.5 mL min−1. The gradient elution program started with 40% B, increased B to 90% up to 10 min remaining in that
condition for 5 min and returned to the initial condition (40% B) within
the next 4 min. The total time of analysis was 20 min at a flow rate of
500 μL min−1. Wavelengths from 220 to 600 nm were employed to
monitor the targeted compounds in diode array detector (DAD). The
source and operating parameters were optimized as follows: capilar
voltage = 3.2 kV, source temperature = 150 °C, desolvation tempera-
ture (N2) = 350 °C, desolvation gas flow = 600 L h−1, and mass range from
m/z 150 to 800 in the full-scan mode.

1.4. Study protocol

Patients were randomized to one of two groups: C. longa or placebo.
A randomization list was generated at www.sealedenvelope.com, in
blocks of random size (4 or 6). The list was generated by a researcher
who did not take part in randomization or patient assessment. The list
was uploaded to a software for allocation concealment. Researchers,
clinicians, and patients were all concealed and blinded to allocation.

The dose of powdered roots of C. longa recommended by the World
Health Organization (WHO) for adults is 1.5–3.0 g/day (WHO, 1999),
which corresponds to 30–60 mg/kg/day, considering an average adult
with 1.73 m2 of body surface area. In Brazil, ANVISA recommends a
dose of 2.5–5.0 mL of a 10% tincture, up to three times a day (BRASIL,
2011), corresponding to a dose of 6–36 mg/kg/day of the powdered
roots, considering an adult with 1.73 m2 of body surface area. We opted
to use an approximated dose of 30 mg/kg/day. Therefore, patients received either *C. longa* powdered roots or placebo (approximately 30 mg/kg/day, twice daily) for 6 months, as follows: 7–10 years-old, 500 mg/day; 11–14 years-old, 750 mg/day; and 15–18 years-old, 1000 mg/day. These doses yielded approximately 20, 30, and 40 mg/kg/day of curcuminoids, respectively. There were no protocol deviations.

**1.4.1. Clinical assessment**

Clinical and demographical data were collected from medical records and interviews. All patients were categorized for asthma severity and asthma control (primary outcome) according to GINA-2016 (Global Initiative for Asthma, 2016). They also underwent a pulmonary function test (spirometry) for determination of forced expiratory volume in 1 s (FEV1).

All data were collected in standardized paper forms and then typed into REDCap (Research Electronic Data Capture, https://projectredcap.org) forms. Our instance of REDCap is hosted in a cloud server provided by USP (InterNuvem, https://internuvem.usp.br) as infrastructure-as-a-service (IaaS). Briefly, REDCap allows safe, regulation-compliant storage of research data (Harris et al., 2009). Paper forms were stored in a locked room at the hospital and were destroyed.

**1.5. Statistical analysis**

There are no methods for computing sample sizes for multinomial outcomes. In a simpler approach, assuming a binary outcome (disease control yes/no) and considering the maximum difference between these proportions at baseline and after 6 months, if 20% of patients have a well-controlled disease and one wishes to take this proportion to 50%, with 80% power and 5% significance, a total of 17 patients per group would need to be included. We opted to augment this number and planned to enroll 48 patients (24 per group).

The primary outcome was disease control, according to GINA-2016 (Global Initiative for Asthma, 2016): frequency of respiratory symptoms, nighttime awakenings, and use of SAβAA for symptom control, and interference with normal activity. The secondary outcome was FEV1.

The clinical outcomes and determination of FEV1 were assessed by a single researcher, blinded to allocation, who was trained by specialists on asthma. Randomization was done by another researcher, who did not assess outcomes.

Results were expressed as means ± standard deviations, medians (ranges), or counts (proportions). Comparisons between the two treatment groups were made with Fisher’s exact test, Student’s *t*-test, or Mann-Whitney’s *U* test, as appropriate, using softwares Stata SE 14.2 (StataCorp, College Station, TX) and Prism 6.0 (GraphPad, LaJolla, CA). A significance level of 5% was adopted. Interim analyses were not planned. All analyses were per-protocol.

**2. Results**

Phytochemical analysis showed that the powdered roots of *C. longa* contained 45.31 ± 3.1 mg/g of curcumin and 6.94 ± 0.4 mg/g of demethoxycurcumin (Table 1 and Fig. 1).

Between February 2013 and November 2015, 55 patients met inclusion criteria and were randomized. Of these, 21 were excluded (38%), because of loss to follow-up, hindering a total of 34 patients completing the study, 17 in each group (Fig. 2). Their baseline characteristics are shown in Table 2. Patients that received *C. longa* were lighter, shorter, and leaner than those receiving placebo. However, z-scores for height-for-age and BMI-for-age were not different between
There was no difference in asthma severity between the two groups.

All patients were on nasal plus inhaled corticosteroids (fluticasone, mometasone, budesonide or beclometasone). Oral anti-histamines (loratadine, desloratadine, cetirizine, or levocetirizine) were used by 28 patients. Inhaled LABA (formoterol) was used by 26 patients, and SAβAA (salmeterol) was used by 42 patients. Another two patients used oral leukotriene inhibitors and two patients used oral corticosteroid (prednisolone). There were no significant differences on treatment regimens between the two groups.

Overall, both groups experienced amelioration of their frequency of symptoms (Fig. 3A) and interference with normal activity (Fig. 3C), but no differences were found between the groups. However, despite also ameliorating in the placebo group, patients receiving C. longa experienced less frequent nighttime awakenings (Fig. 3B), less frequent use of SAβAA (Fig. 3D), and, consequently, better disease control (Fig. 3E) after 3 and 6 months of treatment. None of the treatments affected FEV₁ after 6 months (Fig. 3F). Of note, no patients receiving C. longa reported use of SAβAA many times a day or had uncontrolled disease, conversely to the placebo group.

Only one patient, from placebo group, reported adverse symptoms (nausea), which resolved after adjusting the time of medicine intake. No other side effects were reported in any of the groups.

3. Discussion

We have shown that the powdered roots of C. longa, administered to children and adolescents with asthma, in addition to the standard treatment, compared to placebo, led to less frequent nighttime awakenings, less frequent use of SAβAA, and better disease control after 3 and 6 months.

In animal models of asthma, administration C. longa or curcumin resulted in significant anti-inflammatory and antioxidant effects (Shakeri et al., 2017; Shakeri and Boskabady, 2017), and ameliorated bronchial hyper-responsiveness (Karaman et al., 2012; Oh et al., 2011). C. longa and curcumin attenuate allergic airway inflammation and hyper-responsiveness through inhibition of cyclooxygenase 2 (COX-2), lipoxigenase (LOS), nitric oxide inducible synthase (iNOS), decreasing levels of inflammatory cytokines (Jagetia and Aggarwal, 2007; Jurenka, 2009), inhibition of NF-κB activation (Oh et al., 2011), inhibition of the via Wnt/β-catenin signaling (Yang et al., 2017), activation of nuclear factor-E2-related factor 2/haem oxygenase (HO)-1 signaling pathway (Liu et al., 2015), regulation of CD4+CD25+ (Ma et al., 2013) and inhibition of Notch1-GATA3 signaling pathway (Chong et al., 2014). There is also evidence that intranasal curcumin prevents airway inflammation, obstruction, and remodeling in acute and chronic models of asthma in animals by regulating MAPKinase activation (p38, Erk and JNK) and prostaglandin D2 release (Chauhan et al., 2014; Subhashini et al., 2016a, 2016b, 2013). Curcumin also inhibited smooth muscle cell proliferation in the airways, in vitro and in vivo, possibly by up-regulating the expression of caveolin-1 and blocking the activation of the ERK pathway (Zeng et al., 2013).

Surprisingly, despite the encouraging preclinical evidence of effectiveness, clinical studies are scarce. In a population-based survey, 2478 Chinese older adults (aged 55 years or more) were assessed for curey (in which turmeric is a major ingredient) intake, smoking status and pulmonary function. A curry intake of at least once monthly was significantly associated with better pulmonary function in multivariable analysis controlling for gender, age, anthropometric measures, smoking status, occupational exposure, history of asthma/chronic obstructive pulmonary disease and intake of other supplements. The effect was more pronounced among smokers (Ng et al., 2012).

In a preliminary clinical study, 15 adult patients with stable, persistent asthma with evidence of allergic sensitization were randomized to receive curcumin (1000 mg, twice daily, n = 9) or placebo (n = 6), for 6 months. The treatment did not affect post-bronchodilator FEV₁ (primary endpoint), nor asthma control test scores, rescue use of bronchodilators, doses of corticosteroids, exhaled nitric oxide (NO), serum IgE, or blood counts of leukocytes or eosinophils (Kim et al., 2011). In another, bigger study, 77 patients with asthma were enrolled in a randomized clinical trial to receive curcumin (500 mg, twice daily, for 30 days) or nothing, in addition to standard treatment. Treatment with curcumin resulted in higher FEV₁ measurements, without any adverse events (Abidi et al., 2014).

Curcumin was also studied for the treatment of allergic rhinitis, in which 241 patients with perennial allergic rhinitis were enrolled in a randomized, double-blind clinical trial that compared the effect of oral curcumin or placebo, for 2 months, on nasal symptoms and nasal airflow resistance, and on production of cytokines. Patients receiving...
Curcumin experienced less nasal symptoms (sneezing and rhinorrhea) and lower nasal airflow resistance, and lower concentrations of interleukins (IL)-4, IL-8, tumor necrosis factor alpha (TNF-α), and higher concentrations of IL-10 and intercellular adhesion molecule (ICAM) (Wu and Xiao, 2016).

Curcumin is a very promising drug, since it has minimal toxicity, and no adverse effects were reported with doses up to 12 g/day (Bisht and Maitra, 2009; Jagetia and Aggarwal, 2007; Patil et al., 2009; Wongcharoen and Phrommintikul, 2009). Many products sold over-the-counter advertised as curcumin contain, in fact, a mixture of curcuminoids (Patil et al., 2009). There have been some concerns regarding its bioavailability (Jurenka, 2009), but it can be increased up to 20-fold with concomitant administration of piperine (from black pepper, *Piper nigrum* L.). Interestingly, the best bioavailability of curcumin was...
obtained when mixed with other compounds found in the roots of C. longa (Kiefer, 2007).

The major limitation of our study was the high rate of patients lost to follow up (21/55 patients, 38%). The dropouts were similar between the two treatment groups, and excluded patients were not different from those retained. We did not assess the reasons for dropping out.

Another limitation is that we used a low dose of C. longa roots. One can speculate that, should a larger dose be used, differences on other aspects could be observed. Finally, our results should be generalized with caution to asthmatic patients in other settings since our population consisted mostly of patients with moderate and severe asthma.

4. Conclusion

In conclusion, the powdered roots of C. longa, administered to children and adolescents with moderate and severe asthma, in addition to the standard treatment, compared to placebo, led to less frequent nighttime awakenings, less frequent use of SABAAs, and better disease control after 3 and 6 months.

Authors’ contributions

• GM participated in study design, data collection and patient assessment.

• DA participated in data collection and patient assessment.

• JMS participated in study design, data collection and patient assessment.

• JSC participated in chemical analysis of the powdered plant material.

• PRJ participated in study design and writing and reviewing the final manuscript.

• AMSP participated in study design, preparation of the powdered plant material, chemical analysis of the powdered plant material and writing and revising the final manuscript.

• FC participated in study design, ethics, randomization, data analysis and writing and revising the final manuscript.

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