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ORIGINAL ARTICLE

Curcuma longa extract reduces inflammatory and oxidative stress biomarkers in osteoarthritis of knee: a four-month, double-blind, randomized, placebo-controlled trial

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Abstract
Background and purpose Curcuma longa L. (CL), an Indian herb, has been used to treat many disorders because of its wide spectrum of pharmacological activities. It has been shown to exhibit anti-oxidant and anti-inflammatory properties, and is being used as herbal remedy since ancient times. Osteoarthritis of knee (KOA) is a chronic painful disorder in which prolong use of non-steroidal anti-inflammatory drugs (NSAIDs) or steroids may result into many serious side effects; hence, there is a need to develop herbal drugs, having good analgesia without side effects. Therefore, we planned to evaluate the efficacy of CL in KOA.
Methods The study was designed as a randomized, double-blind, placebo-controlled trial in patients of KOA. After obtaining ethical clearance and written informed consent, a total of 160 patients of KOA were randomly enrolled into two groups to receive either CL extract or placebo along with the standard drug regimen. The patients were assessed on day 0, day 60, and day 120. On the days of their visit, the clinical prognosis was assessed by visual analog scale (VAS) and Western Ontario and McMaster Universities (WOMAC) Osteoarthritis index. On these days, the radiographs were also taken for Kellgren and Lawrence grading and blood samples were collected for assessing the changes in levels of IL-1β and biomarkers of oxidative stress, such as reactive oxygen species and malondialdehyde (MDA).
Results Over all significant improvement was observed in the patients of CL extract group as compared to placebo group. Clinically, the VAS and WOMAC scores became better, and simultaneously, the levels of biomarkers, viz., IL-1β, ROS, and MDA, were also significantly ($p < 0.05$) improved.
Conclusion It may be concluded that on chronic administration, CL suppresses inflammation and brings clinical improvement in patients of KOA, which may be observed by decreased level of IL-1β and VAS/WOMAC scores, respectively. At the same time, CL decreases the oxidative stress also.

Keywords Osteoarthritis of knee · *Curcuma longa* L. · WOMAC · Inflammatory biomarkers

Introduction

Osteoarthritis (OA) is a progressively debilitating, inflammatory disorder of the synovial joints. It is characterized by degradation of extracellular matrix macromolecules and decreased expression of chondrocyte protein, resulting in damage of cartilage, severe joint pain and restriction of movements (Goldring Goldring and Otero 2011). Among the joints of the body, the knee is most commonly involved and the incidence of osteoarthritis of knee (KOA) has been found to be increasing during last three decades (Ambrosia 2005; Das and Farooqi 2008; Allen et al. 2010). Moreover, the age of development of the disease has reduced in recent times (Bhatia et al. 2013). Several comorbidity factors have been

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associated with KOA including but not limited to previous injury, knee-bending occupations (Esser and Bailey 2011).

Therapy for KOA includes non-steroidal anti-inflammatory drugs (NSAIDs)/steroids, exercise, physiotherapy, weight relieving braces, and total knee arthroplasty (Barron and Rubin 2007; Wang et al. 2004). Modern drugs mainly relieve the symptom pain, while the damaging inflammatory process not significantly affected (Bjordal et al. 2004). NSAIDs are one of the most commonly used medications for the treatment of KOA. However, prolonged use of NSAIDs presents side effects on the kidneys and gastrointestinal system. Similarly, therapy with steroids has its known drawbacks and side effects. Furthermore, it has also been observed that in the course of progress of KOA, once the joint structures are damaged, the repair becomes increasingly difficult. Therefore, preventive strategies at an earlier stage are considered to be the best treatment for KOA (Michael et al. 2010).

Hence, an application of alternative systems of medicine with no side effects for the treatment of KOA is becoming more essential. Medicinal plants are an important source of active compounds, which have little or no side effects and a very high therapeutic index (Dharmananda 1998). All over the world, these traditionally used herbal agents are being evaluated on scientific parameters and are being advocated for OA (Altman and Marcussen 2001; Madhu et al. 2013).

Curcuma longa L. (Family Zingiberaceae), commonly known as Turmeric or 'Haridra' in India, is an Asian herb. Haridra is mentioned in 'Charaka Samhita' (Indian traditional system of medicine) and is officially mentioned in the Ayurvedic Pharmacopoeia of India (Kaviratna and Sharma 1913; Ayurvedic Pharmacopoeia of India 1989). It contains a series of curcuminoids which are alkaloidal in nature, having 90 % curcumin as main active ingredient (Roth et al. 1998). *Curcuma longa* L. (CL) has a long history of use for its anti-inflammatory and anti-oxidant effects (Chandra and Gupta 1972; Sharma 1976; Selvam et al. 1995; Scartezzini and Speroni 2000). It has been suggested that curcumin acts by both direct and genomic activity on proinflammatory cytokines (Jurenka 2009). Proinflammatory (PI) cytokines are important in cell signaling and promote systemic inflammation; they are produced predominantly by activated macrophages and are involved in the upregulation of inflammatory reactions (Amico et al. 2015). IL-1β, a PI cytokine, plays a central role in the pathophysiology of cartilage damage and degradation (Ji et al. 2016). Previous studies have shown that the levels of IL-1β are raised in KOA patients compared to controls (Verma and Dalal 2013; Shahine and Elhadidi 2014).

Reactive oxygen species (ROS) are highly reactive transient chemical species or free radicals, such as nitric oxide, superoxide, and hydroxyl anions which are produced by normal cellular biochemical reactions. Production of ROS is central to the progression of many inflammatory diseases. These are produced by cells that are involved in the host-defense response and promote endothelial dysfunction by oxidation of crucial cellular signaling proteins. ROS acts both as a signaling molecule and a mediator of inflammation. When the production of ROS exceeds the capacity of the body's anti-oxidant defense, oxidative stress (OS) develops (Mittal et al. 2014); Harma and Erel 2003). These are produced in excess by abnormal metabolism of chondrocytes and by senescence of cartilage, ultimately leading to the development of KOA (Martin et al. 2004; Yudoh et al. 2005). The prime targets of the free radicals are the polyunsaturated fatty acids in cell membranes and their interaction results in lipid peroxidation (LPO) (Lopaczynski and Zeisel 2001). Lipid peroxides are unstable and decompose to form reactive carbonyl compounds such as MDA. A recent study has also shown that the formation of ROS along with MDA is increased in different grades of KOA patients in parallel to the severity of the disease (Srivastava et al. 2015). Thus, it is evident that the levels of above-mentioned biomarkers are altered in patients of KOA. Hence, the study was planned with two aims:

Primarily, to observe the effect of CL extract on clinical improvement in patients of KOA as assessed by VAS and WOMAC.
Secondarily, to correlate between clinical improvement and biomarkers of oxidative stress and inflammation.

Methods

Trial design

The study was designed as a single centre, two-arm double-blind, randomized, placebo-controlled parallel group clinical trial conducted at King George's Medical University, Lucknow, India. The effect of CL extract was compared with placebo in patients with primary KOA according to the CONSORT statement for reporting RCTs (Moher et al. 2010).

The study was approved by the Institutional Ethics Committee, King George's Medical University (Ref. code:57 E.C.M. IIB/P12) and registered in Clinical Trial Registry of India (CTRI/2015/12/006438). All procedures performed in the study were in accordance with the ethical standards of the institutional ethics committee and with the 1964 Helsinki declaration and its later amendments.

Patient selection and eligibility criteria

Study was carried out on the patients who reported in the out patients' Department of Orthopaedics in King George's

Medical University for OA of the knee joint. The criteria for establishing a patient suffering from KOA were according to the guidelines proposed/issued by 'The American College of Rheumatology' (Altman et al. 1991).

Inclusion criteria

The patients within the age group of 40–80 years of both sexes, who were suffering from primary KOA according to the above-mentioned eligibility criteria who accepted to participate, were included in the study.

Exclusion criteria

The patients less than 40 years and more than 80 years of age and those suffering from rheumatoid arthritis, diabetes mellitus, renal insufficiency, hepatic disease, cardiovascular disease, gout, pregnant women or with any other systematic disease were excluded from the study.

Radiographic OA of the knee was classified according to the Kellgren–Lawrence (KL) grading scale (Kellgren and Lawrence 1957). This scale involves the following grades:

Grade 1—Doubtful narrowing of the joint space and possible osteophytic lipping.
Grade 2—Definite osteophytes and possible narrowing of the joint space.
Grade 3—Moderate multiple osteophytes, definite narrowing of the joint space, some sclerosis and possible deformity of the bone contour.
Grade 4—Large osteophytes with marked narrowing of the joint space, severe sclerosis and definite deformity of the bone contour.

Study products

The extract from rhizomes of CL, developed and registered as 'Haridra' by Himalaya Drug Company Bangalore, India was used in this trial.

The formulation/manufacturing of the Turmeric A (CL extract) capsules (Batch no. 1210001FD) and Turmeric B/Placebo capsules (Talc, batch no. 1210002FD) was carried out as per the principles of current Good Manufacturing Practices (cGMP) and quality parameters as per the pharmacopeial standards.

Each capsule composed of CL extract, which was standardized and extracted using organic solvent and then evaporated at low pressure to obtain a semisolid containing curcuminoids. The extract contained equal to or more than 95 % of total curcuminoids. The standardized CL extract was blended with rhizome powder uniformly and was processed by wet granulation method. The wet granules formed were dried in fluidized bed dryer and sizing was done to obtain uniform size of granules and the same was filled in vegetable hydroxypropyl methyl cellulose (HPMC) capsule shells. The final capsules were filled into the high density polyethylene (HDPE) containers, which served as primary packing.

The high-performance thin layer chromatography (HPTLC) finger print analysis was done; chromatogram of standard curcumin and CL extract are shown in Figs. 1 and 2a, b, respectively. Peak ratio of curcumin: demethoxy curcumin: bisdemethoxycurcumin was 0.39:0.20:0.06.

Study protocol

After obtaining ethical clearance and written informed consent from all individual participants, a total of 160 patients who were suffering from KOA were enrolled in the study.

Randomization

Eligible patients were enrolled on 'first come first serve' basis and were assigned into a treatment group, i.e., CL extract or placebo based on a computerized randomization schedule. After recruitment into the study, every patient was randomly allocated to receive either CL extract 500 mg or placebo 500 mg capsules which were to be taken along with the standard treatment of Diclofenac 50 mg/day as and when required for 4 months.

78 patients took CL extract along with Diclofenac twice a day and 82 patients took placebo capsules along with Diclofenac twice a day for 4 months. Both CL extract and placebo were procured in similar looking capsules from 'The Himalaya Drug Company', Bangalore, India.

Patients in both the treatment groups were evaluated clinically, radiologically and for biochemical changes as well as

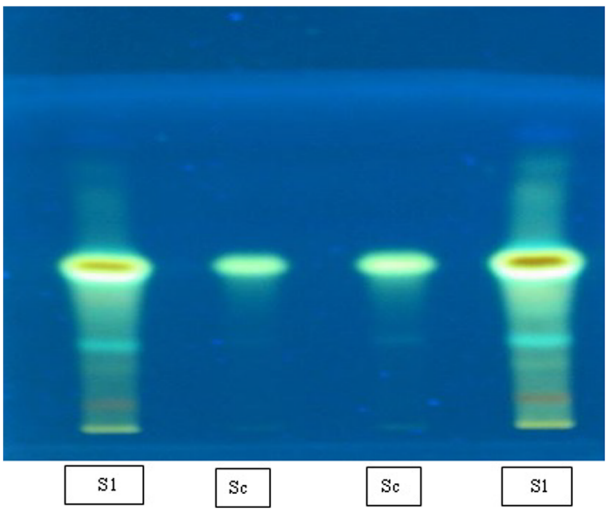


Fig. 1 HPTLC chromatogram of standard curcumin (Sc) and *Curcuma longa* extract (S1) showing the presence of curcumin

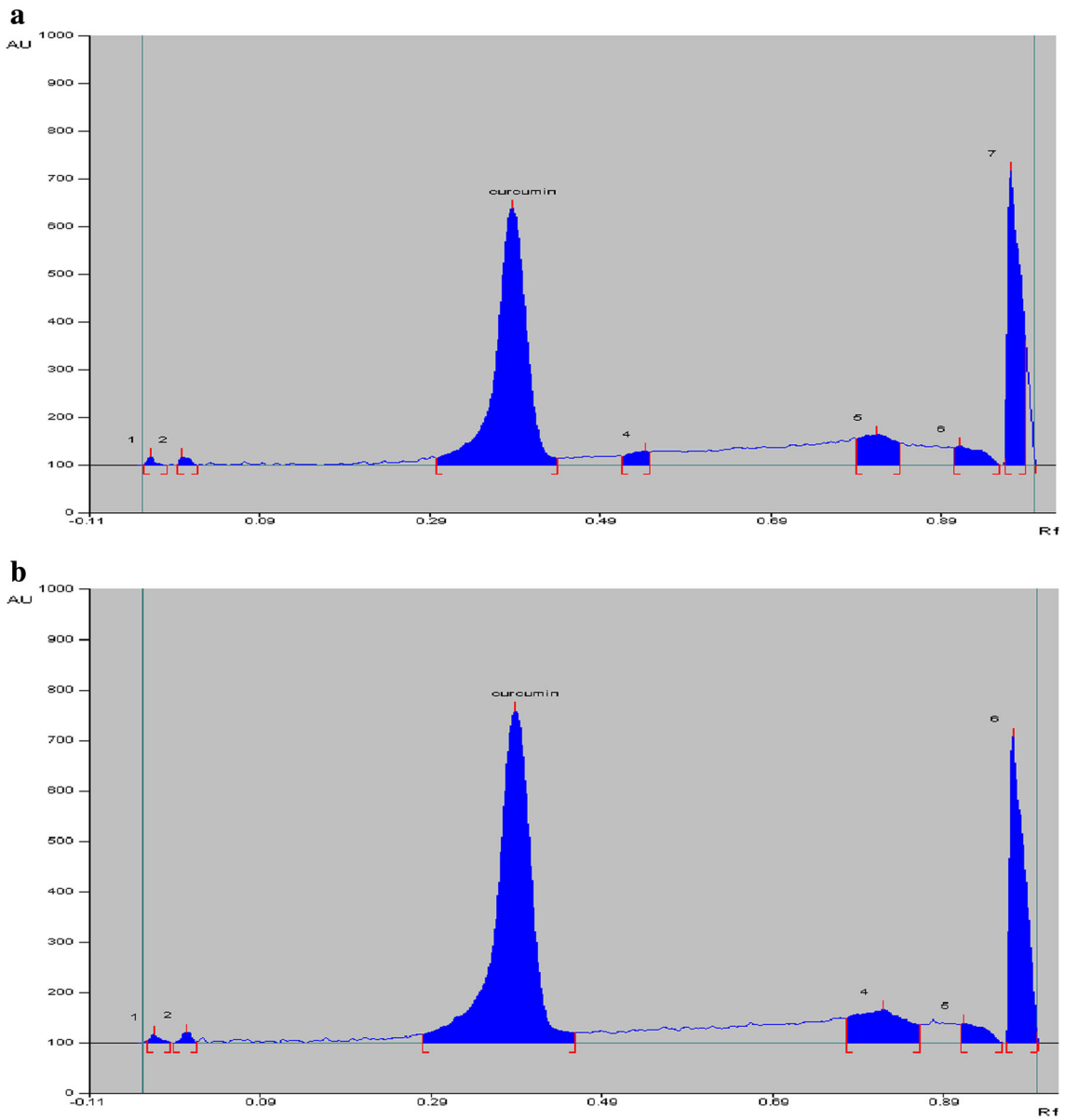


Fig. 2 **a** HPTLC chromatogram showing the peak of standard curcumin. **b** HPTLC chromatogram of CL extract (sample) showing the peak of curcumin

clinical outcomes on day 0, after 2 months and after fourth months. Blood samples along with the radiographs of the knee joints were also obtained from the patients on each three visits, for biochemical estimations and clinical prognosis status.

The dose of CL extract was decided based on the previous clinical studies, wherein CL showed significant symptomatic relief in KOA patients (Madhu et al. 2013; Pinsornsak and Niempoog 2012).

Sample size estimation

Based on the results of an RCT conducted in the past (Belcaro et al. 2010), we excepted to achieve 37 % reduction (effect size: D) of CL extract in WOMAC score in north Indian KOA patients in a 4 month trial in a double-blind manner. To detect this, 1 SD (standard deviation), 5 % margin of error ($\alpha = 0.05$), and 90 % power ($1 - \beta = 0.90$) are required to get the sample size of 80 subjects per group. Hence, a total of 160 patients were proposed to be enrolled in study.

Outcome measures

All efficacy assessment parameters were evaluated for each visit. The primary assessment parameters were visual analog scale (VAS) and Western Ontario and McMaster

Universities Osteoarthritis index (WOMAC) scores improvement level at day 0, 60, and 120, respectively. The secondary assessment parameters were the levels of the biomarkers', such as IL-1β, ROS, and MDA at day 0, 60, and 120, respectively.

Measurement of knee pain by VAS

The severity of pain was measured on VAS. It is a 10-cm horizontal line which contains word descriptions at each end, ranging from "0 to 10" ("0" indicating "no pain" and "10" indicating "unbearable pain"). Pain was assessed by the individual patient themselves by marking "no pain, mild pain, moderate pain, and severe pain" on the pain chart on each visit (Burckhardt and Jones 2003).

WOMAC score

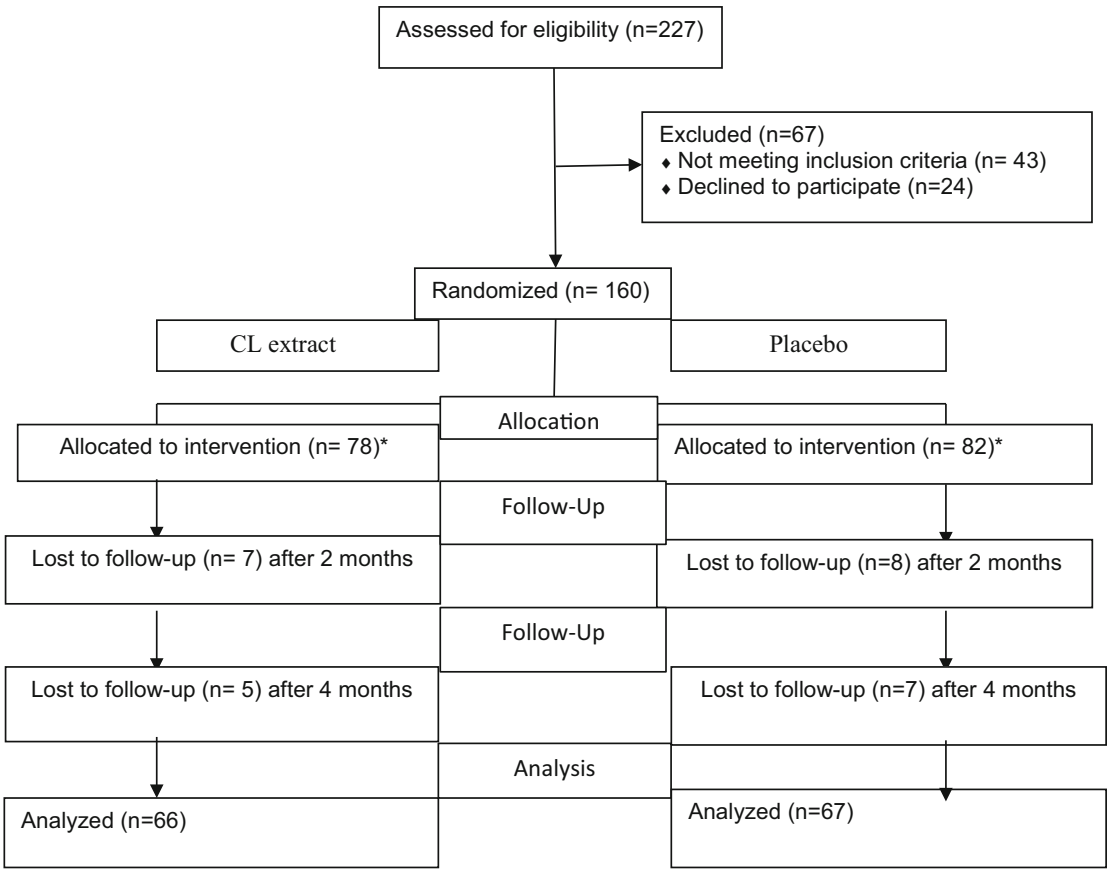
The functional status of KOA patients was evaluated using the WOMAC scores (Likert Version-3.0) (Bellamy et al. 1988). The index consists of three subscales: pain, stiffness and physical function (PF). A higher score on the WOMAC scale represents poorer function or greater pain; the score is directly proportional to the severity of disease. WOMAC was used for functional assessment with 24 questions (Q) to grade: pain (Q1–5), stiffness (Q6–7), and physical functional difficulty (Q8–24). The patient's response was graded qualitatively (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = extreme) with a maximum score of 96.

Laboratory investigations

For biochemical estimations, blood samples were allowed to clot and then centrifuged at 3000 rpm for 30 min to get serum which was stored at −80 °C and analyzed within 1 month. Furthermore, serum analysis was done to measure the levels of MDA and IL-1β.

Chemicals and reagents

2′,7′-dichlorofluorescein diacetate, Histopaque-1077 solution and phosphate-buffered saline (Sigma-Aldrich, St. Louis, MO, USA), thiobarbituric acid (Loba Chemei, India), glacial acetic acid, and trichloroacetic acid (Biobasic, India) and standard curcumin (CDH Pvt, Ltd. New Delhi, India).



*Intention to treat (ITT)

Table 1 Demographic characteristics of the patients

	CL extract ($n = 78$)	Placebo ($n = 82$)	p value
Age in years	50.23 ± 8.08	50.27 ± 8.63	0.97[a]
Gender, no. (%)			
Male	25 (32.1)	32 (39.0)	0.35[b]
Female	53 (67.9)	50 (61.0)	
KL grade			
1	7 (9.0)	4 (4.9)	
2	11 (14.1)	19 (23.2)	0.37[b]
3	32 (41.0)	34 (41.5)	
4	28 (35.9)	25 (30.5)	
BMI (kg/m^2)	28.32 ± 5.06	27.40 ± 5.76	0.28[a]

[a] Unpaired t test

[b] Chi-square test

Biochemical estimations

Estimation of intracellular ROS

2′,7′-dichlorofluorescein diacetate is a stable non-fluorescent, cell permeable compound, which on penetrating the cell is converted to $DCFH_2$ by intracellular esterases which is trapped within the cell and is stable for a few hours. The de-esterified product on oxidation by ROS is converted to the highly fluorescent 2′,7′dichlorofluorescein (DCF) and upon excitation at 488 nm emits green fluorescence which is proportional to the intracellular level of ROS. Thus, change in DCF fluorescence reflects mainly the intracellular accumulation of ROS (Boldyrev 2000).

Briefly, 1 ml of heparinized blood was carefully layered over 1-ml density gradient histopaque-1077 solution (Sigma-Aldrich), and then, it was centrifuged for 30 min at 300 $\times g$. The interface band of peripheral blood mononuclear cells (PBMCs) containing lymphocytes was isolated and washed with phosphate-buffered saline (PBS) and centrifuged at 3000 rpm. The supernatant was discarded, and the PBMCs so obtained were incubated with (10 μM) 2′,7′dichlorofluorescein diacetate (DCF-DA) dye for 30 min at 37 °C in dark.

A minimum of 10,000 events were acquired and lymphocyte population was focused on an ASSIST calibrated Image Stream X Mark II flow cytometer (AMNIS Corporation, Seattle, USA). RAW image files (rif) were acquired and adjusted for spectral overlap using IDEAS analysis software (AMNIS v.6.1.602).

Evaluation of serum MDA

The quantitative measurement of LPO in the terms of MDA equivalent was measured using TBARS assay according to the modified method (Ohkawa et al. 1979; Wade and van Rij 1988).

200 µl of trichloroacetic acid (TCA) (25-g TCA in 10 ml distilled water) was added to 1 ml of serum. The mixture was centrifuged at 1000 $\times g$ for 10 min and the precipitate was reacted with 1 ml of 0.67 % TBA (w/v). The samples were heated at 90 °C for 30 min. After centrifugation, the absorption of MDA-TBA chromogen was measured at 532 nm on UV-spectrophotometer; using 1,1,3,3-tetramethoxy propane as standard. The results are expressed as nmol/ml using molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Estimation of serum IL-1β

The quantitative estimation of serum IL-1β was done using a commercially available Enzyme Linked Immunosorbent Assay (ELISA) kit, according to the manufacturer's protocol (Diaclone, Human serum ELISA Kit) with sensitivity of <5 pg/ml.

Statistical analysis

The results are presented as mean ± SE. Chi-square test was used to compare the categorical variables, and an unpaired t test was used to compare the discrete variables between the groups. Paired t test was used to compare the mean change in discrete variables from day 0 to day 60, day 60 to day 120, and day 0 to day 120. Study analysis was adjusted for the covariables, such as age, gender, and BMI. A p value <0.05 was considered to be significant. All the analysis was carried on SPSS 16.0 version (Chicago, Inc., USA).

Results

The demographic characteristics of the patients are given in Table 1, which shows that the mean age of the patients was 50.27 ± 8.63 for CL extract and 50.23 ± 8.08 for placebo group. In this study, number of female patients was 53 and 50 for CL extract and placebo group, respectively, while male patients were 25 and 32 for CL extract and placebo group, respectively. Radiographs of the knee of enrolled patients were taken, and the patients were graded according to KL grading scale revealing severity of the disease. On the basis of KL grade; KL-I = 7 and 4 patients, KL-II comprised of 11 and 19 patients, KL-III = 32 and 34 patients and KL-IV = 28 and 25 patients were there in group A and group B, respectively. The mean BMI of the patients was 28.32 ± 5.06 for test group and 27.40 ± 5.76 for placebo group patients.

Table 2 Effect of treatment on VAS and WOMAC scores between the groups

Parameters	Group A ($n = 78$)	Group B ($n = 82$)	p value[a]
VAS score			
Day 0	7.94 ± 0.13	7.66 ± 0.14	0.15
Day 60	4.96 ± 0.07	6.00 ± 0.11	0.0001*
Day 120	4.03 ± 0.08	5.11 ± 0.14	0.0001*
WOMAC score			
Pain			
Day 0	15.10 ± 0.31	15.29 ± 0.26	0.64
Day 60	11.19 ± 0.26	12.05 ± 0.21	0.01*
Day 120	9.48 ± 0.17	10.16 ± 0.16	0.06*
Stiffness			
Day 0	5.55 ± 0.21	5.31 ± 0.12	0.53
Day 60	4.51 ± 0.21	4.70 ± 0.23	0.54
Day 120	4.08 ± 0.17	4.16 ± 0.18	0.73
PF			
Day 0	54.03 ± 0.68	50.99 ± 0.68	0.008
Day 60	41.28 ± 0.51	45.11 ± 0.37	0.0001**
Day 120	32.14 ± 0.40	33.88 ± 0.50	0.008*

* Significant ($p < 0.05$), ** ($p < 0.0001$)

[a] Unpaired t test

Table 3 Effect of treatment on biochemical parameters between the study groups

Parameters	CL extract ($n = 78$)	Placebo ($n = 82$)	p value[a]
IL-1β (pg/ml)			
Day 0	126.4 ± 19.94	131.5 ± 19.79	0.85
Day 60	65.61 ± 21.59	74.83 ± 22.31	0.76
Day 120	21.11 ± 1.176	35.82 ± 7.53	0.55
ROS (MFI)			
Day 0	3798 ± 1507.77	2584 ± 671.74	0.001*
Day 60	2553 ± 775.67	2144 ± 1275.97	0.001*
Day 120	1200 ± 864.08	2197 ± 1378.90	0.0001*
MDA (nmol/ml)			
Day 0	5.03 ± 0.16	5.15 ± 0.14	0.57
Day 60	3.85 ± 0.12	5.00 ± 0.11	0.0001*
Day 120	3.69 ± 0.12	4.91 ± 0.11	0.0001*

* Significant ($p < 0.05$)

[a] Unpaired t test

Tables 2 and 5 and Fig. 4 show the primary outcome parameters of the study, i.e., VAS and WOMAC scores. The levels of VAS (4.96 ± 0.07 and 4.03 ± 0.08) and WOMAC score which included variables like pain (11.19 ± 0.26 and 9.48 ± 0.17), stiffness (4.51 ± 0.21 and 4.08 ± 0.17) and PF (41.28 ± 0.51 and 32.14 ± 0.40) were also significantly ($p < 0.05$) reduced in the test drug group than placebo group at day 60 and 120, when compared with baseline values. Moreover, the secondary outcome parameters, i.e., biochemical analysis of CL extract and placebo group patients are shown in Tables 3 and 6 and Figs. 3 and 4. There was significant ($p < 0.05$) reduction in the levels of IL-1β (65.61 ± 21.59 and 21.11 ± 1.176), ROS (2553 ± 775.67 and 1200 ± 864.08), and MDA (3.85 ± 0.12 and 3.69 ± 0.12) in the test drug group at day 60 and day 120, respectively, when compared with baseline values. The effect of CL extract and placebo treatment on clinical parameters is shown in Table 4. Adverse effects in CL extract group were 2 in 78 patients and in placebo group were 4 in 82 patients as detailed in Table 7.

Discussion

The study was envisaged primarily to evaluate the efficacy of CL extract in patients of KOA. Simultaneously, a correlation between severity of the disease and the levels of IL-1β, ROS, and MDA (biomarkers of oxidative stress) was investigated. Thus, this double-blind, randomized, placebo-controlled clinical trial was designed.

The incidence of OA is increasing, where the global prevalence of KOA has become 3.8 % among population between age 50–80 years; incidence being higher in females than in males (Cross et al. 2014). As the knee is the most affected (41 %) synovial joint (Cushnaghan and Dieppe 1991), the maximum research has been carried out on KOA; hence, we also targeted KOA in our study, more so due to easy availability of the patients.

In our study, we found that there was overall clinical improvement by CL in all three parameters of the WOMAC score (Pain, Stiffness, and PF) and VAS in the patients of KOA as compared to the placebo treated patients. This finding is in concurrence with the findings of many other workers (Belcaro et al. 2010; Kuptniratsaikul et al. 2014). Moreover, apart from clinical improvement, there was a decrease in disease-related biomarkers; the biomarker of inflammation (IL-1β) and OS (ROS and MDA) showed significant reduction. Various workers have worked with CL on KOA, but they have not estimated such biomarkers (Pinsornsak and Niempoog 2012; Madhu et al. 2013).

The clinical improvement in WOMAC score and VAS may be subjective, but the levels of biomarkers are certain parameters depicting status of anti-inflammatory activity and oxidative stress. It has been established that there is a rise in these markers in various diseases and lowering of these markers strongly correlates with the disease level (Mateen et al. 2016; Attur et al. 2015).

We evaluated the effect of CL extract on biochemical parameters, viz., IL-1β, ROS, and MDA and found that there was a significant reduction in the levels of these biomarkers in the CL extract group in all four grades of

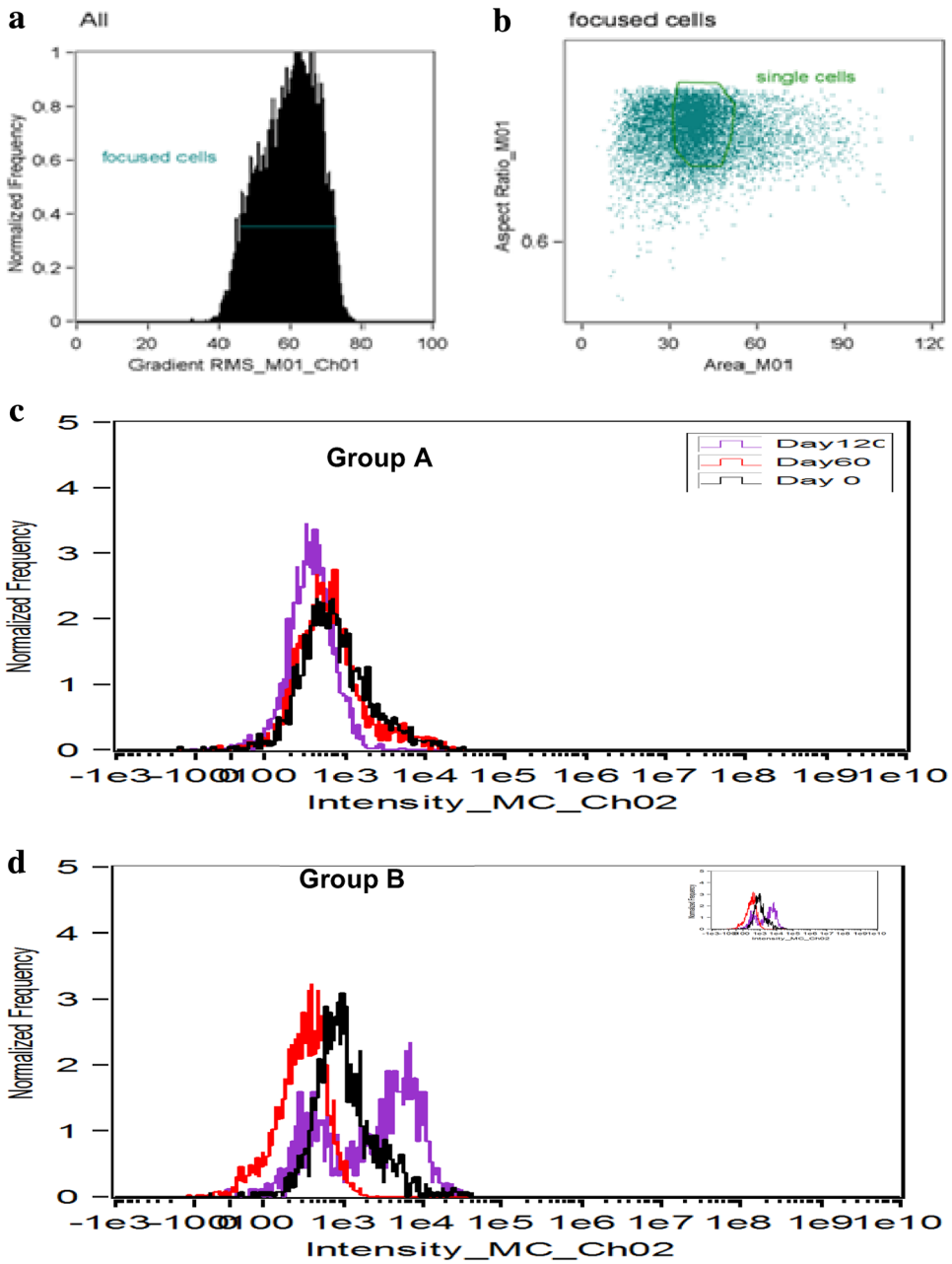


Fig. 3 Measurement of intracellular ROS production **a** and **b** are the gating strategies for focused cells and single cell population (lymphocyte), respectively. **c** and **d** the quantitative estimation of intracellular ROS formation in the two treatment groups, and its subsequent level at day 0, 60, and 120, respectively

patients as compared to patients who received placebo. OS is related to inflammation (Martin et al. 2004); hence, it can be concluded that CL not only improves clinical parameters but also reduces OS and inflammatory processes related with KOA.

Madhu et al. (2013) have used curcuminoids free CL extract in a dose of 1000 mg/day, while Belcaro et al. (2010) have used a complex of curcumin in dose of 200 mg. It is claimed by the later workers that the complex increases bioavailability of curcumin, while the adverse events if any are decreased to a great extent. Madhu et al. (2013) have not estimated any biomarker in their study, while Belcaro et al. (2010) evaluated IL-1β and found reduction in elevated levels as has been observed in our study also. Paracetamol was given as rescue medicine, by Madhu et al. (2013); it is not clear whether given regularly or on SOS basis. In the study conducted by Belcaro et al. (2010), one group of patients was given *'Best possible treatment'* and other group received *'Best possible treatment'* plus the complex of curcumin (Meriva). However,

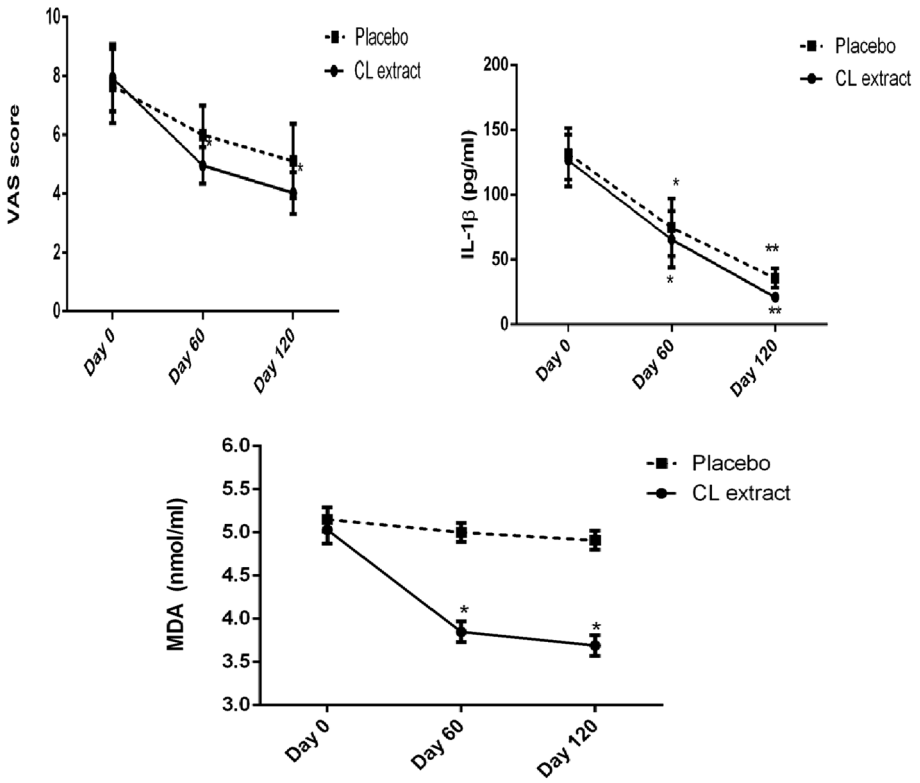


Fig. 4 Effect of treatment on levels of VAS, IL-1β and MDA at day0, day 60, and day 120

both sets of workers have noted that the need for rescue medication was decreased. During our study a fixed regimen of diclofenac 50 mg BD and omeprazole 20 mg once day was given. As far as AE are concerned, very few AE were reported by the patients and these too were quite mild and of benign in nature. Other workers have also reported mild AEs in their studies (Kuptniratsaikul et al. 2014; Madhu et al. 2013).

The biomarkers were found to be significantly raised in the subjects of all four grades in our study on day 0, i.e., at the time of commencement of the study. Similarly, other scientists have found raised levels of ROS in KOA (Srivastava et al. 2015) and in other disorders also (Amico et al. 2015; Sarkar et al. 2005) and the level of MDA has also been reported to be elevated by some workers in OA and other inflammatory disorders (Paliwal et al. 2012).

Our study showed that the treatment with CL brought a decrease in baseline values of IL-1β, ROS, and MDA after 2 months' treatment. This decrease was further reduced as the treatment was continued up to 4 months. Therefore, this study shows that CL is able to decrease biomarkers of inflammation.

KL grading scale is an established radiological method of grading the KOA patients according to radiological findings

Table 4 Clinical assessment parameters among treatment groups expressed as number (*n*) of patients and percentages (%)

Parameters	CL extract ($n = 78$)	Placebo ($n = 82$)	p value[1]
Presence of joint crepitation			
Day 0	30 (100)	35(100)	
Day 60	15 (50)	32 (91.4)	
Day 120	12 (40)	28 (80)	
Number (% reduction)	18 (60)	7 (20)	0.001*
Presence of joint stiffness			
Day 0	25 (100)	22 (100)	
Day 60	15 (60)	19 (86.3)	
Day 120	09 (36)	15 (68.1)	
Number and % reduction	16 (64)	7 (31.8)	0.027*
Joint effusion			
Day 0	29 (100)	27 (100)	
Day 60	16 (72.7)	21 (77.7)	
Day 120	07 (24.1)	17 (63)	
Number and % reduction	22 (75.8)	10 (37)	0.003*

* Significant ($p < 0.01$)

Table 5 Comparison of mean change from day 0 to day 60 and day 120 in VAS and WOMAC scores

	CL extract ($n = 78$)		Placebo ($n = 82$)	
	Mean difference	p value	Mean difference	p value[a]
VAS score				
Day 0 to day 60	2.97 ± 0.12	0.0001*	1.65 ± 0.15	0.0001*
Day 0 to day 120	3.91 ± 0.14	0.0001*	2.54 ± 0.21	0.0001*
Day 60 to day 120	0.93 ± 0.11	0.001*	0.89 ± 0.18	0.0001*
WOMAC score				
Pain				
Day 0 to day 60	3.91 ± 0.34	0.0001*	3.24 ± 0.33	0.0001*
Day 0 to day 120	5.61 ± 0.34	0.0001*	5.13 ± 0.33	0.0001*
Day 60 to day 120	1.70 ± 0.34	0.001*	1.89 ± 0.33	0.0001*
Stiffness				
Day 0 to day 60	1.00 ± 0.18	0.0001*	0.36 ± 0.17	0.03*
Day 0 to day 120	1.32 ± 0.21	0.0001*	0.36 ± 0.17	0.001*
Day 60 to day 120	0.32 ± 0.17	0.04*	0.36 ± 0.17	0.03*
PF				
Day 0 to day 60	12.74 ± 0.70	0.0001*	5.87 ± 0.99	0.0001*
Day 0 to day 120	21.88 ± 0.78	0.0001*	17.11 ± 1.0	0.0001*
Day 60 to day 120	9.14 ± 0.55	0.0001*	11.23 ± 0.58	0.0001*

* Significant

[a] Paired t test

Table 6 Comparison of mean change from Day 0 to Day 60 and Day 120 in biochemical parameters

	CL extract ($n = 78$)		Placebo ($n = 82$)	
	Mean difference	p value	Mean difference	p value[a]
IL-1β (pg/ml)				
Day 0 to day 60	60.81 ± 28.40	0.035*	56.64 ± 27.30	0.041*
Day 0 to day 120	44.5 ± 21.70	0.043*	39.01 ± 23.51	0.101
Day 60 to day 120	105.30 ± 20	0.0001**	95.65 ± 19.68	0.0001**
ROS (MFI)				
Day 0 to day 60	1245 ± 732	0.001*	440 ± 604	0.01*
Day 0 to day 120	2598 ± 643	0.0001**	401 ± 707	0.01*
Day 60 to day 120	1353 ± 89	0.001*	53 ± 103	0.13
MDA (nmol/ml)				
Day 0 to day 60	1.17 ± 0.10	0.0001*	0.15 ± 0.08	0.08
Day 0 to day 120	1.34 ± 0.11	0.0001*	0.24 ± 0.09	0.01*
Day 60 to day 120	0.16 ± 0.04	0.001*	0.09 ± 0.33	0.01*

* Significant ($p < 0.05$), ** ($p < 0.001$)

[a] Paired t test

Table 7 Adverse effects (AEs) reported in the two treatment groups

AEs	CL extract ($n = 78$)	Placebo ($n = 82$)
Dyspepsia	1	2
Nausea/vomiting	1	1
Constipation	0	1
Total no. of patients with AEs	2	4

of the joint. In this study, it was used to grade the patients at the beginning and twice during study period. It was observed that the radiographic appearance of the joints was not improved even after 4 months treatment; however, significant symptomatic relief was observed after 2 months of treatment as assessed by VAS and WOMAC scores. This relief was further significantly increased after 4 months of treatment. This explains that although radiographically there was no change in the appearance of damaged joint, however, as there was arrest of inflammatory process and OS, the patients were relieved from pain and inflammation.

Conclusion

This study showed that adjuvant therapy of CL extract along with Diclofenac produces overall significant improvement in patients of KOA. It also improves the quality of life in patients and can be taken as herbal supplement. Thus, it is proposed that in the patients of KOA, CL should be given as soon as the diagnosis is made. Any NASID, if required may be given for a short period but CL may be prescribed for long durations, without fear of damaging GIT or kidneys of patient.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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REVIEW

Efficacy of Turmeric Extracts and Curcumin for Alleviating the Symptoms of Joint Arthritis: A Systematic Review and Meta-Analysis of Randomized Clinical Trials

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ABSTRACT Although turmeric and its curcumin-enriched extracts have been used for treating arthritis, no systematic review and meta-analysis of randomized clinical trials (RCTs) have been conducted to evaluate the strength of the research. We systemically evaluated all RCTs of turmeric extracts and curcumin for treating arthritis symptoms to elucidate the efficacy of curcuma for alleviating the symptoms of arthritis. Literature searches were conducted using 12 electronic databases, including PubMed, Embase, Cochrane Library, Korean databases, Chinese medical databases, and Indian scientific database. Search terms used were ''turmeric,'' ''curcuma,'' ''curcumin,'' ''arthritis,'' and ''osteoarthritis.'' A pain visual analogue score (PVAS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) were used for the major outcomes of arthritis. Initial searches yielded 29 articles, of which 8 met specific selection criteria. Three among the included RCTs reported reduction of PVAS (mean difference: −2.04 [−2.85, −1.24]) with turmeric/curcumin in comparison with placebo ($P < .00001$), whereas meta-analysis of four studies showed a decrease of WOMAC with turmeric/curcumin treatment (mean difference: −15.36 [−26.9, −3.77]; $P = .009$). Furthermore, there was no significant mean difference in PVAS between turmeric/curcumin and pain medicine in meta-analysis of five studies. Eight RCTs included in the review exhibited low to moderate risk of bias. There was no publication bias in the meta-analysis. In conclusion, these RCTs provide scientific evidence that supports the efficacy of turmeric extract (about 1000 mg/day of curcumin) in the treatment of arthritis. However, the total number of RCTs included in the analysis, the total sample size, and the methodological quality of the primary studies were not sufficient to draw definitive conclusions. Thus, more rigorous and larger studies are needed to confirm the therapeutic efficacy of turmeric for arthritis.

KEYWORDS: • ***arthritis*** • ***curcuma*** • ***osteoarthritis*** • ***pain visual analogue score*** • ***systematic review*** • ***turmeric***

INTRODUCTION

THE TERM ARTHRITIS is derived from the Greek words ''artho'' and ''itis,'' meaning joint and inflammation, respectively. Arthritis is a form of joint disorder characterized by chronic inflammation in one or more joints that usually results in pain and is often disabling.[1,2] Arthritis includes more than 100 different forms: the most common form is osteoarthritis, but other forms include rheumatoid arthritis, psoriatic arthritis, and related autoimmune diseases.[1,2] Although the causes of these diseases are different, their symptoms and treatments are similar. As osteoarthritis is a degenerative joint disease, the number of people with arthritis is also growing with the increase in the aging population.[1] The worldwide prevalence of knee osteoarthritis increased 26.6% from 1990 to 2010, and it affects about 9.6% of men and 18% of women more than 60 years of age.[3] The occurrence of osteoarthritis increases with age due to the decreased capacity to suppress inflammation, age-related sarcopenia, and increased bone turnover.[1] Rheumatoid arthritis is a systemic inflammatory and destructive joint disease with a prevalence of about 1–2% of the adult population worldwide.[2]

Although arthritis is associated with inflammation and pain, the exact cause of arthritis remains uncertain, and there is no treatment for its fundamental causes. The major goal of arthritis treatment is to reduce joint pain induced by inflammation in the joints, daily wear and tear of joints, and muscle strains.[4] The existing pharmaceuticals for treating arthritis are analgesics, steroids, and nonsteroidal anti-inflammatory drugs (NSAIDs), which reduce the symptoms such as severe pain and inflammation.[5] Classical NSAIDs are cyclooxygenase (COX) inhibitors that inhibit prostaglandin and thromboxane synthesis, thereby reducing inflammation.[5]

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New NSAIDs selectively inhibit COX-2 and are usually specific to inflamed tissue, which decreases the risk of peptic ulcer.[5] However, their long-term use cannot be sustained due to inadequate pain relief, immune disturbances, and serious gastrointestinal and cardiovascular adverse events.[6] Therefore, herbal therapies with anti-inflammatory properties and minimum side effects are needed for the treatment of arthritis, including rheumatoid arthritis and osteoarthritis, especially after the withdrawal of many Food and Drug Administration-approved anti-inflammatory drugs.[7]

Curcuma longa and *Zingiber officinale*, both of which belong to the Ziangiberaceae family, are potential alternative medicines for arthritis.[8,9] They have been used as seasonings in many ethnic cuisines in various countries such as Bangladesh, India, and Pakistan. They have long been used as anti-inflammatory treatments in traditional Chinese and Ayurvedic medicines.[10] The effective components of *Z. officinale*: gingerols, shogaols, zingerone, and paradol, and ginger itself have been reported to exert anti-inflammatory effects by inhibiting COX-1 and COX-2, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and 5-lipoxygenase (5-LOX).[11] Several systematic reviews of clinical trials have shown that ginger may reduce the subjective experience of pain in some conditions such as muscular diseases.[12] In addition, turmeric extracts have activities similar to ginger although they have different effective compounds. Several studies have evaluated the efficacy of turmeric extracts for the treatment of musculoskeletal disorders.[13]

Although turmeric belongs to the Zingiberaceae family, turmeric contains different bioactive components, mainly curcumin and demethoxycurcumin, bis-demethoxycurcumin, and turmeric essential oils. When used as an alternative medicine or dietary supplement, turmeric is typically used as an extract that is standardized to 80–95% curcuminoids, primarily curcumin. Turmeric and its derivatives have anti-inflammatory activities. Unlike ginger, turmeric and curcumin do not modulate COX-1 activity,[14,15] but modify NF-κB signaling, proinflammatory cytokines such as interleukin production and phospholipase A2, COX-2, and 5-LOX activities. Curcumin also modulates the expressions of various transcription factors involved in energy metabolism such as signal transducer and activator of transcription, peroxisome proliferator-activated receptor-γ, activator protein-1, cAMP responding element binding protein, estrogen response element, and others.[15] As a result, turmeric and its components have been reported to exert beneficial effects on osteoarthritis, type 2 diabetes, and dyslipidemia. Turmeric is better tolerated than ginger and pepper due to being less hot and spicy. Therefore, it is important to conduct a systematic review of the antiarthritis effects of curcuma.

The purpose of this review was to systemically evaluate all randomized clinical trials (RCTs) of turmeric and curcumin for treating arthritis symptoms and to elucidate the efficacy of curcuma for alleviating the symptoms of arthritis. To the best of our knowledge, this is the first systematic review and meta-analysis of RCTs on the efficacy of turmeric for arthritis symptoms.

METHODS

Data sources and selection criteria

The following electronic databases were searched: PubMed, Embase, Cochrane Library, Korean databases such as DBpia, the Research Information Service System (RISS), the Korean Information Service System (KISS), Chinese medical databases such as China National Knowledge Infrastructure (CNKI) and the Chinese Scientific Journals Database, the Indian Medical Journals and the Indian Journals. Dissertations were also included. The search was conducted in the databases using proper languages of English, Korean, and Chinese. The following keywords of Medical Sub Headings (MeSH) were used as search terms: ''curcumin,'' ''curcuma,'' ''turmeric,'' ''*Curcuma domestica*,'' ''*Curcuma Longa*,'' ''arthritis,'' ''osteoarthritis,'' ''randomized,'' ''controlled trial,'' and ''clinical trial.'' In the systematic review, all RCTs were included from the available databases (as far back as 1966 in PubMed) up to April, 2016, that had examined the effects of turmeric (Curcuma) and curcumin on arthritis.

Article evaluation and selection

Two independent reviewers (J.W.D. and M.Y.) screened the articles. In the first screening, the related articles were identified by the titles and abstracts of the articles and the relevant articles were retrieved in full text and validated for inclusion in the systematic review. The third reviewer (S.P.) independently validated the selected articles.

Eligibility criteria for studies used in this review

All prospective randomized clinical studies using turmeric (*C. longa* and the synonym *domestica*) and curcumin for the treatment of arthritis were included in this systematic review. Exclusion criteria included *in vitro* studies, *in vivo* studies in nonhuman species, studies that were only published in abstract form or included insufficient data to properly evaluate the outcomes, nonclinical trial studies, and studies in which arthritis was not the primary outcome measured, and then we eliminated the duplicates. A flow diagram of the article selection process is shown in Figure 1. Although no language barriers were imposed, all studies included in this review were written in English. Dissertations about randomized clinical studies were also included.

Subjects and intervention

Subjects included in the studies were mostly middle-age and elderly men and women, although the ages varied among the studies. Subjects were recruited with degenerative primary knee osteoarthritis[16–23] and rheumatoid arthritis[24] with mild-to-moderate severity according to the American Rheumatism Association criteria. Subjects in most studies had pain scores ≥5 of 10 on the numerical rating scale.[25] Inclusion criteria are summarized by study in

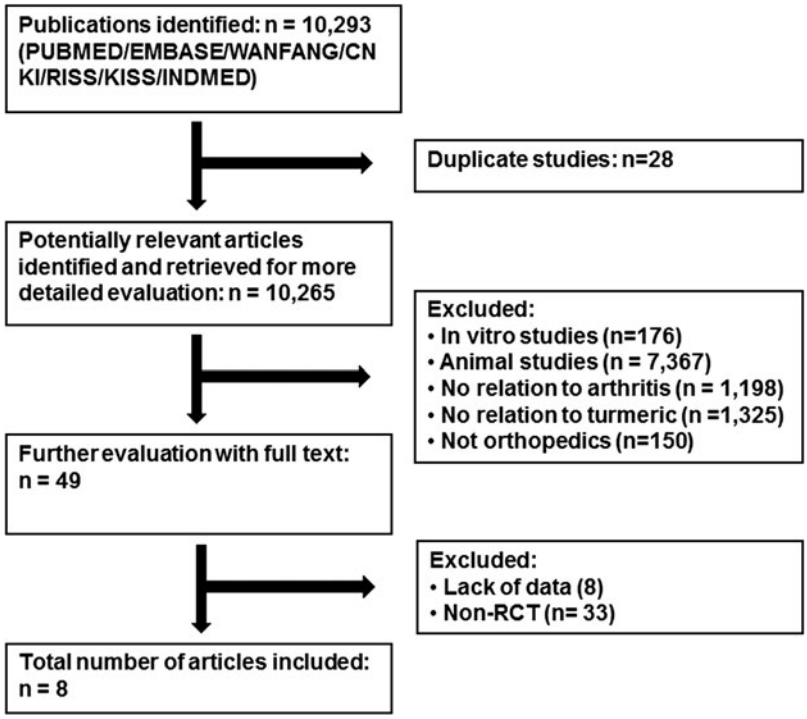

FIG. 1. Flowchart of the selection process of the randomized clinical trials for systematic review.

Table 1. Two studies did not include exclusion criteria.[20,21] Exclusion criteria of subjects were somewhat different among the other studies but four studies excluded the patients who had conditions that would prevent the use of treatment protocols such as abnormal liver or renal function, history of peptic ulcer, allergy to curcumin, curcuminoids, or other drugs used in the study such as ibuprofen, diclofenac sodium, and glucosamine.[16–18,24] Several studies excluded subjects with conditions that would interfere with the outcome assessments or act as confounders in the study, including secondary osteoarthritis, candidates for surgical joint replacement or any other surgical treatment, presence of heart, renal, and liver failure, using corticosteroids with doses more than 10 mg/day during the preceding 3 months, history of psychological disorders, and intraarticular injections during the preceding 3 months.[17,19,22–24] Other exclusions included pregnancy or lactation,[19,23,24] body mass index >25,[19,23] severe bone or joint deformity,[23] history of infections resulting in hospitalization,[24] recent use of anti-inflammatory drugs, or abuse of drugs or alcohol.[24] The subjects were instructed not to use any medications or herbs other than those provided by the study during the experimental periods.

The studies included in the systematic review utilized turmeric extracts and its components, mostly curcuminoids, and in one study polysaccarides, which were considered as the primary effective component for osteoarthritis. Ethanolic extracts of turmeric were used in two studies,[16,18] and water extracts rich in polysaccharides were used in one study.[22] In the remaining five studies,[17,19–21,24] curcumin or curcumin-containing mixture was considered as treatments (Table 1).

Outcome measures

Osteoarthritis and rheumatoid arthritis have similar symptoms such as pain, tenderness, swelling, and stiffness. The severity of arthritis was determined by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[26] and pain visual analogue score (PVAS)[27] for pain. WOMAC is a standardized index for the assessment of the severity of osteoarthritis symptoms. PVAS is a tool widely used to measure pain. The validity and reliability of WOMAC and PVAS have been established.[26] WOMAC consisted of subclasses 5 items for pain, 2 items for stiffness, and 17 items for physical functioning. Each item was rated from 0 to 4 and the total scoring for pain, stiffness, and physical function was calculated by adding each item for the categories. However, one study[22] measured only five items for pain. Subjects answered a perception of pain intensity on a 10 cm horizontal scale in the PVAS assessment and the severity was represented by the scores of 0–10. In WOMAC and PVAS, a lower score indicated less severe symptoms. WOMAC for pain and PVAS were used as outcome measures for meta-analysis.

Other outcome measures used in the studies were to evaluate function of joints and stiffness when pain was determined by PVAS. In addition, pain associated with daily tasks such as running and walking upstairs,[16,21,24] clinician global impression of change,[22] and Lequesne's pain functional index (LPFI)[17] was measured.

Quality assessment of the articles

The Cochrane tool was used to assess quality of the RCT articles included in this systematic review by determining

Table 1. Summaries of Subjects' Inclusion Criteria and Intervention Strategies

First author (year, country, reference)	*Study design* / *Gender* (n)[a]	*Number and characteristics of patients/diseases*	*Inclusion criteria*	*Preparation of turmeric extracts and curcumin (powder)*	*Experimental intervention (type, dose, and duration)*	*Control intervention (type, dose, and duration)*	*Treatment groups* (n)[b]
Kuptniratsaikul (2009, Thailand, 16)	RCT with two parallel groups M ($n=21$) & F ($n=86$)	107 subjects age 50+ (CR 61.4 ± 8.7 IB 60.0 ± 8.4 years) OA	1. Age >50 years 2. Morning stiffness 3. Crepitus with motion 4. Pain score of >5 of 10	Ethanolic extract of turmeric and powdering (mainly curcuminoids)	*Curcuma domestica* extract capsules: 250 mg curcuminoids 4×/day 6 weeks	Ibuprofen: 400 mg/tablet twice daily 6 weeks	1.CR ($n=45$) 2. IB ($n=46$)
Chandran (2012, India & USA, 24)	RCT with three parallel groups M ($n=7$) and F ($n=38$)	45 subjects Age 18–65 (CR 47.9 ± 8.6 CR+DI 47.0 ± 16.2 DI 48.9 ± 10.8) RA	1. Age 18–65 years 2. Diagnosed with RA (functional class I or II) and disease activity score >5.1	Curcumin	Curcumin: 500 mg/capsule twice daily Curcumin 500 mg+ diclofenac sodium 50 mg/capsule twice daily 8 weeks	Diclofenac sodium 50 mg/capsule twice daily 8 weeks	1. CR ($n=15$) 2. DI ($n=15$) 3. CR+DI ($n=15$)
Pinsornsak (2012, Thailand, 21)	RCT with two parallel groups M ($n=13$) and F ($n=62$)	107 Subjects Age 38–80 (years, 45–54: $n=16$ 55–64: $n=24$ 65–74: $n=30$ More than 75: $n=5$) OA	1. More than 38 years of age 2. <30 minutes of joint morning stiffness 3. Crepitus on active motion 4. Bony tenderness 5. Bony enlargement 6. No palpable warmth of synovium	Curcumin powder	Diclofenac 25 and 250 mg curcumin Twice daily 3 months	Diclofenac 25 and 250 mg placebo Twice daily 3 months	1. CR+DI ($n=44$) 2. DI ($n=44$)
Madhu (2013, India, 22)	RCT with four parallel groups M ($n=37$) and F ($n=83$)	120 subjects Age 40+ (TR 56.6 ± 10.6 TR+GS 58.2 ± 9.3 PL 56.8 ± 10.0 GS 56.8 ± 8.0 years) OA	1. Age 40+ 2. Diagnosis of knee OA 3. Duration of pain was at least 6 months 4. Radiological evidence of OA grades 2 and 3	Polar extract of turmeric rich in polysaccharides and powdering (Turmacin™)	Turmeric extract: 500 mg/capsule twice daily Turmeric extract 500 mg+GS 375 mg/capsule twice daily 42 days	Placebo (cellulose) 500 mg/capsule twice daily GS 375 mg/capsule twice daily 42 days	1. PL ($n=29$) 2. TR ($n=29$) 3. GS ($n=28$) 4. TR+GS ($n=24$)

(*continued*)

Table 1. (Continued)

First author (year, country, reference)	*Study design* / *Gender (n)*[a]	*Number and characteristics of patients/diseases*	*Inclusion criteria*	*Preparation of turmeric extracts and curcumin (powder)*	*Experimental intervention (type, dose, and duration)*	*Control intervention (type, dose, and duration)*	*Treatment groups (n)*[b]
Belcaro (2014, Italy, 19)	RCT with two parallel groups M ($n=61$) and F ($n=63$)	124 subjects Age: no age limits (CR ± GS 56.6 ± 4.7 CN+GS 55.8 ± 5.8 years) OA	1. To perform the treadmill walking test 2. To understand the WOMAC questionnaire	Meriva: natural curcuminoid mixture (20%), phosphatidylcholine (40%), and microcrystalline cellulose (40%). Curcuminoid mixture contains curcumin (75%), demethoxycur-cumin (15%), and bisdemethoxycurcumin (10%)	Meriva 500 mg+ Regenasure (glucosamin) 500 mg Each 1 capsule/day 4 months	Chondroitin sulfate 400 mg+ glucosamine 500 mg 1 capsule/day 4 months	1. CR+GS ($n=63$) 2. GS ($n=61$)
Panahi (2014, IRAN, 17)	RCT with two parallel groups M ($n=9$) and F ($n=31$)	53 subjects Age less than 80 years (CR 57.3 ± 8.8 PL 59.1 ± 17.3 years) OA	1. Age less than 80 years 2. Degenerative primary knee OA 3. Bilateral OA	Curcuminoids (C3®complex) with Bioperine pepper extract	C3®complex 500 mg Bioperine 5 mg 3 ×/day 6 weeks	Placebo Inert starch three/day 6 weeks	1. CR ($n=21$) 2. PL ($n=19$)
Nakagawa (2014, Japan, 20)	RCT with two parallel groups M ($n=9$) and F ($n=41$)	50 subjects Age 40+ (CR 71.9 ± 5.3 PL 66.1 ± 7.2) OA	1. Age 40+ 2. Knee OA 3. Kellgren–Lawrence grades of II or III upon radiographic classification	Water dispersible powder of curcumin	Theracurmin (180 mg/day, curcuminoids) 6 capsules/day 8 weeks	Placebo (starch, dextrin, and maltose) 6 capsules/day 8 weeks	1. CR ($n=18$) 2. PL ($n=23$)
Kuptniratsaikul (2014, Thailand, 18)	RCT with two parallel groups M ($n=35$) and F ($n=296$)	331 subjects Age ≥50 (CR 60.3 ± 6.8 IB 60.9 ± 6.9) OA	1. More than 50 years of age 2. Had primary OA 3. Pains scale ≥5 out of 10	Ethanolic extract of turmeric and powdering: curcuminoids (75–85%)	Curcumin extracts 1500 mg/day 4 weeks	Ibuprofen 1200 mg/day 4 weeks	1. CR ($n=171$) 2. IB ($n=160$)

CN, chondroitin; CR, curcumin; DI, diclofenac sodium; GS, glucosamine; IB, ibuprofen; OA, osteoarthritis; PL, placebo; RA, rheumatoid arthritis; RCTs, randomized clinical trials; TR, turmeric extract; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

[a]The number of subjects to be recruited initially.

[b]The number of subjects who had participated until the end of the study.

the risk of bias (ROB).[28] This validated tool consists of the following eight categories: (1) random sequence generation, (2) allocation concealment, (3) blinding of participants, (4) assessor blinding, (5) reporting drop out or withdrawal, (6) intention to treat, (7) selective outcome reporting, and (8) other potential bias. Each category was scored as H, high ROB, U, uncertain ROB, or L, low ROB. Three independent reviewers (M.Y., S.P., and J.W.D.) performed the quality assessment, and disagreement on scores was resolved through discussion.

Data analysis

The data used for the meta-analysis were continuous variables of PVAS and arthritis symptoms (WOMAC for pain), and means and standard deviations were used for the meta-analysis. However, two studies[20,21] were not included in the meta-analysis since they did not provide the means and standard deviations in the articles. Standard mean differences and 95% confidence intervals (CIs) were calculated for a PVAS and WOMAC using the Cochrane Collaboration's software (RevMan Version 5.0 for Windows; The Nordic Cochrane Centre, Copenhagen, Denmark). Since the studies showed small variations in clinical heterogeneity between each study such as age, dose of curcuma, and treatment duration, the studies had low heterogeneity. However, in some meta-analysis, PVAS and WOMAC for pain were pooled together since both indexes presented the intensity of pain but their scales were different. When both indexes were pooled, random effect models were used. In addition, the heterogeneity was quantitatively confirmed with the heterogeneity analysis (Cochran's Q) of studies in meta-analysis. In the heterogeneity test, the heterogeneity of the included studies was considered to be low at $I^2 \leq 25\%$, moderate at $I^2 \leq 50\%$, high at $I^2 \leq 75\%$, and very high at $I^2 > 75\%$.[29] When I^2 value was low, the fixed effect model was selected and otherwise the random model was used. The meta-analysis was conducted with data from parallel-group design studies between curcuma and placebo treatments or pain killer treatment. Funnel plots were used to detect reporting biases for this systematic review since the number of studies included was less than 10.

Subgroup analysis

Eight studies were included in the systematic review, but all could not be included in the meta-analysis since their control groups were either placebo or pain killer, and the data formats were not matched with each other. Six studies provided means and standard deviation of PVASs,[16,17,20–22,24] whereas four studies gave those of WOMAC.[17,18,22,23] Thus, six and four studies were included in the meta-analyses of PVAS and WOMAC, respectively. The subgroup analysis could not be done for this study. Fortunately, the dosage of curcumin or turmeric was similarly assigned among the studies: it was prescribed with about 1 g/day without giving other pain killers and about 500 mg/day with pain killers. However, the experimental periods varied among the studies and ranged from 4 weeks to 4 months. It is better to conduct meta-analysis with subgroups according to short-term and long-term studies. Although the meta-analysis could not be performed according to the duration of the study, the adverse effects were reviewed with the duration of the study.

RESULTS

Summary of included studies

A total of 10,293 studies were found in the initial electronic searches from PubMed, Embase, WANFANG, CNKI, RISS, KISS, and IndMED, and 28 duplicates were removed. From the remaining 10,265 studies, those potentially not related RCT studies about curcumin and arthritis were removed and the details of the removed studies were as follows: 176 *in vitro* studies, 7367 animal experiments, 1198 no relation to arthritis, 1325 no relation to turmeric or curcumin, and 150 nonarthritis orthopedic-related studies (Fig. 1). Further evaluation of the full texts resulted in the elimination of 8 studies with lack of data and 33 studies due to non-RCT. Finally, eight RCTs met the inclusion criteria (Fig. 1). The eight RCTs were used for the systematic review.[16–18,20–24] The subject characteristics and intervention strategies of the included RCTs are summarized in Table 1 and intervention results and adverse events are organized in Table 2.

Middle-age and elderly adults of both genders were mainly included in the selected studies (Table 1). Subjects in the selected studies had arthritis with either PVAS greater than 5 or with morning stiffness lasting less than 30 min, indicating moderate symptoms of osteoarthritis (Table 1). Subjects were excluded when they had the following problems: allergy to curcuminoids and pain killers such as ibuprofen that were used as the control group, secondary arthritis, current use of any immunotherapy, including corticosteroids, liver damage, serious chronic metabolic diseases other than arthritis such as diabetes, inflammatory disorders, heart, renal, and liver failure, and severe arthritis to be a candidate for surgical joint replacement.

Six of the studies were performed in Middle East and Asian countries and two studies were conducted in the United States and Italy as given in Table 2. Six RCTs,[16–21,23] one RCT,[24] and one RCT[22] had two-arm, three-arm, and four-arm parallel design, respectively. The three-arm parallel design study included the groups of curcumin only, curcumin+diclofenac sodium, and diclofenac sodium groups, and the four-arm parallel design study consisted of curcumin only, curcumin+glucosamine, glucosamine only, and placebo. Six RCTs contained curcumin powder and placebo groups[17,20–24] and four studies used pain killers such as ibuprofen, diclofene, or glucosamine as a control group.[16,18,22,24] The dosage of curcumin was varied in different studies within 100–2000 mg/day and the curcumin or turmeric was provided one to four times a day with up to 500 mg per time. One study[22] used a polysaccharide-rich extract that contained no curcumin (Table 2).

Table 2. Summaries of Intervention Results and Adverse Events

First author (year), reference	*Intervention dose & duration*	*Main outcomes for meta-analysis*	*Treatment results*	*Control results*	*Other results*	*Author's conclusions*	*Adverse events*
Kuptniratsaikul (2009), 16	Curcumin 250 mg 4×/day 6 weeks	PVAS	CR 2.7 ± 2.5 $P = .2$	IB 3.8 ± 2.5 $P = .2$	Pain on stairs TR 3.1 ± 1.5 IB 3.8 ± 2.4	CR safe and effective, similar to IB	CR 1, IB 3
Chandran (2012), 24	Curcumin 500 mg or DI 50 mg 2×/day 8 weeks	PVAS	CR 27.5 ± 9.4 $P < .05$ CR+DI 34.3 ± 26.7, $P < .05$	DI 39.2 ± 20.1 $P < .05$	CRP CR 5.34 ± 4.12 $P < .05$ CR+DI 6.66 ± 6.87, $P > .05$ DI 3.35 ± 2.5 $P > .05$	Curcumin provide significant improvement for RA	CR mild throat fever and infection DI itching, eye swelling and dim vision
Pinsornsak (2012), 21	Curcumin 1000 mg+ diclofenac 75 mg 3 months	PVAS	CR+DI 3.19 $P < .001$ from baseline	DI+PL 3.55 $P < .001$ from baseline	Pain score CR+DI 81.99 $P < .001$ DI+PL 84.49 $P < .001$	Curcumin had additive effects with DI	Minor hair loss and renal distress
Madhu (2013), 22	Turmeric polysaccharide extract 500 mg glucosamine 375 mg 2×/day 42 days	PVAS WOMAC for pain	PVAS TR 19.5 ± 17.8 TR+GS 36.3 ± 29.0 WOMAC TR 27.1 ± 16.1 TR+GS 36.2 ± 27.7 $P < .05$ for all	PVAS PL 46.0 ± 20.8 GS 29.3 ± 20.6 WOMAC PL 47.9 ± 12.6 GS 34.9 ± 19.5	CGIC TR 2.21 ± 1.80 PL 4.72 ± 1.27 TR+GS 3.37 ± 2.41, $P < .05$ GS 3.32 ± 1.78 $P < .05$	Curcumin significantly effective for pain and reduced need for medication	N.R.
Belcaro (2014), 19	Curcumin phospholipid +glucosamine 500 mg each 1×/day 4 months	WOMAC for pain	CR+GS 6.8 ± 2.0, (compared with baseline) $P < .05$	CN+GS 10.2 ± 2.2, (compared with baseline) $P < .05$	WOMAC total index CR+GS 36.3 ± 5.0 CN+GS 64.2 ± 7.3 Karnosfki index CR+GS 93.4 ± 6.4 CN+GS 79.6 ± 6.6 $P < .05$	CR+GS more effective than CN+GS	N.R.
Panahi (2014), 17	Curcumin 500 mg+ Bioperine 5 mg 3×/day 6 weeks	WOMAC for pain PVAS	WOMAC CR 37 ± 19 $P < .001$ PVAS CR 6.1 ± 2.9 $P < .001$	WOMAC PL 57 ± 12 PVAS PL 9.4 ± 3.4	LPFI CR 7.8 ± 3.6 PL 12 ± 4 $P = .013$ WOMAC total index CR 25 ± 13 PL 40.6 ± 12.6 $P = .001$	Results support efficacy of CR for OA	CR 3, PL 4 with intestinal symptoms
Nakagawa (2014), 20	Curcumin 180 mg/day 8 weeks	PVAS decline in score	CR −0.40 as compared with baseline, compared with PL, $P = .023$	PL −0.22 as compared to baseline	No. of subjects using pain killer CR 32% PL 60% $P = .0252$	CR more effective than PL	No serious adverse events
Kuptniratsaikul (2014), 18	Curcuma extract 1125–1275 mg CR/day 4 weeks	WOMAC for pain	CR 3.17 ± 1.98 $P < .01$ (compared with baseline)	IB 3.25 ± 2.11 $P < .01$ (compared with baseline)	WOMAC total score IB 3.23 ± 1.97 CR 3.36 ± 2.04	CR equally effective as IB but fewer side effects	Both groups mild intestinal symptoms

CGIC, clinician global impression of change; Karnosfki index, Karnosfki Performance Scale index; LPFI, Lequesne's pain functional index; N.R., not reported; PVAS, pain visual analogue score.

Table 3. Risk of Bias in the Studies Included in the Systematic Review

Author (year)	Random sequence generation	Allocation concealment	Patient and practitioner blinding	Assessor blinding	Selective outcome reporting	Reporting drop out or withdrawal	Intention-to-treat analysis	Other potential bias	Reference
Kuptniratsaikul *et al.* (2009)	H	L	L	H	L	U	H	U	16
Chandran *et al.* (2012)	L	H	L	U	U	U	L	L	24
Madhu *et al.* (2013)	U	L	H	U	H	L	L	U	22
Panahi *et al.* (2014)	H	L	L	H	L	U	L	U	17
Kuptniratsaikul *et al.* (2014)	H	L	L	H	L	U	L	U	18
Belcaro *et al.* (2014)	H	H	H	H	L	L	L	U	19
Nakagawa *et al.* (2014)	L	L	L	L	U	L	L	U	20
Pinsornsalz *et al.* (2012)	L	U	H	L	U	L	U	U	21

H, high risk; L, low risk, U, uncertain.

Risk of bias

Table 3 shows the ROB assessment for the included RCTs. Among eight RCTs, four RCTs were classified as high quality[17,18,20,23] and four RCTs had a moderate quality.[16,21,22,24] Three studies used a proper method for randomization of the subjects such as coin flipping and computer-generated random numbers and the allocation of the groups was concealed to the subjects and also practitioners,[17,20,24] but the remaining studies did not describe how the subjects were randomized and allocated. Six RCTs used blinding of patients and practitioners,[16,18,20,22–24] but the remaining two RCTs did not mention their blindness to the practitioners. The drop-out rates were not high and they were not different between experimental and placebo groups. Two studies did not report the drop-out rates.[23,24]

Outcomes

The symptoms of arthritis, including osteoarthritis and rheumatoid arthritis, were mainly pain and inflammation and the symptoms were classified as pain, stiffness, swollenness, and movement. Most of the studies measured the severity of arthritis symptoms by pain, stiffness, and function, and the severity was scaled by PVAS and WOMAC. In four studies, the severity of pain was scaled by PVAS[16,20,21,24] and by WOMAC.[18,23] Two studies measured both scales.[17,22] As a result, three sets of meta-analysis were performed to compare PVAS between curcuma and placebo,[17,22,24] PVAS between curcuma and pain medicine,[16,18,22,24] and WOMAC between curcuma and placebo.[17,22,23] Since WOMAC for pain was used for meta-analysis, six results of pain index were combined for comparing between curcumin and placebo from four studies.[17,19,22,24]

Since two studies[20,21] did not provide means and standard deviation for PVAS or WOMAC at the end of the study, six studies were used for meta-analysis. Furthermore, since the studies measured either PVAS[16,24] or WOMAC[18,23] or both,[17,22] and the control group was either placebo[17,23] or pain medicine[16,18] or both,[22,24] three combinations were made for meta-analysis. Figure 2A provided the pooled results of PVASs between curcumin and placebo groups from the meta-analysis of three studies.[17,22,24] The PVAS was much lower in the curcumin group than in the placebo groups (overall mean differences and CI: −2.04 and −2.85, −1.24; $P < .00001$). In addition, the pooled PVAS and WOMAC pain score from five results for three studies[17,22,24] were much lower in the curcuma group than in the placebo group (overall mean differences and CI: −15.26 and −26.94, −3.77; $P = .009$; Fig. 2B). Figure 2C shows the pooled results of PVAS and WOMAC score between curcumin and pain medicine groups from five studies.[16,18,19,22,24] The pooled pain indexes of PVAS and WOMAC from five studies were not significantly different between the curcumin and commercially available pain medicine such as ibuprofen, diclofenac, and glucosamine (overall mean differences and CI: −1.89 and −4.13, 0.35; $P = .10$). These results suggested that curcumin (about 1 g/day) might have similar effects as analgesic medicines.

Since the symptoms of osteoarthritis were evaluated mainly by pain, the outcome measures were quantitatively determined by the severity of pain. Most studies used PVAS or pain index in WOMAC (Table 2). Some studies used different methods: frequencies of pain killer used during the study were decreased[17,20,22]; the distance to walk for 6 min, pain on level walking, pain on stars, and time spent on 100 m walk were not significantly different between the curcumin and ibuprofen groups.[16,18] In addition to pain levels, the functional changes in the joints were important factors to determine the severity of arthritis. Functional changes can be measured on the WOMAC subscales such as morning stiffness and function, LPFI, Karnosfki Performance Scale index, and Japanese Knee Osteoarthritis Measure (JKOM).

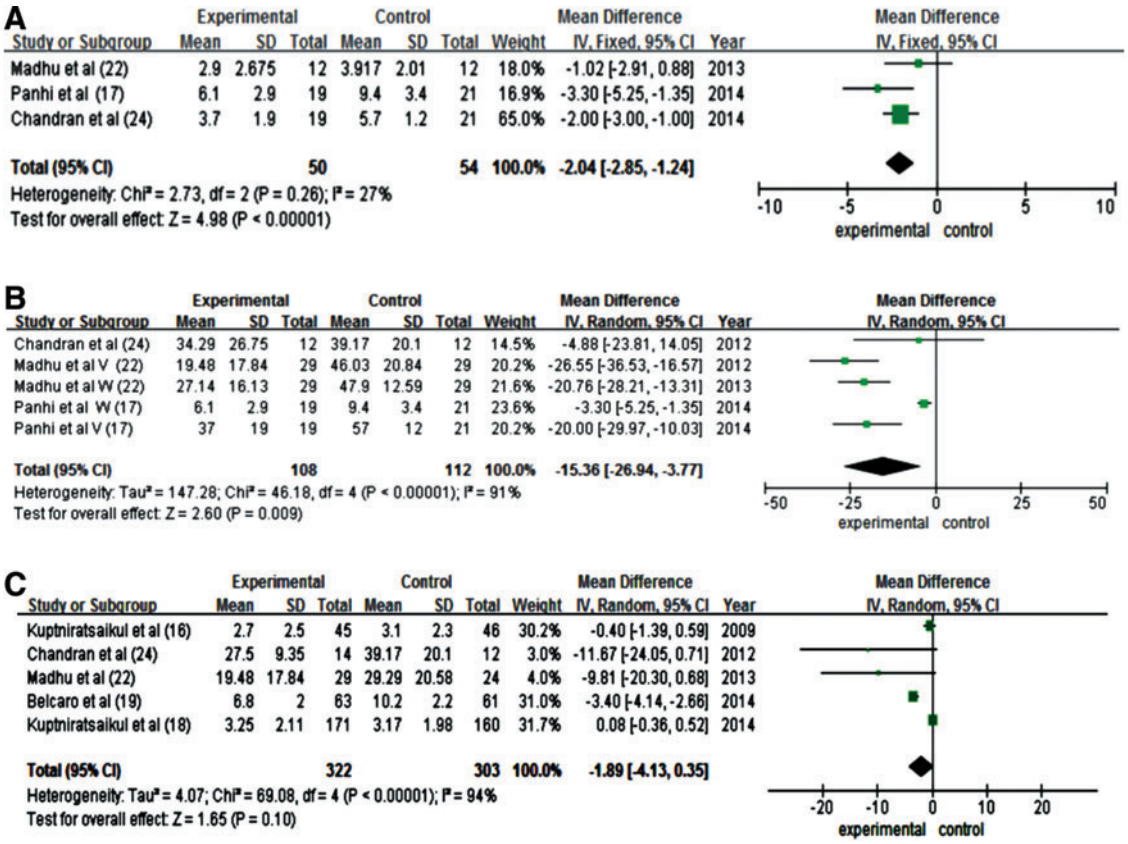

FIG. 2. Forest plot of the meta-analysis for the scores of arthritis severity. **(A)** Mean differences in PVAS between curcuma and placebo. **(B)** Mean differences in PVAS and WOMAC between curcuma and placebo. **(C)** Mean differences in PVAS and WOMAC between curcuma and pain medicine. Each study is identified by first author and year. PVAS, pain visual analogue score; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index. Color images available online at www.liebertpub.com/jmf

Total scores of WOMAC subscales were also lower in the curcumin group than in the placebo group.[17,19] LPFI was significantly reduced in the curcumin group in comparison with the placebo group,[17] whereas JKOM decreased in the curcumin group more than in the placebo group, but it was not significantly different[20] (Table 2). Karnosfki Performance Scale index was increased in the curcumin group in compared with the chondroitin group, indicating that the performance was improved with curcumin (Meriva).[19] These results suggested that curcumin improved joint function measured by morning stiffness, movements, and other clinical assessments in comparison with the placebo group. However, it did not indicate the fundamental improvement of arthritis symptoms.

Publication bias

A symmetrical funnel plot was produced by this meta-analysis (the hollow circles in Fig. 3), which indicated there was no publication bias.

Adverse events

Six RCTs[16–18,20,21,24] reported adverse effects in both the control and experimental groups. Curcumin containing herbs and curcumin showed mild fever and throat infection, gastrointestinal symptoms, hair loss, tachycardia, hypertension, and redness of tongue (Table 2). However, other control groups such as placebo and pain medicine (ibuprofen and diclofenac) also showed similar adverse effects such as gastrointestinal symptoms, itching, swelling around eyes and face, dimness of vision, unwell feeling, and renal dysfunction. Two RCTs did not report any adverse effects[22,23] (Table 2). These reports indicated that the adverse symptoms were not limited to the curcumin containing herbs and curcumin up to about 1200 mg/day. Thus, turmeric preparations and curcumin were considered to be safe at doses not exceeding 1200 mg/day for up to 4 months.

DISCUSSION

Although the exact biochemical cause of osteoarthritis remains unknown, it is associated with inflammation in articular cartilage, which can cause abnormal joint structure in the knee and hip and it is accompanied with pain. The most common treatments are analgesics and NSAIDs.[5] However, the drugs have serious adverse events in the gastrointestinal tract and cardiovascular system.[6] Therefore, herbal treatments that can mitigate the pain and inflammation have been investigated as potential primary or adjunct therapies for

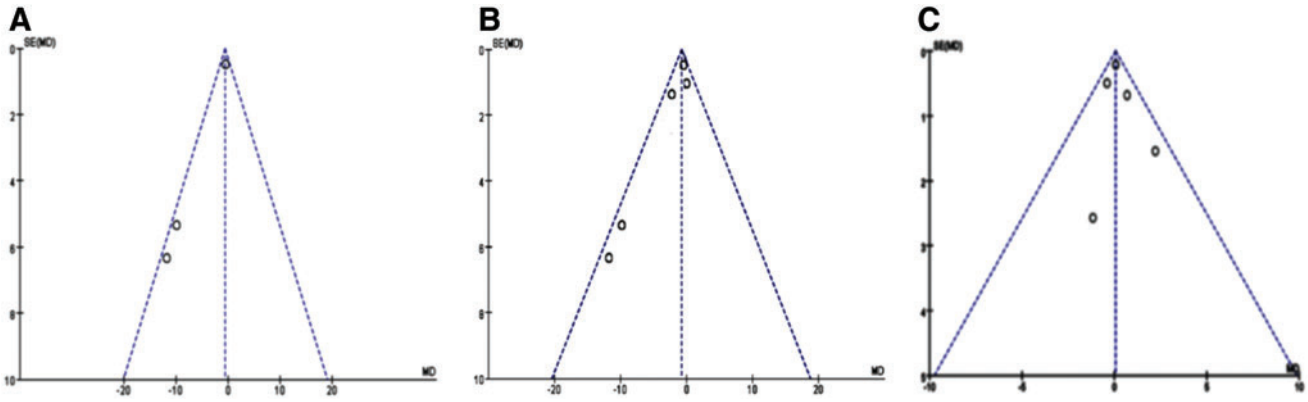

FIG. 3. Funnel plot of the meta-analysis for the scores of arthritis severity. **(A)** Three results to measure PVAS as arthritis severity between curcuma and place. **(B)** Four results to measure and PVAS and WOMAC as arthritis severity between curcuma and placebo. **(C)** Five results to measure PVAS and WOMAC as arthritis severity between curcuma and pain medicine. The *hollow circles* represent the studies in the meta-analysis. Color images available online at www.liebertpub.com/jmf

relieving arthritis symptoms. This systematic review and meta-analysis provided scientific evidence that 8–12 weeks of standardized turmeric extracts (typically 1000 mg/day of curcumin) treatment can reduce arthritis symptoms (mainly pain and inflammation-related symptoms) and result in similar improvements of the symptoms as ibuprofen and diclofenac sodium. Therefore, turmeric extracts and curcumin can be cautiously recommended for alleviating the symptoms of arthritis, especially osteoarthritis. However, the sample sizes (45–124) of the studies included in this review were insufficient to be conclusive, and some studies represented moderate quality. Further high-quality RCT studies with more subjects are needed to confirm the therapeutic efficacy of turmeric and curcumin for arthritis.

The article by Pinsornsak and Niempoog[21] was not included in the meta-analyses because its design did not permit its data to be merged with any of the other studies. That RCT was a comparison of diclofenac (75 mg/day) with or without curcumin (1000 mg/day). Both groups made significant improvements over the 3-month course of the study, but although the group that included curcumin seemed to improve more, there was no significant differences between groups. Since diclofenac is an NSAID, it is possible that its mechanisms of action are similar to those of curcumin and the redundancy of action resulted in little additional benefit. The authors also suggested that the lack of statistical significance might have been influenced by the drop-out rate of 9% due to difficulty in traveling for follow up in the rural area. They also suggested that the dose may have been too low; however, other studies included in this review found significant improvements at lower dosages. However, the design of this study did not permit a determination of the effectiveness of curcumin alone.

The study by Madhu *et al.*[22] was unique in using a turmeric extract that contained only polar substances, especially polysaccharides, and no curcumin. This study had four groups: placebo, turmeric, chondroitin sulfate, and turmeric plus chondroitin sulfate. Turmeric and chondroitin sulfate both provided significant benefits by both PVAS and WOMAC score, with turmeric performing significantly better. However, combining turmeric and chondroitin provided no added benefit, which may be due to redundant effects as already suggested for curcumin and diclofenac. The most important contribution of this study, however, may be that it demonstrated potent anti-inflammatory and/or analgesic benefits for turmeric components other than curcumin.

Osteoarthritis is exacerbated by the activation of NF-κB, which is initiated by a host of stress-related stimuli, including proinflammatory cytokines, excessive mechanical stress, and extracellular matrix degradation products.[4,30] These actions reduce the amount of articular cartilage in the joints and wear out the bones near the joints to induce pain and difficulty in movements. As a result, osteoarthritis treatment focuses on relieving pain and swelling, improving joint mobility and stiffness, increasing the strength of the joints, and minimizing the disabling effects of the disease.[31] Thus, the severity of arthritis is mostly measured by PVAS and WOMAC as symptomatic end-point results in RCTs.

The approved drugs commonly used to treat arthritis, such as NSAIDs, have adverse effects, and alternative treatments have been investigated. NSAIDs increase the risk of gastrointestinal bleeding, vascular adverse events, and allergic responses.[32] Symptomatic slow-acting drugs for osteoarthritis such as glucosamine sulfate, glucosamine hydrochloride, chondroitin sulfate, hyaluronic acid, avocado soybean unsaponifiables, and diacerein are common alternative medicines for treating osteoarthritis symptoms.[33–36] In systematic reviews, glucosamine and diacerein were found to reduce pain but did not alleviate joint space narrowing.[33,36] In addition, they also caused some gastrointestinal and metabolic disturbances, although the adverse effects were less than NSAIDs.[37] People with impaired glucose tolerance or insulin resistance are more likely to exhibit severely impaired glucose metabolism with glucosamine treatment for osteoarthritis.[33,37] Therefore, these drugs cannot be used for long-term treatment, although osteoarthritis is a chronic long-term disease. Herbal medicine

is often recommended for osteoarthritis treatment. Herbal and complementary therapies are safer to use and can be taken for longer periods, but they are also subject to widespread advertising and attractive, but unsubstantiated, claims that are often made for many natural products. Promising therapeutic agents for treating osteoarthritis can be compounds that block NF-κB signaling.[38] Several candidates for naturally occurring NF-κB inhibitors are phytochemicals such as flavonoids and catechins from green tea, rosehip, curcumin, and resveratrol.[38,39]

Turmeric (*C. longa*) has a long history of safe use as food and it has long been used as in anti-inflammatory treatment in traditional Chinese and Ayurvedic medicine.[30] Turmeric contains a yellow-pigmented fraction that mainly consists of curcuminoids. The principal ingredient of curcuminoids is curcumin, which is reported to have beneficial effects on osteoarthritis, type 2 diabetes, and dyslipidemia due to its antioxidant and anti-inflammatory activities. However, the systemic bioavailability of curcumin is known to be poor.[40] Several studies have reported that curcumin concentrations are extremely low or nonexistent in serum and tissues at 1, 2, 3, and 4 h after taking a single oral dose of 500–8000 mg in humans, and also after long-term oral administration of 440–2200 mg curcumin or curcuma extracts per day.[41–43] This is associated with the low stability of curcumin in aqueous solution at physiological pH, and within 30 min, curcumin is degraded into trans-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal, ferulic aldehyde, ferulic acid, feruloyl methane, vanillin, vanillic acid, and other dimerization end products.[44,45] The metabolites of curcumin are present in high concentrations in the circulation after curcumin consumption. These curcumin metabolites may be responsible for the anti-inflammatory and antioxidant activities that reduce the symptoms of metabolic diseases including osteoarthritis.[44,45] However, Gupta *et al.* reported that curcumin is low but detectable in the circulation as the forms of glucuronide and sulfate conjugates in the patients with oral consumption of 8 g/day curcumin for more than 2 months.[46] Thus, curcumin itself can be a therapeutic agent for relieving arthritis.

Korea Food and Drug Safety administration has declared turmeric roots as ''generally regarded as safe.'' Turmeric and curcumin have been found to be safe and tolerable in human clinical trials and systematic reviews.[47] No long-term studies with curcumin have revealed toxic or adverse effects.[48] However, some clinical studies in humans with high doses (8–12 g) of curcumin have shown a few side effects, with some subjects reporting mild nausea or diarrhea.[49] The studies used in this systematic review and meta-analyses used several types of turmeric and curcumin preparations and all appeared to provide efficacy for treating arthritis.

Recently, high doses of curcumin was found to alter iron metabolism by chelating iron and suppressing the protein hepcidin, potentially causing iron deficiency in susceptible patients.[40] However, overall, the dosage required to improve osteoarthritis was less than 2000 mg/day and this dosage did not show any noticeable adverse effects in this review. Thus, turmeric and curcumin can be safely used as a therapeutic agent for osteoarthritis.

To the best of our knowledge, this is the first systematic review and meta-analysis of RCTs on the effectiveness of turmeric extract or curcumin for arthritis. Although the present meta-analysis of RCTs suggested that oral administration of curcumin reduced arthritis symptoms, as measured by PVAS and WOMAC, as much as pain medicine, it is difficult to recommend curcumin and turmeric as a good therapeutic agent for arthritis due to the limitations of the RCT studies included in this systematic review. The limitations of the studies are as follows: first, the number of RCTs ($n=8$) and sample sizes ($n=45$–124) of the primary studies are low. RCTs had either a placebo control or pain medicine control, and they also utilized different end-point measurements such as PVAS and/or WOMAC. Thus, total sample size of each meta-analysis was low: PVAS for curcuma and placebo was 60 curcuma and 62 placebo; WOMAC score for curcuma and placebo was 308 curcuma and 291 placebo; PVAS for curcuma and pain medicine was 258 curcuma and 242 pain medicine. Furthermore, there were various turmeric preparations, some designed to increase absorption, that complicate drawing firm conclusions about the most effective preparation method and dose. However, this is also a strength of the study because it demonstrates that turmeric contains multiple functional compounds and their metabolites that have efficacy for arthritis. In addition, the RCTs included in the systematic review had overall low-to-moderate ROB. Four RCTs were classified as high quality[17,18,20,23] and four RCTs had a moderate quality.[16,21,22,24] Some studies did not report randomization of the subjects and allocation of the groups,[17,20,24] whereas two RCTs did not mention their blindness to the practitioners.[17,21] In addition, two RCTs did not report drop-out rates and reasons for withdrawals from the trials.[23,24] However, it is difficult to detect bias resulting from authors not publishing negative results that are considered uninteresting, so there is still some possibility of publication bias.

In conclusion, although the studies used in this meta-analysis do not have sufficient number of subjects to permit a definitive recommendation for the use of curcumin as a treatment for arthritis, they do provide a compelling justification for its use as a dietary adjunct to conventional therapy. Furthermore, they also provide sufficient evidence to support larger clinical trials that could eventually lead to its acceptance as a standard therapy for many forms of arthritis and possibly other inflammatory conditions.

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AUTHOR DISCLOSURE STATEMENT

James W. Daily is the President of Daily Manufacturing, Inc. that manufactures dietary supplements. The other authors have no conflicts of interest.

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Enhanced metabolic bioavailability of tetrahydrocurcumin after oral supplementation of a γ-cyclodextrin curcumin complex

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ABSTRACT

Tetrahydrocurcumin, curcumin's main metabolite, exhibits properties similar to curcumin with superior effectiveness in certain categories. However, as curcumin's bioavailabilty and *in vivo* conversion are low, formulations yielding appreciable tetrahydrocurcumin concentrations are required to convey potential health benefits either through tetrahydrocurucmin itself, complementary or synergistic to curcumin.

Here, we conducted a study with humans orally receiving γ-cyclodextrin complexed curcumin for 12 weeks daily and analyzed the metabolic bioavailability of tetrahydrocurcumin. Notably, supplementation yielded threefold higher serum tetrahydrocurcumin compared to curcumin already after 4 weeks. In fact, this is in line with previously unpublished data demonstrated here revealing an increased metabolic bioavailability (BA = 39.8) of tetrahydrocurcumin.

Foremost we show that γ-cyclodextrin enhances the oral and metabolic bioavailability of curcumin and tetrahydrocurcumin, respectively. This is likely related to an improved uptake of cyclodextrin complexed curcumin corroborated by enclosed *in vitro* studies, its metabolic turnover and the prolonged plasma half-life of tetrahydrocurcumin.

1. Introduction

Tetrahydrocurcumin (THCC) is a reduced form and main metabolite of curcumin (CC), which is the principal curcuminoid contained in the powdered rhizome of *Curcuma Longa L.* Curcumin has received great attention in traditional medicines and science due to its antioxidant, antiinflammatory, antidiabetic, antiviral, antimicrobial, anticancer, immune regulatory, cardio- and hepatoprotective as well as neuroprotective properties (Hewlings & Kalman, 2017; Xu et al., 2018). At present, several formulated curcumin products are progressively marketed as nutraceuticals for dietary supplementation claiming benefits for human health (Douglass & Clouatre, 2015; Jamwal, 2018; Stohs, Ji, Bucci, & Preuss, 2018). However, despite its biological activities, hydrophobic curcumin is practically insoluble in water limiting its bioavailability. Furthermore, it is chemically instable and prone to rapid metabolism after oral ingestion. Therefore, questions of the benefit of dietary supplementation of curcumin have been raised (Nelson et al., 2017).

The majority of orally ingested unformulated curcumin is excreted in the feces indicating a low intestinal absorption. A minor proportion is subject to metabolic conversion in the intestines and the liver as well as through gut microbiota (Fig. 1) (Dei Cas & Ghidoni, 2019; Ireson et al., 2002). Briefly, human hepatic and intestinal phase I enzymes reduce curcumin to dihydro- and tetrahydrocurcumin while phase II enzymes attach glucuronic acid and sulfates to increase the solubility of curcumin and its catabolites for excretion (Holder, Plummer, & Ryan, 1978).

Obviously, tetrahydrocurcumin is a central metabolite of curcumin (Ireson et al., 2002). With regards to its physicochemial and pharmakokinetic properties, it is more stable under physiological conditions (Pan, Huang, & Lin, 1999) and has more than twofold longer plasma half-lifes than CC (Vijaya Saradhi et al., 2010). Furthermore, some studies state that THCC is easily absorbed through the gastrointestinal

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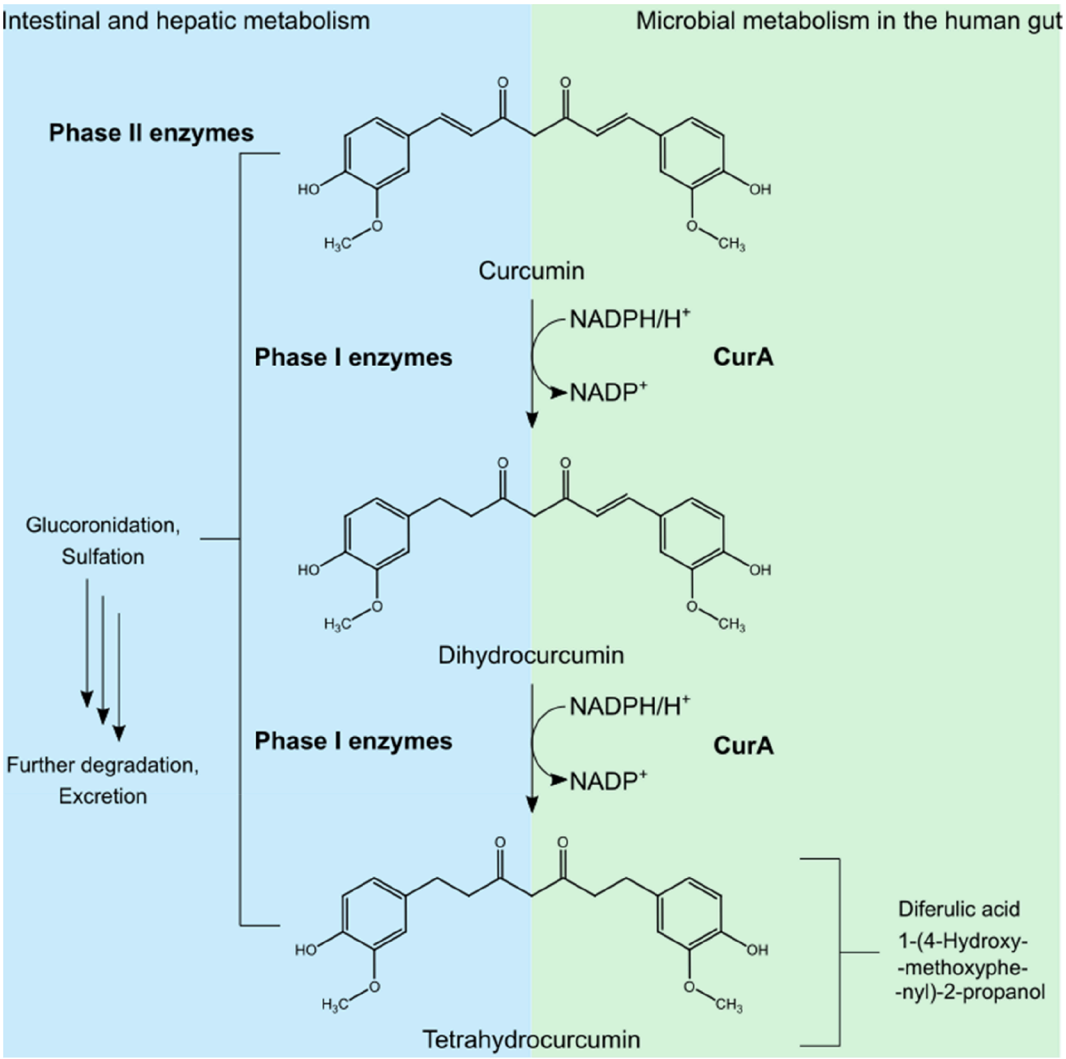

Fig. 1. Human and gut mircobial metabolism of orally ingested curcumin. A major portion of orally ingested unformulated curcumin is excreted in the feces. A minor portion of curcumin is subject to catabolism mediated by human and gut microbial enzymes. The polyphenol is mainly catabolized by intestinal and hepatic xenobiotic enzymes. Among these, phase I enzymes reduce curcumin to dihydrocurucmin and tetrahydrocurucmin in a NADPH dependent manner. In parallel, phase II enzyes attach glucuronic acid and sulfates to curcumin, dihydrocurumin and tetrahydrocurcumin, which increases their solubility and facilitates urinary excretion. In addition to the xenobiotic metabolism of humans, gut microbia such as *E. coli* express CurA, which is a specific enzyme to reduce curcumin to tetrahydrocurumin via dihydrocurcumin in an NADPH dependent manner. In addition, gut microbiota such as *E. coli* are able to further break down tetrahydrocurucmin yielding dihydroferulic acid and 1-(4-Hydroxy-3-methoxyphenyl)-2-propanol.

tract (Han, Deng, Wang, Liu, & Yang, 2016; Yodkeeree, Garbisa, & Limtrakul, 2008), which was corroborated by permeabilization studies of unformulated and formulated THCC in Caco-2 cells (Sermkaew, Wiwattanawongsa, Ketjinda, & Wiwattanapatapee, 2013).

Regarding its biological activities, THCC has been attributed inferior to equal potency (Aggarwal, Deb, & Prasad, 2014), while several comparative studies suggest mainly antioxidant and antidiabetic effects that are superior to CC. (Table 1). The potent antioxidative properties of the reduced curcuminoid THCC are likely related to its beta-diketone moiety (Sugiyama, Kawakishi, & Osawa, 1996). Cell and animal studies have shown a greater efficacy of the molecule to diminish reactive oxygen species (ROS) and oxidative damage induced by organic radicals, oxidizing agents (Somparn, Phisalaphong, Nakornchai, Unchern, & Morales, 2007; Sugiyama et al., 1996), chloroquine (Pari & Amali, 2005), $CuSO_4$ (Naito et al., 2002), iron-nitrilotriacetate (Fe-NTA) (Osawa, 2007), radiation (Khopde et al., 2000) and streptozotocin (Pari & Murugan, 2007d). From a molecular point of view, THCC appears to interplay with GSH (Kadoma & Fujisawa, 2007) and increases non-enzymatic antioxidants such as vitamin C, E and GSH (Nakmareong et al., 2011; Osawa & Kato, 2005; Pari & Amali, 2005; Pari & Murugan, 2007d). Furthermore, it potently upregulates enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), gluthatione peroxidase (GPx) and glutathione S-transferase (GST) (Murugan & Pari, 2007a; Osawa & Kato, 2005; Pari & Amali, 2005; Pari & Murugan, 2007d).

With regards to its antidiabetic effects, Pari and Murugan have shown that THCC decreases blood glucose levels (Murugan & Pari, 2007a, 2007b; Pari & Murugan, 2007a, 2007b, 2007d), plasma glycoproteins (Murugan & Pari, 2007a; Pari & Murugan, 2007b), tissue hexose, hexosamine, fucose (Pari & Murugan, 2007b), hepatic and renal markers (Murugan & Pari, 2007b) as well as collagen cross-linking (Pari & Murugan, 2007c) more efficiently than CC. In addition, THCC was further able to increase the activity of Na^+/K^+, Ca^{2+}, Mg^{2+} ATPases as well as hemoglobin (Murugan & Pari, 2007a), tissue sialic acid (Pari & Murugan, 2007b) and plasma insulin levels (Murugan & Pari, 2007a, 2007b; Murugan, Pari, & Rao, 2008; Pari & Murugan, 2007a, 2007b). Notably, THCC enhanced the insulin binding affinity of erythrocytes by increasing the number of receptor sites and the insulin binding affinity

Table 1
Efficacy of tetrahydrocurcumin superior to curcumin highlighting molecular modes of action. Arrows up and down respectively indicate reduction / enhancement or up- / downregulation.

Biological Activity	Molecular target
Antioxidative	• ↓ ROS (Khopde et al., 2000; Murugan & Pari, 2007a; Naito et al., 2002; Nakmareong et al., 2011; Osawa, 2007; Osawa & Kato, 2005; Pari & Amali, 2005; Pari & Murugan, 2007d; Somparn et al., 2007; Sugiyama et al., 1996; Suzuki et al., 2005) ↓ AAPH, DPPH induced LPO (Somparn et al., 2007) ↓ Chloroquine induced LPO (Pari & Amali, 2005) ↓ $CuSO_4$ induced LPO (Naito et al., 2002) ↓ Fe-NTA induced oxidative damage (Osawa, 2007) ↓ Radiation induced LPO (N_2O conditions) (Khopde et al., 2000) ↓ Streptozotocin induced LPO (Pari & Murugan, 2007d) ↓ TBHP induced LPO (Sugiyama et al., 1996) ↑ GSH (Nakmareong et al., 2011; Osawa & Kato, 2005; Pari & Amali, 2005; Pari & Murugan, 2007d) ↑ Non-enzymatic antioxidants (vitamin C, E) (Pari & Amali, 2005) ↑ SOD, CAT, GPx (Murugan & Pari, 2007a; Pari & Amali, 2005; Pari & Murugan, 2007d), ↑ GST (Murugan & Pari, 2007a; Osawa & Kato, 2005; Pari & Murugan, 2007d)
Antidiabetic	• ↓ Blood glucose (Murugan & Pari, 2007a, 2007b; Pari & Murugan, 2007a, 2007b, 2007d) ↓ Glycoproteins (Murugan & Pari, 2007a; Pari & Murugan, 2007b) ↑ Hemoglobin (Murugan & Pari, 2007a) ↑ Plasma insulin (Murugan & Pari, 2007a, 2007b; Murugan et al., 2008; Pari & Murugan, 2007a, 2007b) ↑ Tissue sialic acid, ↓ hexose, ↓ hexosamine, ↓ fucose (Pari & Murugan, 2007b) ↓ Collagen cross-linking (Pari & Murugan, 2007c) ↑ Cellular insulin binding sites / affinity (Murugan et al., 2008) ↑ Na^+/K^+, Ca^{2+}, Mg^{2+} ATPase (Murugan & Pari, 2007a) ↓ Hepatic and renal markers (Murugan & Pari, 2007b)
Antihyperlipidemic	• ↓ Serum cholesterol, liver cholesterol, triglycerides, free fatty acids, phospholipids (Pari & Murugan, 2007a) ↓ HMG-CoA (Pari & Murugan, 2007a) ↓ VLDL, LDL (Pari & Murugan, 2007a) ↑ HDL (Pari & Murugan, 2007a)
Antiinflammatory	• ↓ cPLA2 (Dileep et al., 2011; Hong et al., 2004) ↓ Carragen induced TAK1, COX-2, NFκB (Zhang et al., 2018)
Immune modulatory	• ↓ Histamine (Suzuki et al., 2005)
Neuroprotective	• JNK (Begum et al., 2008), AChE (Arunkhamkaew et al., 2013)

Abbreviations: ROS: reactive oxygen species, LPO: lipid peroxidation; AAPH: 2,2′-azobis-(2-amidinopropane)-dihydrochloride; DPPH: 2,2-diphenyl-1-picrylhydrazyl; NTA: nitrilotriacetic acid; TBHP: tertbutylhydroperoxid; GSH: reduced glutathione; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; GST: glutathione S transferase; HMG-CoA: 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; VLDL: very low density lipoprotein; LDL: low density lipoprotein; HDL: high density lipoprotein; cPLA2: cytosolic phospholipase A2; TAK1: transforming growth factor β activated kinase-1; COX-2: cyclooxygenase-2; NFκB: nuclear factor-κB; 3 β; JNK: c-Jun N-terminal kinase; AChE: acetylcholinesterase.

(Murugan et al., 2008).

In a study with streptozotocin induced diabetic rats, THCC showed significant antihyperlipidemic effects greater than observed for CC by minimizing serum and liver cholesterol, triglyceride, phospholipid and low density lipoprotein (LDL) while increasing high density lipoprotein (HDL) levels (Pari & Murugan, 2007a). Observed effects on cholesterol were likely related to a decreased 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA) activity, which was corrobated by Prasanth et al. (2012).

Only a few studies have indicated antiinflammatory, immune modulatory (Suzuki et al., 2005) and neuroprotective modes of action that were superior to the ones of curucmin. Nevertheless, it is noteworthy that THCC posseses a higher *in silico* binding affinity to the hydrophobic substrate channel of cytosolic phospholipase A2 (cPLA2), which is responsible for arachidonic acid release (Dileep, Tintu, & Sadasivan, 2011). In contrast, Hong et al. showed that the antiinflammatory effect of curcuminoids is rather related to the inhibition of cPLA2 phoshorylation (Hong et al., 2004). Furthermore, a recent study of Zhang et al. (2018) conveyed that THCC is more potent than CC in selectively inhibiting cyclooxygenase-2 (COX-2) expression and nuclear factor-κB (NFκB) suppression via transforming growth factor β activated kinase-1 (TAK1), even though Aggarwal et al. concluded that COX-2 and NFκB are only targeted by CC and not THCC (Aggarwal et al., 2014). Finally, THCC could potentially aid in the prevention and treatment of neurodegenerative diseases by regulation of stress-activated c-Jun N-terminal kinase (JNK) (Begum et al., 2008) and inhibition of acetylcholinesterase (AChE) (Arunkhamkaew, Athipornchai, Apiratikul, Suksamrarn, & Ajavakom, 2013).

Commercial manufacturers and scientist have invented innovative formulations to increase the solubility and stability of curcumin while decelerating its rapid metabolism (Douglass & Clouatre, 2015; Jamwal, 2018; Stohs et al., 2018). However, because curumin is still rather unstable and reactive once released into the body (Nelson et al., 2017), it has been postulated that the "introduction of THC[C]" is necessitated (Aggarwal et al., 2014). Some studies even state that the degradation to THCC among other metabolites may solely be responsible for curcumins biological activities doubting that curcumin itself should be considered a lead compound (Nelson et al., 2017).

Recently, a β-cyclodextrin tetrahydrocurucmin complex with slighlty enhanced THCC bioavailability in rats (BA = 6.8 (Gopi & Jacob, 2016)) has been shown to alleviate symptoms of rheumatoid arthritis patients (Jacob et al., 2019). Based on the biological activity of CC and THCC, we suppose that formulations increasing the bioavailability of CC and metabolic conversion to THCC might convey superior health benefits by complementary or synergistic effects of both molecules.

In the following and for the first time, we present human data from different clinical studies on the enhanced metabolic bioavailability of THCC after oral supplementation of a γ-cyclodextrin curcumin formulation and serve an explanation for increased serum tetrahydrocurucmin levels based on an elevated water solubilty. Additional experiments on *E. coli* that is known for its curcumin reducing ability confirm that γ-cyclodextrin efficiently increases curcumin uptake and tetrahydrocurcumin production.

2. Materials and methods

2.1. Metabolic bioavailability of THCC in humans according to Purpura et al.

Serum THCC is stemming from the metabolism of orally supplemented curcumin. We therefore introduce the term metabolic bioavailability (BA), which relates blood THCC levels to the ingested amount of CC. Data on the metabolic BA of THCC after single oral supplementation of CAVACURMIN® compared to standard turmeric extract and a phytosome as well as an essential oil formulation have not been published formerly by Purpura et al. (2018). These are given in the following with permission of the authors. Serum curcuminoid levels of 12 human volunteers that participated in the one-center, double-blinded, cross-over study were analyzed. Subjects received a single dose of 6 capsules of CAVACURCMIN®, the phytosome and the essential oil formulation corresponding to a curcumin intake of 348 mg, 354 mg and 355 mg (m(curcumin, formulation)), respectively. Serum curcumin levels were compared to the ones obtained after ingestion of standard turmeric extract containing m = 1774.2 mg curcumin (m(curcumin, standard). For further details on the study design, blood sampling, sample preparation and analysis of serum curcuminoid levels, please refer to Purpura et al. (2018).

THCC was analyzed in parallel to the other curcuminoids via HPLC-

MS/MS using electron spray ionization. The mass to charge ratio (m/z) of the THCC parent and daughter ions respectively were 373.2 kg/C and 137.1 kg/C.

The relative metabolic bioavailability and the metabolic bioefficiency of THCC were respectively calculated according to equation (1) and (2) for each formulation and based on Douglass et al. (2015) The respective curcumin loads of CAVACURCMIN®, the phytosome and the essential oil formulation were 17.5%, 18.5% and 79.4%.

$$RelativeMetabolicBioavailability(THCC) = \frac{AUC_{0-12h}(\text{THCC, formulation})}{AUC_{0-12h}(\text{THCC, standard})} \cdot \frac{m(curcumin, standard)}{m(curcumin, formulation)} \quad (1)$$

$$MetabolicBioefficiency(THCC) = RelativeMetabolicBiovailability(THCC) * Load \quad (2)$$

with $AUC_{0\text{-}12h}$(THCC, formulation) being the area under the plasma concentration time curve measured for the observational period from the time point of ingestion of the respective formulation until 12 h, $AUC_{0\text{-}12h}$(THCC, standard) being the respective area under the plasma concentration time curve measured after ingestion of standard turmeric extract, m(curcumin, standard) being the actual amount of curcumin ingested by supplementation of standard turmeric extract and m(curcumin, formulation) being the respective actual amounts ingested by supplementation of the respective formulations.

2.2. Human serum curcuminoid levels and safety of long-term dietary intervention of CAVACURMIN®

Samples of the clinical study performed in here with CAVACURCMIN® were additionally measured for curcumin and THCC content 12 h after last intake of study products. The study design is summarized in brief as follows. Results focus on the analysis of curcuminoids and safety endpoints assessed during the study.

2.2.1. Study design and subjects

The study was performed as randomized, double blind, placebo-controlled parallel design. 61 overweight and obese subjects were allocated to intervention (21 male and 40 females). 5 subjects dropped out, 56 completed the study successfully. Inclusion criteria for subjects participating the study are summarized as follows: age between 35 and 70 (body mass index: 28–40 kg/m^2; body fat women $\geq$ 36%, body fat men $\geq$ 23%); no pregancy (women); no blood donation within 4 weeks prior to screening; no participation in clinical trial using investigational product within 4 weeks prior to sreening; no allergy to ingredients of study preparations; non-smoker; no alkohol or drug abuse; no history or presence of significant cardiovascular disease; no fat malabsorption; no gall bladder resection; no history of gastrointestinal diseases or conditions; no known human immunodeficiancy virus (HIV), hepatitis B or C infection; no use of drugs and dietary supplements with antiinflammatory or antioxidative properties, vitamins, mineral supplements in the period from 2 month prior to the study until and during the study; no chronic intake of lipid/glucose modifying agents, proton pump inhibitors, substances affecting blood coagulation.

The study was part of BTS965/16 (registration number: DRKS00010789) and the protocol was approved by the Institutional Review Board (IRB) of Landesärztekammer Baden-Württemberg. Prior to the dietary intervention, all volunteers were screened and their informed consent was obtained after written and oral explanation of the aims, methods, benefits and potential hazards of the study.

2.2.2. Study materials

Study volunteers received a daily total of 6 capsules of either γ-cyclodextrin complexed curcumin (CAVACURMIN®, Wacker Chemie AG, Germany) or cellulose that were both packed in blisters. This correspondend to an actual daily curcuminoid intake of 360 mg (336.8 mg curcumin (CC), 20.9 mg demethoxycurcumin (DMCC), 2.3 mg bisdemethoxycurcumin (BDMCC)). Subjects were asked to consume three capsules of CAVACURMIN® or cellulose twice daily in the morning and evening 30 min prior to a meal over a period of 12 weeks. During the whole study, subjects were further asked to abstain from meals with curry and tumeric, to avoid additional intake of curcuminoides by normal food.

2.2.3. Study procedure

Blood samples were were collected after overnight fasting of at least 10 h. On the day before each visit at week 4, 8 and 12, subjects received a standardized dinner consisting of farmhouse bread with cream cheese and cucumber of freely choosable quantitites. On the day of each visit, safety assessment and determination of the blood curcuminoid concentrations (tetrahydrocurcumin, curcumin, demethoxycurcumin and bisdemethoxycurcumin) were performed after 12 h of fasting and as described in the following chapters.

2.2.4. Safety assessment

Safety evaluation of the dietary intervention included tolerability assessment, analysis of blood routine parameters, vitals sign analysis and report of adverse events. Tolerability was assessed after 4 and 12 weeks and individually rated by subjects as “well tolerated”, “slightly unpleasant” and “very unpleasant”. Blood routine parameters were determined before and at each visit at Synlab Medizinisches Versorgungszentrum Leinfelden and within 24 h after blood sampling. The analysis included a differentiated haemogram, liver enzymes (GPT: glutamat-pyrvate transaminase, GOT: glutamic oxaloacetic transaminase, γ-GT: γ-glutamyl transferase, AP: alkaline phosphatase), fat status (total cholesterol, LDL, HDL, cholesterol and triglycerides), creatinine, uric acid and fasting glucose. Vital sign analysis included the measurement of blood pressure and heart rate in sitting position under fasting conditions. During the whole study intervention, subjects were asked to document all adverse events (AE) in a diary. All distinctive features (adverse events / serious adverse events defined in accordance to ICH-GCP Guidelines) were inquired and documented in case report forms (CRFs). Seriousness criteria were defined according to ICH-GCP. Safety data are presented in supplementary file.

2.2.5. Blood sample preparation and curcuminoid determination

For the determination of curcuminoid levels (THCC: tetrahydrocurcumin, CC: curcumin, DMCC: demethoxycurcumin, BSMCC: bisdemethoxycurcumin), blood plasma samples of the CAVACURMIN® group were taken at each visit. For sample preparation, an aliquot of blood plasma was diluted 1:1 with sodium acetate buffer (pH4.5). Glucoronidase treatment was performed for 60 min at 37 °C and stopped by addition of acetic acid, water and cooling down of the mixture. Next, samples were extracted using ethyl acetate, ultrasound treatment and centrifugation. The upper organic layer was finally transferred, dried with nitrogen, reconstituted (45% 0.1% formic acid in water / 55% acetonitrile) and filtered through spin cups prior to liquid chromatography tandem mass spectroscopic analysis (LC-MS/MS: XEVO TQ-S Micro, Waters Cooperation, USA, column: Acquity UPLC BEH C18 1.7 µm, 2.1 × 50 mm, Waters Cooperation, USA, solvent: 55% 0.1% formic acid in water / 45% acetonitrile, volume of injection: 5 µL, column temperature: 30 °C, PDA-detector wavelength range: 210 nm – 500 nm). The limits of quantification for THCC, CC, DMCC and BMCC were 2.18 ng/ mL, 0.91 ng/ mL, 0.16 ng/ mL and 0.13 ng/ mL, respectively. The limits of detection for CC, THCC, DMCC and BMCC were 0.27 ng/ mL, 0.66 ng/ mL, 0.05 ng/ mL and 0.04 ng/ mL, respectively. Quantification was performed with external calibration in plasma samples.

Table 2
Determination of the THCC bioavailability after single oral supplementation.

Formulation	$AUC_{0\text{-}12h}$ / ng·h·mL^{-1}	c_{max} / ng·mL^{-1}	t_{max} / h	Relative metabolic BA (THCC)	Relative BA(CC) (Purpura et al., 2018)	Metabolic Bioefficiency (THCC)
Standard (5 × dose)	108.4 ± 68.0	16.4 ± 9.1	6.1 ± 4.0	1.0	1.0	1.0
Standard (normalized)	21.3 ± 13.3	3.2 ± 1.8	6.1 ± 4.0	1.0	1.0	1.0
CAVACURMIN®	846.5 ± 265.5	152.5 ± 42.5	1.8 ± 0.8	39.8	85.0	7.4
Essential oil	34.7 ± 13.1	5.9 ± 2.5	9.0 ± 3.3	1.6	1.7	1.3
Phytosome	213.5 ± 109.3	35.4 ± 24.4	4.7 ± 4.0	9.9	9.0	1.8

Note that the respective curcumin loads of CAVACURCMIN®, the phytosome and the essential oil formulation calculated for the metabolic bioefficiency of THCC were 17.5%, 79.4% and 18.5%.
Abbreviations: $AUC_{0\text{-}12h}$: area under the curve for the time frame 0 h to 12 h; c_{max}: maximum observed tetrahydrocurcumin concentration; t_{max}: time of maximum observed tetrahydrocurcumin concentration; BA: bioavailability.

2.3. In vitro studies

2.3.1. In vitro solubility experiments

Sodium taurocholate (NaTC) was dissolved in LB medium to achieve final concentrations of 0 mM, 6 mM and 10 mM. Having added an excess of curcuminoids in absence of γ-cyclodextrin (-γCD) or in the form of CAVACURMIN® (+γCD), the mixture was incubated for 30 min at 35 ℃ while shaking. Finally, the resultant suspensions were filtrated using a 0.2 µm filter, diluted twofold with a 20 mM NaTC solution to measure curcumin solubility by HPLC.

2.3.2. E. Coli CC to THCC conversion experiments

20 mg curcuminoids or 92 mg of CAVACURMIN® equaling the same amount curcuminoids were added to 20 mL LB medium containing variable amounts of NaTC (0 mM, 6 mM, 10 mM). After shaking at 35 ℃ for 30 min, the resultant suspensions were filtrated through a 0.2 µm filter. 4 mL of each filtrate were added to about 2.9×10^5 *E.coli* and the solutions were incubated again for 60 min at 35 ℃ while shaking at 160 rpm. Subsequently, 300 µL of each suspension were collected and THCC was extracted with 300 µL acetonitrile : water = 40 : 60 including 0.05% trifluoroacetic acid. After centrifugation at 10,000 rpm for 15 min, the supernatant was collected, filtered through a 0.2 µm filter and analyzed by HPLC. The extraction procedure was repeated twice.

2.3.3. Comparison of the curcumin solubility and conversion efficiency to THCC of CAVACURMIN® and other commercially available formulations

CAVACURMIN® and commercially available formulations based on soy lecithin (phytosome), gum ghatti and essential oils of turmeric were added to LB medium including 6 mM NaTC. After shaking at 35 ℃ for 30 min, the resultant suspensions were filtrated through a 0.2 µm filter and diluted twofold with 20 mM NaTC solution to measure curcumin solubility by HPLC.

The conversion rate of curcumin to THCC using the soy lecithin formulation was determined as described above for CAVACURMIN®. Note that we did not analyze the conversion of CC to THCC by *E. coli* using the gum ghatti and the essential oil-based formulations, as the solubility of CC was found to be very low with these complexes.

2.3.4. HPLC analysis of curcumin and THCC

A Shimadzu HPLC system (LC2010C, Shimadzu Corporation, Kyoto, Japan) equipped with a Sun Fire C18 column (5 µm, 4.6 mm i. d. × 100 mm) was used for the determination of the curcumin and THCC concentrations. Prior to analyis, the column temperature was allowed to reach 30 ℃. A mobile phase of acetonitrile : water = 40 : 60 including 0.05% trifluoroacetic acid was used at af flow rate of 1.0 mL/ min. Curcumin or THCC was detected using a photodiode array (PDA) detector at 280 nm or 430 nm.

2.3.5. Statistical analysis

Statistical analysis was performed using Graphpad Prism version 8.4.2. (Graphpad Software, Inc, USA). Two-sided statistical tests were performed using a significance level of 0.05.

3. Results

3.1. Metabolic bioavailability of THCC in humans according to Purpura et al.

Purpura et al. have shown an increased bioavailability of curcumin (BA = 84.8) after single oral intake of CAVACURMIN® compared to standard turmeric extract. In addition, the bioavailability of curcumin for the cyclodextrin complex was ~ 10 fold and ~ 50 fold higher than observed for a phytosome (BA = 8.9) and essential oil-based (BA = 1.7) formulation (Purpura et al., 2018).

Serum THCC levels that were concurrently analyzed are presented in the following with permission of Purpura et al. CAVACURMIN® yielded a considerable metabolic BA(THCC) of 39.8 compared to the standard, which was respectively 4.0 and 24.9 fold higher than for the phytosome

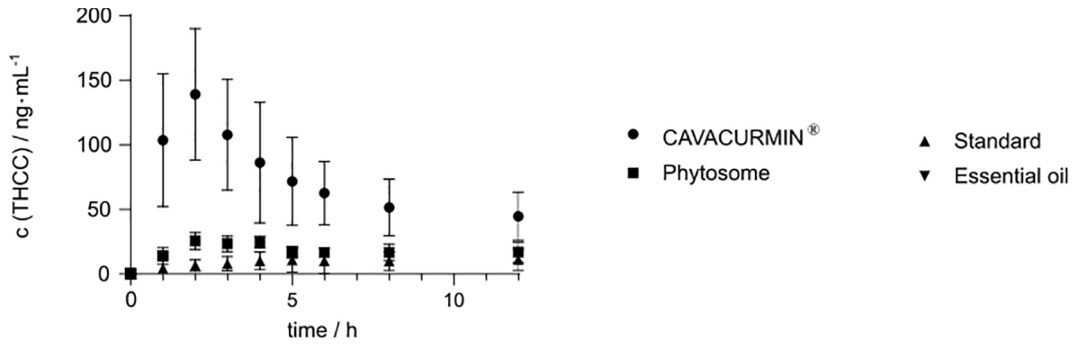

Fig. 2. Mean THCC plasma concentration time curve. Plasma tetrahydrocurcumin levels were determined after oral supplementation of CAVACURMIN®, an essential oil-based and phytosome formulation compared to standard turmeric extract. Data are expressed as mean ± standard deviation of individual measurements of 12 patients under investigation.

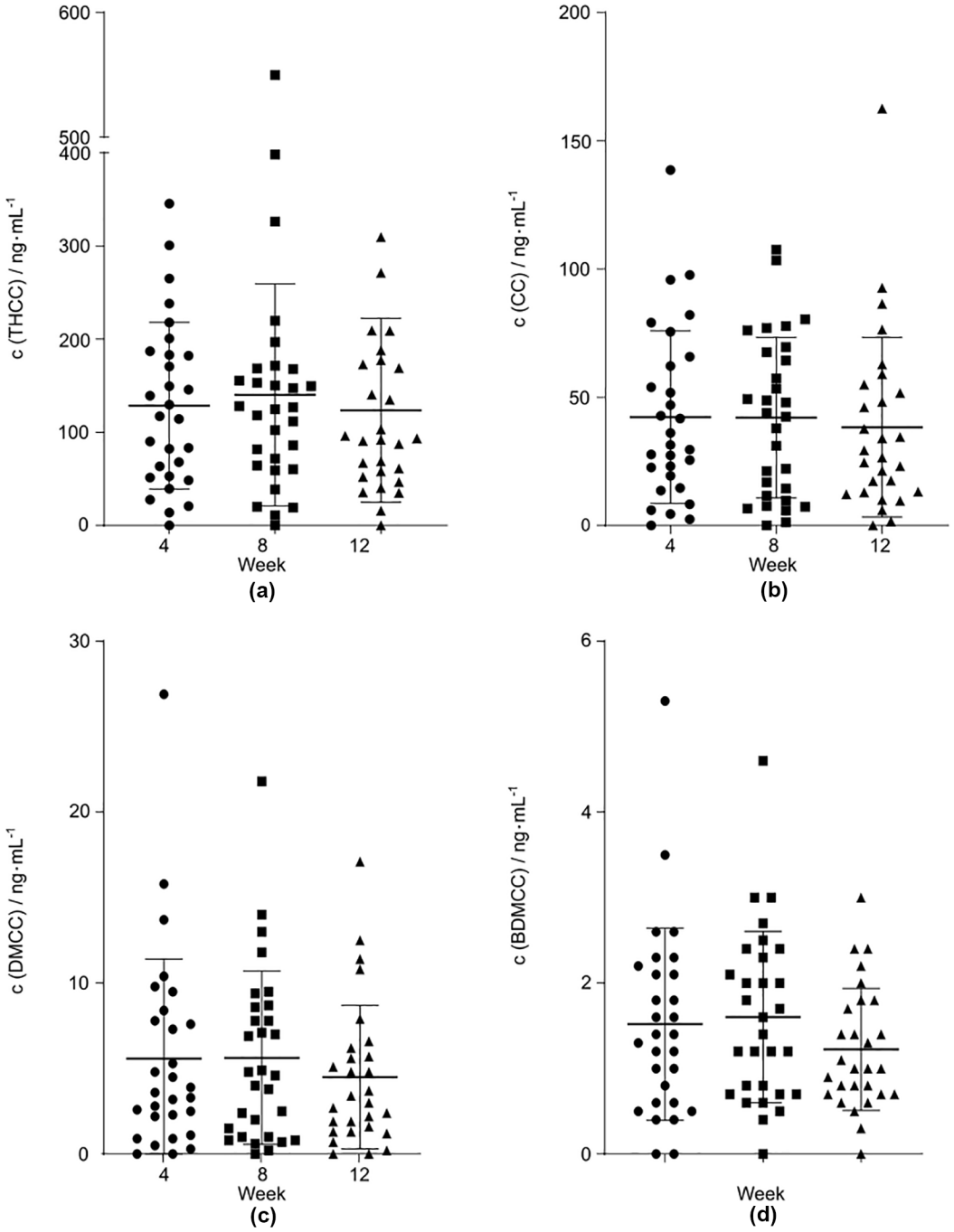

Fig. 3. Mean curcuminoid concentrations in human serum after long-term daily oral supplementation of CAVACURMIN®. (**a**) serum tetrahydrocucrumin, (**b**) curcumin, (**c**) demethoxycurcumin and (**d**) demethoxycurcumin blood serum concentrations measured 12 h after last intake of CAVACURCUMIN® after 4 weeks (dots), 8 weeks (squares) and 12 weeks (triangels) daily oral supplementation. Mean values ± standard deviations are indicated by horizontal solid lines.

(metabolic BA = 9.9) and essential oil-based (metabolic BA = 1.6) formulation (Table 2). Note that Purpura et al. supplemented subjects with a fivefold higher dose of standard turmeric extract (5 × dose) compared to the formulations in order to measure reasonable curcuminoid levels. For an easier comparison in Table 2, the $AUC_{0\text{-}12h}$ and the c_{max} were therefore normalized by the factor m(curcumin, standard) divided by m(curcumin, CAVACURMIN®).

Note that metabolic BA values were calculated with respect to the ingested amounts of curcumin. These were m = 1774.2 mg, m = 348.0 mg, m = 355.2 mg and m = 354.0 mg respectively for the standard turmeric extract, CAVACURMIN®, the essential oil and the phytosome formulation. The bioefficiencies of THCC for CAVACURMIN®, the essential oil and phytosome formulation respectively were 7.4, 1.3 and 1.8. A graphical illustration of the pharmacokinetic analysis is displayed in Fig. 2 showing a maximum achievable plasma THCC concentration of $c_{max} = 152.5 \pm 42.5\ \text{ng}\cdot\text{mL}^{-1}$ at $t_{max} = 1.8 \pm 0.8$ h after oral intake of CAVACURMIN®. Respective values for the standard (5 × dose), the essential oil and the phytosome formulation were $c_{max} = 16.4 \pm 9.1\ \text{ng}\cdot\text{mL}^{-1}$ at $t_{max} = 6.1 \pm 4.0$ h, $c_{max} = 5.9 \pm 2.5\ \text{ng}\cdot\text{mL}^{-1}$ at $t_{max} = 9.0 \pm 3.3$ h and $c_{max} = 35.4 \pm 24.4\ \text{ng}\cdot\text{mL}^{-1}$ at $t_{max} = 4.7 \pm 4.0$ h.

Note, that data have not been published formerly, because we intended to validate the unexpectedly high THCC serum concentrations by another independent study as follows in the next chapter.

Table 3
Comparison of serum curcuminoid levels of single* and long-term oral supplementation. Serum curcuminoid levels of Purpura et al.* at time point $t = 12$ h are compared to the average serum curcuminoid concentration measured after long-term supplementation of CAVACURMIN® (mean of data from week 4, 8 and 12).

	c ($t = 12$ h) / ng·mL^{-1} (THCC)	c ($t = 12$ h) / ng·mL^{-1} (CC)	c ($t = 12$ h) / ng·mL^{-1} (DMCC)	c ($t = 12$ h) / ng·mL^{-1} (BDMCC)
Purpura et al. (6 capsules)	44.7 ± 18.8	14.9 ± 8.8	2.0 ± 1.4	0.5 ± <0.1
Long-term suppl. (3 capules)	139.5 ± 129.2	45.0 ± 50.4	5.9 ± 7.9	1.6 ± 1.5

Abbreviations: c ($t = 12$ h): Plasma curcuminoid concentration at $t = 12$ h; THCC; tetrahydrocurcumin; CC: curcumin, DMCC: demethoxycurcumin; BDMCC: bisdemethoxycurcumin.

3.2. Human curcuminoid serum levels during long-term supplementation of γ-cyclodextrin complexed curcumin

Blood curcuminoid levels including tetrahydrocurcumin, curcumin, demethoxycurcumin and bisdemethoxycurcumin were determined before the start of the intervention (baseline), at each visit at 4, 8 and 12 weeks after at least 10 h of fasting before supplementation and after 12 h of last intake of the curcumin study preparation. The measured curcuminoid levels should be comparable to the last measurement performed by Purpura et al. (see Fig. 2, $t = 12$ h) with the only difference that subjects received three instead of six capsules (Purpura et al.) 12 h before the assessment of blood curcuminoid levels.

The baseline measurements did not show any curcuminoids thus confirming that subjects were compliant to a curcumiond free diet. Fig. 3 summarizes the mean curcuminoid concentrations measured at each visit for subjects completing the study in the CAVACURMIN® group. Only one outlier was excluded due to non-compliance of product intake i.e. the time between CAVACURMIN® ingestion and measurement was likely < 12 h. Data are presented for n = 28.

The mean serum levels of tetrahydrocurcumin (Fig. 3 **a**), curcumin (Fig. 3 **b**), demethoxycurcumin (Fig. 3 **c**) and bisdemethoxycurcumin (Fig. 3 **d**) after 4 weeks of supplementation are comparable to the ones after 8 and 12 weeks. Therefore, the calculation of the average concentrations measured for all three timepoints for THCC, CC, DMCC and BMCC respectively yielding 139.5 ± 129.2 ng·mL^{-1}, 45.0 ± 50.4 ng·mL^{-1}, 5.9 ± 7.9 ng·mL^{-1} and 1.6 ± 1.5 ng·mL^{-1} is acceptable. Note, that the average serum concentration of THCC was by a factor of more than three and significantly ($p < 0.0001$) higher than the one of CC.

The comparison of the curcuminoid data in Fig. 3 with serum levels in Fig. 2 after 12 h of last CAVACURMIN® intake ($t = 12$ h) indicates that long-term supplementation of CAVACURMIN® increases serum curcuminoid levels by factors of more than three reaching a steady state during the first four weeks (Table 3). However, note that ingested curcumin amounts were about half the amount of ingested curcumin in Purpura et al. (6 capsules ingested), because subjects ingested three CAVACURMIN® capsules in the morning before the assessment of blood curcuminoids (and three afterwards in the evening). Note that values in Table 3 are not corrected for the absolute ingested amounts 12 h before assessment of the curcuminoid levels (Purpura et al.: m(CC) = 348 mg, m(DMCC) = 21.6 mg, m(BDMCC) = 2.4 mg; long-term supplementation: m(CC) = 168.4 mg, m(DMCC) = 10.5 mg, m(BDMCC) = 1.2 mg).

3.3. In vitro solubility experiments

Based on our observation that γ-cyclodextrin complexed curcumin supplementation results in elevated THCC levels in humans, we figured that this might be related to increased curcumin solubility (Hassaninasab, Hashimoto, Tomita-Yokotani, & Kobayashi, 2011). In order to test our hypothesis, we evaluated the solubility of CAVACURMIN® in presence of physiological concentrations of the bile acid sodium taurocholate (NaTC) mimicking conditions in the human gut. As shown in Fig. 4 **a**, the solubility of the curcumin γ-cyclodextrin complex increases at increasing bile acid concentrations.

Next, we tested the ability of *E. coli* that is known for its ability to selectively reduce CC to THCC in presence of NaTC. The results in Fig. 4 **b** indicate that bile acids enhance the uptake of curcumin yielding an up to tenfold higher amount of THCC than observed without NaTC. Furthermore, the optimal concentration for microbial curcumin uptake appears to be 6 mM indicating saturation effects at increasing concentrations.

Finally, we performed the same solubility tests and *E. coli* metabolism studies as above comparing three different curcumin mixtures to

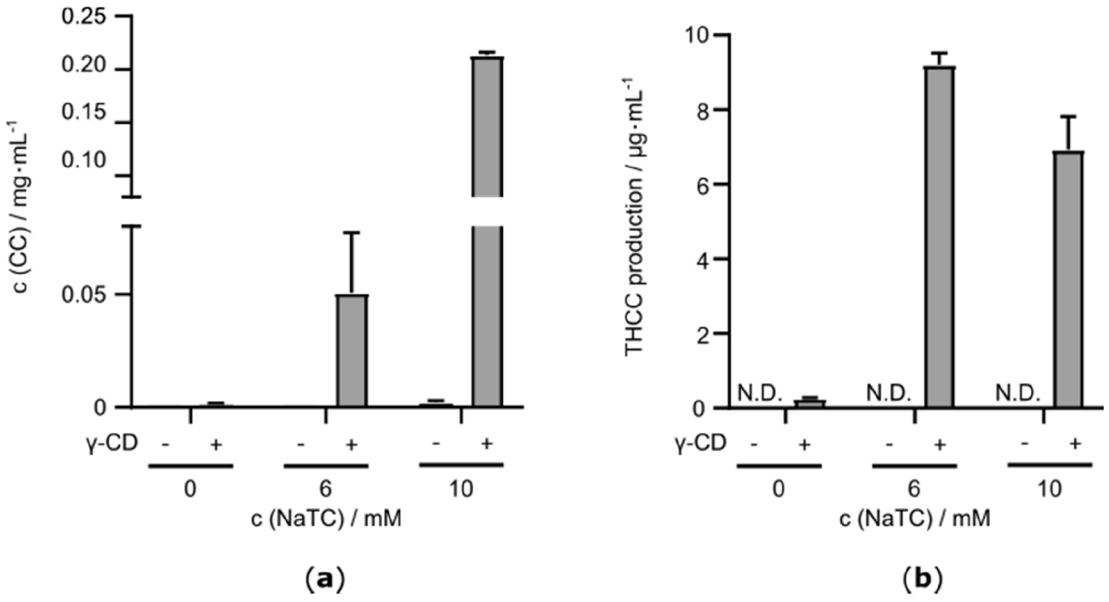

Fig. 4. ***In vitro*** **solubility of curcuminoids in absence and presence of NaTC (a) and** ***E. coli*** **metabolism of CC to THCC in absence and presence of NaTC (b).** Abbreviations: γ-CD: γ-cyclodextrin, N.D.: no determination possible. Data are expressed as mean + standard deviation.

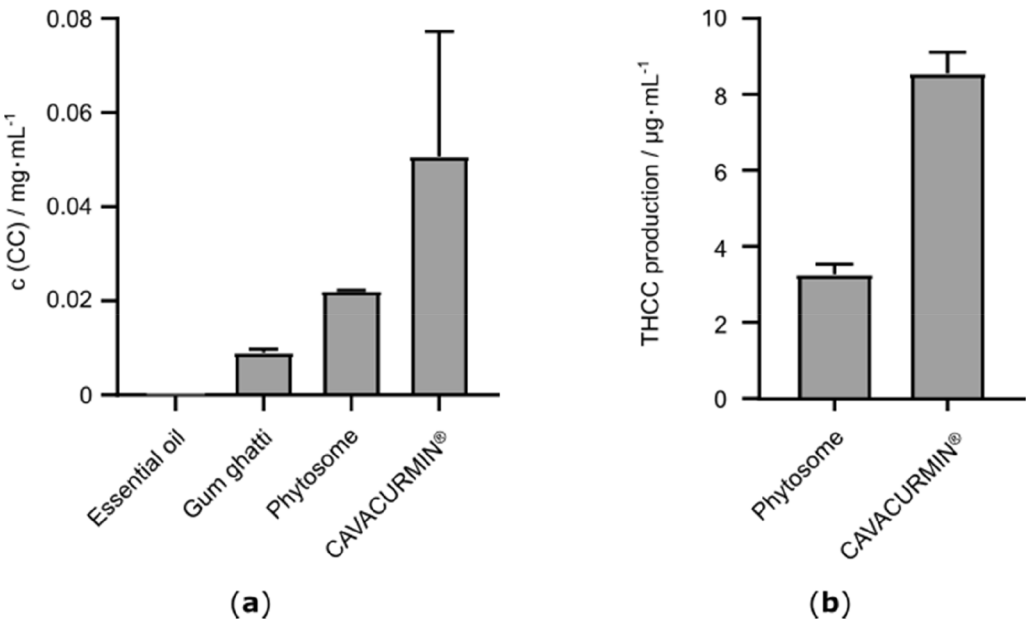

Fig. 5. *In vitro* solubility of curcuminoids for different formulations and comparative *E. coli* metabolism of CC to THCC. (**a**) The solubility of curcuminoids of an essential oil-based, a gum ghatti and a soy lecithin (phytosome) formulation were compared to CAVACURMIN® in LB medium containing 6 mM NaTC. (**b**) Because the solubility of the curcuminoids was low for the essential oil and gum ghatti formulations, *in vitro E. coli* conversion experiments of CC to THCC were only performed for the phytosome formulation and CAVACURMIN®. Data are expressed as mean + standard deviation.

the curcumin γ-cyclodextrin complex. As displayed in Fig. 5 **a**, the solubility of curcumin in the γ-cyclodextrin complex is 2.3, 5.6 and 82.3 fold higher than the soy lecithin, gum ghatti and essential oil formulation, respectively. The THCC prodcution was almost threefold higher for the γ-cyclodextrin complex compared to the soy lecithin formulation (Fig. 5 **b**). Note that we did not analyze the conversion of CC to THCC by *E. coli* using the gum ghatti and the essential oil-based formulations, as the solubility of CC was found to be very low with these complexes.

4. Discussion

Curcumin and its main metabolite tetrahydrocurcumin exhibit several biological activities that could potentially convey beneficial health effects for humans upon dietary supplementation. However, due to its unfavorable physicochemical and pharmacokinetic properties, innovative formulations that increase curcumin's solubility and intestinal uptake while decelerating its metabolism and excretion are desired. Still, valid critical studies are even doubting curcumin's biological activity *in vivo* while showing evidence that metabolites such as tetrahydrocurcumin might be responsible for observed beneficial effects for human health. Therefore, formulations that additionally yield elevated tetrahydrocurcumin serum levels could potentially be either synergistic or complementary to the modes of action of curcumin (Aggarwal et al., 2014).

Here, we showed that γ-cyclodextrin enhances the metabolic bioavailability of tetrahydrocurcumin (BA = 39) more effectively than an essential oil (BA = 1.6) and phytosome (BA = 9.9) formulation compared to standard turmeric extract (Fig. 2 and Table 2). Furthermore, the serum concentrations of THCC were by factors of about three higher than the one of CC in the case of CAVACURCUMIN®.

For verification of elevated THCC levels upon γ-cyclodextrin curcumin supplementation, results were compared with samples generated in a second independent clinical study measuring serum curcuminoid levels after oral supplementation of CAVACURMIN® for a period of 12 weeks. Overall, the intervention with CAVACURMIN® proved to be safe and well tolerated by most subjects (see **supplement**). Predominantly in the beginning of the intake, a few subjects reported gastrointestinal findings known for curcumin. However, the gastrointestinal system seemed to get accustomed to the ingredient, as the frequency of gastrointestinal disorders decreased over time.

With respect to curcuminoid analysis, we observed that THCC serum concentrations were by a factor of more than three higher than the ones of CC (Fig. 3**)** and thus even higher than observed by Purpura et al. (Fig. 2, $t = 12$ h), even though only half the amount of curcumin was ingested 12 h before the last intake. On the one hand, this corroborates that γ-cyclodextrin is increasing the metabolic bioavailability of THCC (among CC, DMCC and BDMCC). On the other hand, the comparison of our data with the ones of Purpura et al. suggest that it requires more than one day of oral supplementation but maximum four weeks to yield curcuminoid (THCC, CC, DMCC, BDMCC) saturation in human blood (Table 3).

In the past, several different formulations have been invented to enhance the bioavailability of curcumin including techniques using liposomes, micelles, synthetic emulsifiers, fibers and nanoparticles (Jamwal, 2018; Stohs et al., 2020; Stohs et al., 2018). A recently published preclinical study in rats presented an increased THCC bioavailability (BA = 6.8) after oral supplementation of chemically reduced tetrahydrocurcumin complexed with β-cyclodextrin (Gopi & Jacob, 2016). However, and to the best of our knowledge, no human study has so far shown enhanced THCC levels upon oral curcumin supplementation. Nevertheless, we believe that formulations other than curcumin γ-cyclodextrin complexes are likely possible to deliver increased systemic THCC concentrations as was observed by using standard turmeric extract as well as phytosome and essential oil formulations in here.

Tetrahydrocurcumin is a main metabolite of curcumin and we observed serum THCC levels that were by a factor of more than three higher than serum CC levels. Our *in vitro* experiments (Fig. 4 and Fig. 5) revealed that γ-cyclodextrin increases the solubility in presence of bile acids present in the intestinal tract. Furthermore, it was shown that this also leads to an elevated production of THCC in *E. coli* (Hassaninasab et al., 2011). In fact, THCC is probably also produced by commensals in the gastrointestinal tract of humans after oral supplementation of curcumin. However, since *E. coli* mainly inhabit the colon (Chalova, Sirsat, O'Bryan, Crandall, & Ricke, 2009) where reabsorption of microbially secreted molecules like THCC is improbable, (Stohs et al., 2020) a major contribution of *E. coli* metabolism leading to elevated THCC levels in humans can be ruled out. Based on evidence in literature (Dei Cas & Ghidoni, 2019; Ireson et al., 2002; Pan et al., 1999; Vijaya Saradhi et al., 2010) our observations are therefore likely explained by an increased solubility of γ-cyclodextrin complexed curcumin allowing enhanced intestinal uptake and xenobiotic metabolism of intestinal and hepatic tissues yielding THCC, which additionally exhibits an elevated plasma

half-life. Nevertheless, further *in vitro* studies with such cells using γ-cyclodextrin complexed curcumin are necessary to corroborate our assumption and elucidate the underlying molecular mechanisms of elevated THCC bioavailability after oral supplementation of this formulation. Finally, it is still under debate whether curcumin, tetrahydrocurcumin, or both convey potential health benefits to humans upon oral supplementation. Therefore, clinical trials with formulated tetrahydrocurucmin for which human data are sparse as well as clinical trials comparing curcumin and tetrahydrocurumin ideally in complex with γ-cyclodextrin are required in the future to clarify potential biological activities. Once proven effective in humans for instance as potential antioxidant (Aggarwal et al., 2014), analgesic or anti-inflammatory substance (Jacob et al., 2019), oral supplementation of distinct target groups with γ-cyclodextrin complexed curcumin delivering tetrahydrocurcumin might be recommendable taking in mind that our study showed long-term safety.

5. Conclusions

In summary, we presented data of two independent human studies for the first time showing that γ-cyclodextrin enhances the bioavailability of curcumin and concurrently the metabolic bioavailability of tetrahydrocurcumin, a major metabolite of curcumin with own biological functionality, to a greater extent than standard turmeric extract, a phytosome and an essential oil formulation. We further provided a potential explanation for elevated serum tetrahydrocurcumin levels. These are likely related to enhanced intestinal uptake of better soluble curcumin, reduction by intestinal and hepatic cells and elevated plasma half-life of tetrahydrocurcumin.

CRediT authorship contribution statement

Christian Hundshammer: Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Christiane Schön:** Conceptualization, Methodology, Formal analysis, Writing - review & editing. **Madoka Kimura:** Formal analysis. **Takahiro Furune:** Formal analysis. **Keiji Terao:** Supervision. **Dana Elgeti:** Writing - review & editing, Supervision, Project administration. **Rachela Mohr:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

D. Elgeti, R. Mohr and C. Hundshammer are employed at Wacker Chemie AG declaring commercial conflicts of interest. M. Kimura, T. Furune and K. Terao are employed at Cyclochem (Japan) declaring commercial conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104410.

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ORIGINAL ARTICLE

Short-term effects of highly-bioavailable curcumin for treating knee osteoarthritis: a randomized, double-blind, placebo-controlled prospective study

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Abstract
Background We previously developed a surface-controlled water-dispersible form of curcumin and named it Theracurmin® (Theracurmin; Theravalues, Tokyo, Japan). The area under the blood concentration–time curve of Theracurmin in humans was 27-fold higher than that of curcumin powder. We determined the clinical effects of orally administered Theracurmin in patients with knee osteoarthritis during 8 weeks of treatment.
Methods Fifty patients with knee osteoarthritis of Kellgren–Lawrence grade II or III and who were aged more than 40 years were enrolled in this randomized, double-blind, placebo-controlled, prospective clinical study. Placebo or Theracurmin containing 180 mg/day of curcumin was administered orally every day for 8 weeks. To monitor adverse events, blood biochemistry analyses were performed before and after 8 weeks of each intervention. The patients' knee symptoms were evaluated at 0, 2, 4, 6, and 8 weeks by the Japanese Knee Osteoarthritis Measure, the knee pain visual analog scale (VAS), the knee scoring system of the Japanese Orthopedic Association, and the need for nonsteroidal anti-inflammatory drugs.
Results At 8 weeks after treatment initiation, knee pain VAS scores were significantly lower in the Theracurmin group than in the placebo group, except in the patients with initial VAS scores of 0.15 or less. Theracurmin lowered the celecoxib dependence significantly more than placebo. No major side effects were observed with Theracurmin treatment.
Conclusion Theracurmin shows modest potential for the treatment of human knee osteoarthritis.

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Introduction

Osteoarthritis, also referred to as degenerative joint disease, is a slow destructive process of the joints that affects millions of people worldwide. Although the exact biochemical cause of osteoarthritis remains unknown, the process usually begins when the joint structures are abnormal or the stress placed on joint surfaces is unusually high. Hip or knee osteoarthritis is a chronic condition that is usually treated with analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs), but these drugs sometimes cause serious gastrointestinal and cardiovascular adverse events, especially with long-term use [1, 2]. Thus, there is a need for disease-modifying agents that not only decrease joint pain but also slow the progression of the condition.

Curcumin is a polyphenol extracted from turmeric, which has been safely used in foods such as curries for a long time [3]. Curcumin is a promising therapeutic food material because of its anti-inflammatory and antioxidative functions, and has long been used as an anti-inflammatory treatment in traditional Chinese and Ayurvedic medicine [3]. Curcumin regulates various biochemical and molecular pathways by modulating several molecular targets, including transcription factors, cytokines,

enzymes, and genes regulating cell proliferation or apoptosis [4]. The anti-inflammatory effect of curcumin seems to be comparable with those of steroidal drugs and NSAIDs such as indomethacin and phenylbutazone [5]. Some studies have shown that curcumin's anti-inflammatory properties are related to the suppression of prostaglandin synthesis through its effect on cyclooxygenase (COX) [6], a key enzyme responsible for the conversion of arachidonic acid to prostaglandins. Curcumin has also been shown to inhibit proteasome activity and induce apoptosis in human colon cancer cells in vitro and in vivo [7]. It has also been suggested that an important mechanism of curcumin is inhibition of NF-kB activation [8], which is a key event in the chronic inflammatory process. Given these findings, curcumin is expected to be effective for a range of diseases related to chronic inflammation, including cancer, cardiovascular disease, metabolic syndrome, Alzheimer disease, osteoarthritis, and other common diseases and aging conditions [3, 4, 9, 10]. Furthermore, it has been reported that curcumin can be a potent inhibitor of the production of inflammatory and catabolic mediators by chondrocytes [10]. Because osteoarthritis and related osteoarticular conditions of synovial joints are characterized by inflammation, curcumin's biological actions in joint tissues may facilitate the development of clinically safe, orally administered therapeutic agents for treating joint diseases.

However, curcumin's poor bioavailability has been an obstacle to realizing its beneficial health effects, because only a small amount of curcumin is absorbed with oral administration [11]. To overcome this bioavailability problem, we previously developed a surface-controlled water-dispersible curcumin and named it Theracurmin® (Theracurmin: Theravalues, Tokyo, Japan) [12]. The absorption efficacy of Theracurmin was investigated and compared with that of curcumin powder. In rats, the area under the blood concentration–time curve (AUC) after oral administration of Theracurmin was found to be more than 40-fold higher than that of curcumin powder. With healthy human volunteers, the AUC of Theracurmin was 27-fold higher than that of curcumin powder. These findings demonstrated Theracurmin's much higher bioavailability than those of currently available curcumin preparations. Thus, Theracurmin is believed to be useful for providing the clinical benefits of curcumin in humans.

The purpose of this study was to determine the clinical effects of orally administered Theracurmin in patients with knee osteoarthritis during 8 weeks of treatment. We hypothesized that Theracurmin ingestion for 8 weeks would improve the symptoms and functional abilities of patients with knee osteoarthritis.

Materials and methods

A randomized, double-blind, placebo-controlled, prospective clinical study was conducted to test our hypothesis in two treatment groups: Theracurmin and placebo. A total of 50 patients with knee osteoarthritis confirmed by radiographic analysis were selected and enrolled in the study. Written informed consent was obtained from all subjects before participation. All procedures were reviewed and approved by the research ethics committee of our hospital, and this study was carried out in accordance with the World Medical Association's Declaration of Helsinki.

The inclusion criteria were primary medial knee osteoarthritis patients over 40 years of age with Kellgren–Lawrence grades of II or III upon radiographic classification. The exclusion criteria were previous knee surgeries, knee injection treatment during the study, knee steroid injections within 2 months before the study, or other steroid administration within 4 weeks before the study. If patients needed NSAIDs during the study, oral celecoxib was prescribed, 2 pills per day (100 mg per pill). The other combined therapy we allowed was pain relief patches.

Theracurmin or placebo was administered orally twice a day for 8 weeks. Subjects in the Theracurmin group took 6 capsules of Theracurmin per day, which contained 180 mg of curcumin. Similarly, subjects in the placebo group took 6 placebo capsules per day, which had a similar shape and color to those of the Theracurmin capsules; the capsules were primarily made of starch, dextrin, and maltose. The subjects were requested to report the number of capsules remaining and celecoxib pills prescribed at their week 2, 4, 6, and 8 visits at our outpatient clinic for the compliance check.

Blood biochemistry analyses were performed before the study and after 8 weeks of each intervention. The patients' knee symptoms were evaluated at 0, 2, 4, 6, and 8 weeks according to the following criteria: the Japanese Knee Osteoarthritis Measure (JKOM) [13], the knee pain visual analog scale (VAS) included in JKOM, and the knee scoring system of the Japanese Orthopedic Association (JOA) [14]. JKOM consists of 25 questions divided into 4 subcategories—pain and stiffness, condition in daily life, general activities, and health conditions—for patient self-assessment, and is based on the World Health Organization's International Classification of Functioning, Disability, and Health. It is validated in the same manner as the Western Ontario and McMaster Universities' Arthritis Index (WOMAC). The JOA scale evaluates four items: ability to walk (30 points), ability to climb up and down stairs (25 points), range of motion (35 points), and joint swelling (10 points). Each knee joint can achieve a maximum score of 100 points on the JOA scale. We used the improved JKOM, VAS, and JOA scores

(the differences between the scores at each time point and those before the study). We evaluated adverse events and the number of celecoxib pills that the subjects needed during the 8-week period.

The two-sample one-sided *t* test was used to perform the statistical analysis of the VAS, JKOM, and JOA scores. The chi-square test was used to analyze the need for celecoxib. The level of statistical significance was set to a *P* value of <0.05.

Results

Drop-outs from the study are shown in Fig. 1. Three subjects (two and one in the Theracurmin and placebo groups, respectively) did not attend follow-up at weeks 1, 2, and 6. The other three patients discontinued participation in the study because of minor side effects. One patient in the Theracurmin group had a feeling of tachycardia and hypertension on day 50, and another patient in the Theracurmin group had redness of the tongue on day 6. In addition, one patient in the placebo group felt unwell on day 7. The other three cases in the Theracurmin group dropped out because they underwent intra-articular injection of hyaluronic acid on weeks 2, 2 (different patient), and 3. There were two Kellgren–Lawrence grade II cases and one Kellgren–Lawrence grade III case. The initial VAS scores in these three cases were 1.0, 0.54, and 0.45, and the improved VAS scores before intra-articular injection of hyaluronic acid were 0, 0.10, and 0.29, respectively. Therefore, we included 41 patients (18 and 23 patients in the Theracurmin and placebo groups, respectively) for further analysis.

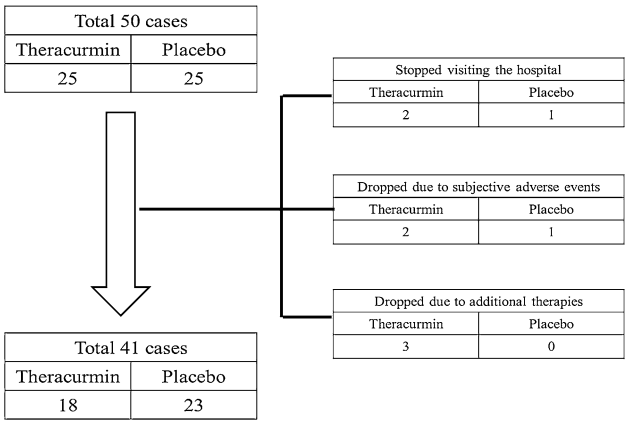

Fig. 1 Study profile, including enrollments and dropouts. Seven patients in the Theracurmin group and two patients in the placebo group dropped out. Therefore, we analyzed 41 patients (18 patients in the Theracurmin group and 23 patients in the placebo group)

The baseline characteristics of the study subjects in the two groups are shown in Table 1. Reflecting the general demographic profile of knee osteoarthritis, the majority of the subjects were female, with 77.8 % in the Theracurmin group and 78.3 % in the placebo group. The patient ages were also comparable between the two groups. The Kellgren–Lawrence criteria grade was used to quantify disease severity in order to effectively randomize disease status upon study entry between the groups. The majority of the subjects had grade II disease, with similar frequencies seen in the two groups. No statistical differences in the baseline characteristics were evident. Compliance was similar in the Theracurmin and placebo groups.

Although the improved VAS scores were not significantly different between the two groups ($P = 0.10$) except

Table 1 Baseline characteristics of the study subjects in the two groups

	Theracurmin	Placebo	Total
Number of participants	18	23	41
Male (%)	22.2 (4/18)	21.7 (5/23)	22.0 (9/41)
Female (%)	77.8 (14/18)	78.3 (18/23)	78.9 (32/41)
Mean (SD) age (years)	71.9 (5.3)	66.1 (7.2)	68.7 (7.0)
Mean (SD) height (cm)	156.4 (7.0)	158.1 (7.6)	157.4 (7.3)
Mean (SD) body weight (kg)	61.5 (8.6)	62.1 (8.0)	61.8 (8.2)
Mean (SD) body mass index (kg/m^2)	25.1 (2.7)	24.8 (2.3)	24.9 (2.4)
Osteoarthritis lesions			
Either left or right only (%)	72.2 (13/18)	56.5 (13/23)	63.4 (26/41)
Both left and right (%)	27.8 (5/18)	43.5 (10/23)	36.6 (15/41)
Kellgren–Lawrence classification for knee osteoarthritis			
II (%)	72.2 (13/18)	87.0 (20/23)	80.5 (33/41)
III (%)	27.8 (5/18)	13.0 (3/23)	19.5 (8/41)
Mean VAS score (SD)	0.52 (0.24)	0.42 (0.25)	0.47 (0.25)
Mean JOA score of each knee (SD)	82.5 (14.3)	84.8 (10.5)	83.8 (12.3)
Mean JKOM total score (SD)	35.5 (22.5)	27.0 (11.7)	307 (17.6)

No statistical differences in the baseline characteristics between the groups were evident

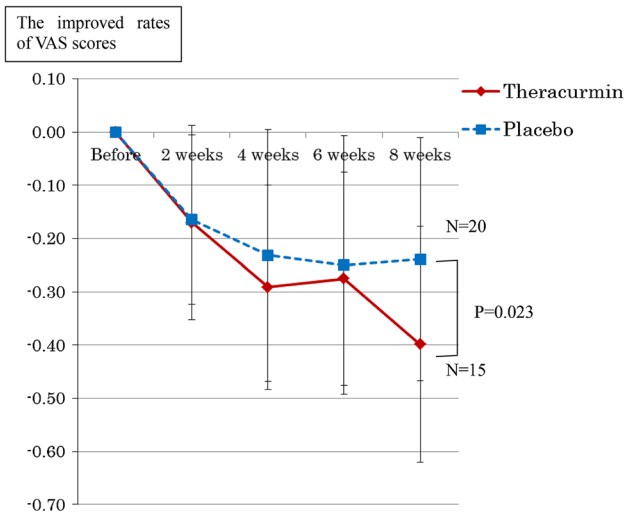

Fig. 2 The improved VAS scores in the two groups are presented as means ± SDs. Except for the patients with initial VAS scores of 0.15 or less, the improved VAS scores were significantly larger in the Theracurmin group than in the placebo group at 8 weeks ($P = 0.023$)

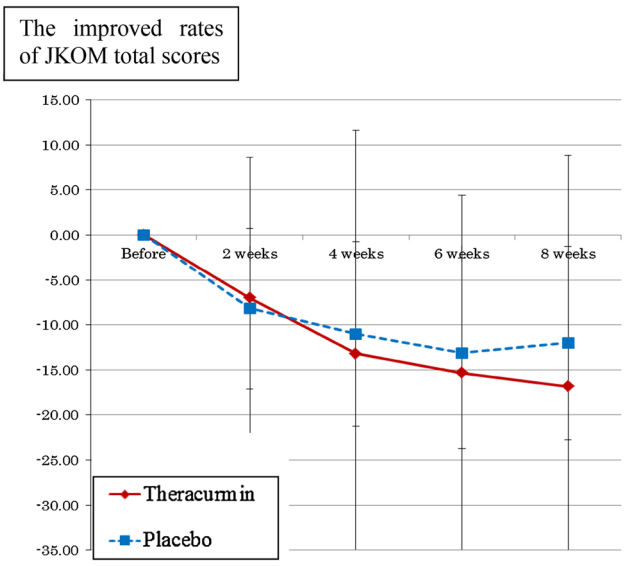

Fig. 3 The improved JKOM total scores for the two groups are shown as the means ± SDs

in the patients with initial VAS scores of 0.15 or less, the improved VAS scores were significantly higher in the Theracurmin group than in the placebo group at 8 weeks (Fig. 2). The mean VAS scores at 0 and 8 weeks in all 41 cases were 0.52 and 0.20, respectively, in the Theracurmin group, and 0.42 and 0.21, respectively, in the placebo group. Except the patients with initial VAS scores of 0.15 or less, the mean VAS scores at 0 and 8 weeks were 0.60 and 0.20, respectively, in the Theracurmin group, and 0.47 and 0.23, respectively, in the placebo group. In each of the two groups there were three patients with initial VAS scores of 0.15 or less.

There were no statistically significant differences in the JKOM scores between the two groups. However, the improved scores in the JKOM total score (Fig. 3) and in each JKOM subcategory tended to be higher in the Theracurmin group than in the placebo group, especially from 6 to 8 weeks in all 41 cases and 35 cases, except in the patients with initial VAS scores of 0.15 or less. There were no statistically significant differences in JOA total and subcategory scores (data not shown) between the two groups.

At 8 weeks only, NSAIDs were needed significantly less in the Theracurmin group than in the placebo group (Fig. 4).

At 8 weeks, all laboratory test results were similar to the baseline scores in both groups with the following exceptions: there were slight increases in triglyceride levels in some patients at week 8 in both groups (two patients in the Theracurmin group and four patients in the placebo group), slight increases in the level of creatinine (one case in the Theracurmin group), uric acid (one case in the placebo

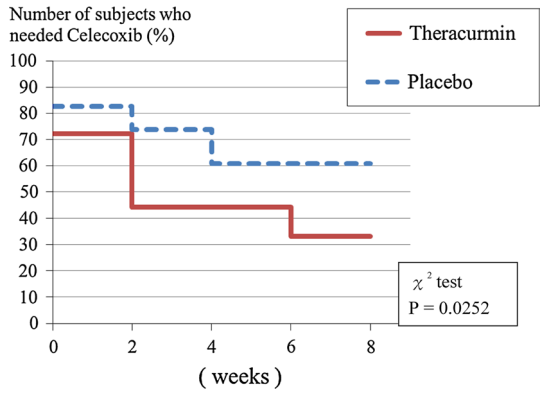

Fig. 4 NSAID necessity in the two groups. At 8 weeks only, the ratio of patients who needed celecoxib was significantly smaller in the Theracurmin group than in the placebo group ($P = 0.0252$)

group), and amylase (one case in the placebo group) as well slight decreases in red blood cells (two cases in the Theracurmin group) and cholinesterase levels (one case in the Theracurmin group). These were all minor changes, and no serious adverse events were observed in either group during this study.

Discussion

In this study, the knee pain VAS scores were significantly lower in the patients treated with Theracurmin than in those treated with placebo at 8 weeks among those who had initial VAS scores of >0.15. The same tendency was seen in

the total JKOM scores and its subcategorical scores, including pain and stiffness in the knees, condition in daily life, general activities, and health conditions. Moreover, Theracurmin significantly lowered celecoxib dependence compared with placebo. These results suggest that Theracurmin may decrease the pain and discomfort of knee osteoarthritis and improve the patient's general condition and quality of daily life. Because osteoarthritis is a slowly progressive and chronic condition that decreases quality of life, agents like Theracurmin, which is also a common food ingredient that is mildly effective with no major side effects, has modest potential for the treatment of human knee osteoarthritis.

The patients in the Theracurmin group took six capsules of Theracurmin per day, and each capsule contained 30 mg curcumin. We confirmed the safety of a Theracurmin dose of 150–210 mg that healthy humans could take in the phase 1 study. Therefore, six 30-mg capsules of Theracurmin per day were used in various clinical studies in our group, and no major side effects were observed.

In this study, the improved VAS scores between both groups were not significantly different, except in the patients with initial VAS scores of 0.15 or less. In each group, there were three patients with initial VAS scores of 0.15 or less. If the initial VAS score was small, the improved VAS score did not increase. This was because, when all 41 cases were considered, the improved VAS scores did not differ significantly between both groups. Three cases in the Theracurmin group dropped out because they underwent intra-articular injection of hyaluronic acid. The initial VAS scores in these three cases were 1.0, 0.54, and 0.45, and the improved VAS scores before intra-articular injection of hyaluronic acid were 0, 0.10, and 0.29. Our results indicate that Theracurmin was ineffective in some cases.

Some studies have suggested that curcumin has an effect on arthritis. Huang et al. [15] showed that curcumin dramatically attenuates the progression and severity of collagen-induced arthritis in mice and suppresses the production of the B-cell-activating factor belonging to the tumor necrosis factor family (a mechanism involved in rheumatoid arthritis). Banji D et al. [16] stated that combination treatment with methotrexate and curcumin had a significant anti-arthritic effect and protected against hematologic toxicity in rats. In addition to these anti-inflammatory effects, there curcumin seems to exert a chondroprotective action. For instance, Yang et al. [17] described novel pharmacological actions of curcumin on chondrocytes stimulated by advanced glycation end products. Kumar D et al. [18] also demonstrated that curcumin regulates the expression and secretion of various matrix metalloproteinases. Both the anti-inflammatory and chondroprotective effects of curcumin provide strong support for the potential effectiveness of curcumin treatment for osteoarthritis, and may explain the results of our present study. Further studies may be needed to show the chondroprotective effect of Theracurmin in knee osteoarthritis.

COX-2 inhibitors have been used as efficient anti-inflammatory agents or for osteoarthritis, although their cardiovascular toxicity can be problematic during long-time use. Lev-Ari et al. [19] reported a synergistic effect of celecoxib and curcumin that resulted in inhibition of the growth of osteoarthritic synovial adherent cells, which may be associated with increased induction of apoptosis, and stated that the synergistic effect was mediated by a mechanism that involved inhibition of COX-2 activity. In our study, Theracurmin decreased the need for the COX-2 inhibitor celecoxib. However, before they discontinued celecoxib, the subjects took both celecoxib and Theracurmin, so it is possible that there was a synergistic effect. Further study would be needed to clarify the best option among celecoxib alone, curcumin alone, and combined celecoxib and curcumin.

There have been three clinical reports that have examined the effects of curcumin in human osteoarthritic or rheumatic diseases. One pilot clinical study evaluated the safety and effectiveness of curcumin alone, diclofenac sodium alone, and their combination in patients with active rheumatoid arthritis [20]. Significantly greater improvement was shown by the curcumin group than by the diclofenac sodium group. More importantly, curcumin treatment was found to be safe and was not associated with adverse events. These results provided the first evidence for the safety and superiority of curcumin treatment in patients with active rheumatoid arthritis. With regard to curcumin's effect on osteoarthritis, there have been two reported studies on the use of Meriva [21, 22], a complex of curcumin with soy phosphatidylcholine phytosomes, which have suggested that curcumin is efficacious for treating knee osteoarthritis. However, although these two studies were promising, both were open-label trials, not double-blind studies or randomized controlled trials with no restriction on NSAIDs, other painkillers, and other treatments prescribed by the general practitioners. The present study, which was a double-blind placebo-controlled trial, showed that even the placebo group showed statistically significant effects on all criteria, including those of the JOA scale, VAS, and JKOM (including all four JKOM subcategories), even though we only allowed celecoxib as a concomitant drug. Therefore, to evaluate the effects of specific compounds on knee osteoarthritis, we strongly believe that double-blind placebo-controlled studies should be conducted, which means that the effect seen in the Meriva studies may not be certain. In contrast, our study clearly showed Theracurmin's effectiveness for treating knee osteoarthritis in a double-blind placebo-controlled study.

Several studies of Theracurmin have been conducted in animals and humans, and it has been suggested to be

effective for treating cancer and cardiovascular conditions. For instance, two papers showing the clinical effectiveness of Theracurmin have been published. One paper suggested that regular endurance exercise combined with daily curcumin ingestion may decrease left ventricular afterload to a greater extent than monotherapy with either intervention alone in postmenopausal women [23]. The other paper showed that curcumin ingestion and aerobic exercise training increased flow-mediated dilation in postmenopausal women, which suggested that both can potentially improve age-related decline in endothelial function [24]. Similar to the present study, there were no major adverse effects of Theracurmin in the two previous studies, in spite of Theracurmin's much higher bioavailability.

Patients with rheumatoid arthritis or osteoarthritis need novel anti-arthritic therapies because of possible gastrointestinal and cardiovascular adverse events caused by current therapies using NSAIDs and COX-2 inhibitors. There have been some in vitro and in vivo studies of arthritis treatment using natural health products or functional food materials. Ahmed et al. [25] suggested that epigallocatechin-3-gallate could decrease synovial hyperplasia, cartilage degradation, and bone resorption by modulating multiple targets in joints. Resveratrol may also prevent intervertebral disc degeneration, osteoarthritis-associated inflammation, chondrocyte apoptosis, and rheumatoid arthritis-related pannus formation, which are desirable goals in the treatment of osteoarthritis and rheumatoid arthritis [26]. In contrast to NSAIDs, curcumin has no gastrointestinal side effects and can even protect the gastric mucosa. Therefore, curcumin is thought to be beneficial in the management of chronic inflammation-related joint disease, including osteoarthritis [27]. However, because assessments of the outcomes of arthritis treatments are usually subjective, it is critical to confirm the effect of a test agent in double-blind placebo-controlled studies. For instance, glucosamine and chondroitin, which have often been used by arthritis patients, were found to be no more effective than placebo, and using then in combination did not decrease joint pain or have an impact on joint space narrowing [28]. In the present double-blind placebo-controlled study, we were able to provide the first evidence of the safety and superiority of curcumin treatment in patients with knee osteoarthritis. We also believe that we were able to obtain these results because of the high bioavailability of Theracurmin, which enhanced the health benefit of curcumin.

The limitations of our study were the small number of patients ($n = 50$) and short duration (8 weeks) of the treatment of knee osteoarthritis. This was a pilot study to determine the effect of Theracurmin on knee osteoarthritis, but we were fortunately able to observe significant outcomes with respect to improved VAS scores and decreased NSAID (celecoxib) necessity. Future studies may be needed to validate our findings in a large number of patients with knee osteoarthritis over a longer duration.

In our evaluations, we used JKOM, which was designed for Japanese patients with knee osteoarthritis, and did not use WOMAC, which is commonly used throughout the world; however, JKOM has been validated and is widely used in Japan.

In conclusion, we conducted a randomized, double-blind, placebo-controlled, prospective clinical study of the efficacy of Theracurmin, which is a highly bioavailable form of curcumin, in patients with osteoarthritis. Theracurmin was significantly effective in deceasing pain and NSAID necessity with no major adverse events. Theracurmin has modest potential for the treatment of human knee osteoarthritis in the future.

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Conflict of interest T. Hashimoto and A. Imaizumi are full-time employees of Theravalues Corporation. C. Tamura is a part-time employee of Theravalues Corporation.

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