Nutrafoods (2022) 2:444-456 DOI 10.17470/NF-022-0053

Original research

Safety and Efficacy of a Proprietary Platform Technology-Based Curcumin on Arthritis, Joint Pain, and Mobility

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that affects the joints, skin, eyes, heart, kidneys, and lungs. Since there is no complete cure for RA, the treatment goals are to alleviate discomfort and prevent or decrease the progression of the disease. Curcumin, the active component of the common spice turmeric, has a wide range of biological activities by modulating several transcription factors and signalling pathways. However, limiting factors of regular curcumin include solubility, absorption in the gut, metabolism, and systemic elimination. BioSOLVE Curcumin® is an improved formulation developed to overcome these problems. In the current study, BioSOLVE Curcumin® was investigated for safety and efficacy in RA complications. For a period of 12 weeks, 24 individuals with RA were given 250 mg of BioSOLVE Curcumin® or as placebo capsules twice a day. ACR20 (American College of Rheumatology-20), VAS (Visual Analogue Scale), and WOMAC (Western Ontario and McMaster) scales were used to analyse the comparative efficacy of the investigational product, BioSOLVE Curcumin®, in active RA. In healthy middle-aged and older individuals with RA, BioSOLVE Curcumin was found to be a safe and effective supplementation for lowering RA symptoms, including pain and stiffness, as well as improving physical function.

Keywords: Rheumatoid arthritis, joint pain, curcumin, double-blind clinical trial, bioavailability, efficacy safety

Introduction

Rheumatoid arthritis (RA) is a chronic, symmetrical, inflammatory autoimmune disease that begins in the small joints and progresses to the skin, eyes, heart, kidneys, and lungs. Morning stiffness of the affected joints for more than 30 minutes, fatigue, fever, weight loss, tender, swollen, and warm joints, and rheumatoid nodules under the skin are common symptoms of RA. This disease typically manifests itself between the ages of 35 and 60, with periods of remission and exacerbation. Joint bone and cartilage are frequently destroyed and tendons and ligaments become weak [1]. RA can also affect young children under the age of 16, which is known as juvenile RA (JRA). JRA is similar to RA except that no rheumatoid factor is present [2, 3, 4, 5]. The majority of the individuals become hard to treat and fail to achieve treatment goals such as clinical remission or low disease activity. Many are unsuitable for new RA treatments due to genetic profile, joint erosion, the presence, or development of autoantibodies, pregnancy, and comorbidities. Disease-modifying anti-rheumatic drugs (DMARD), oral and systemic glucocorticoids, nonsteroidal anti-inflammatory drugs (NSAIDs), and modern biological treatments are currently available to treat RA. These treatments are closely correlated with severe symptoms such as osteoporosis, hypertension, gastrointestinal bleeding, hepatotoxicity, ocular toxicity, myelosuppression, hypersensitivity, and infectious disease risk [6].

The integration of biomolecules such as curcumin, celastrol, thymoquinone, polyphenols, and others has demonstrated high efficacy in treating RA symptoms in a dose-dependent manner. The anti-rheumatic properties are due to the ability of biomolecules to act on inflammatory mediators such as nitric oxide (NO), cytokines, chemokines, adhesion molecules, NF-kB, lipoxygenase (LOX), and arachidonic acid (AA). While these bioactive compounds show great efficacy, there are various obstacles to using them effectively, including poor solubility (which makes absorption from the gastrointestinal system problematic) and a high first-pass metabolism (which results in poor bioavailability).

Curcumin is a bright yellow substance generated by *Curcuma longa* plants. It is the main curcuminoid in turmeric (*Curcuma longa*), which belongs to the *Zingiberaceae* (ginger) family. Curcumin is well-documented for its ability to alleviate RA symptoms. This bioactive molecule is available as an herbal supplement, a pharmaceutical ingredient, a food flavour, and a colourant. Curcumin can affect redox-signalling pathways in cells because it contains multiple functional groups that have antioxidant properties. Curcumin can also stimulate nuclear factor-erythroid-2-related factor 2 (Nrf2), a key transcription that interacts with the antioxidant response element in the regulatory area of numerous genes that code for intracellular antioxidants, cytoprotective, and detoxification proteins. Curcumin is indeed an anti-inflammatory nutritional plant component that reduces all inflammatory mediators, including adhesion molecules and growth factors, chemokines, cytokines, cyclooxygenase-2 (COX-2), tissue factor, inducible nitric oxide, and epigenetic modifications. Curcumin's anti-inflammatory properties are beneficial to inhibit the NF-kB pathway as well as many other pro-inflammatory signalling pathways like activator protein-1 (AP-1), COX-2, early growth response protein 1 (Egr-1), signal transducers, and activators of transcription (STAT), representatives, and mitogen-activated protein (MAP) kinases [7]. Curcumin has also been studied for its anticancer and chemo-preventive properties. Studies have shown that it suppresses cellular proliferation, promotes apoptosis and arrests growth in various stages of the cell cycle (depending on the cell type), and prevents angiogenesis. Curcumin is now being tested in clinical trials

for a range of disorders, including cardiovascular disease, type 2 diabetes, Alzheimer's disease, RA, multiple sclerosis, and a variety of human cancers, due to its various actions.

Curcumin is a nutraceutical/herbal product that has been specially formulated to take advantage of highly bioavailable curcumin for its intended pharmacological effect. While most ingested curcumin is excreted unmetabolized in the faeces, the small amount that is absorbed is extensively converted to its water-soluble metabolites, glucuronides, and sulphates. The solubility of curcumin 95% powder is relatively low, resulting in very low *in vivo* absorption and bioavailability.

The current clinical study was designed and executed in individuals with active RA in the knees, hands, and neck to assess the efficacy and safety of BioSOLVE Curcumin® in comparison to a placebo for 12 weeks. In addition, acute toxicology studies and bioavailability studies of BioSOLVE Curcumin® were carried out in rat models.

Zeus Hygia Lifesciences manufactures and supplies BioSOLVE Curcumin® as an encapsulated curcumin powder derived from 95% curcumin (*Curcuma longa*) extract. BioSOLVE Curcumin® has been carefully designed and developed to explore the maximum benefits of highly bioavailable curcumin for its targeted pharmacological activity. In general, curcumin is limited by its weak solubility and low gut absorption; absorbed less from the gastrointestinal tract and quickly degraded, resulting in faster systemic clearance [8]. BioSOLVE Curcumin® has been developed using a proprietary technology to address the drawbacks of standard curcumin extract enhancing its solubility, bioavailability, and other physicochemical properties.

Based on the available literature published by several researchers worldwide [9], the current clinical study investigates BioSOLVE Curcumin®'s therapeutic effect and safety in RA individuals of the knee, hands, and neck. The test product was given to individuals with active RA for 90 days (3 months) and the efficacy was assessed using subjective questionnaires in terms of ACR20 [10].

In addition, an acute oral toxicity study was conducted in rats to assess the toxicity of the BioSOLVE Curcumin®. The objective of the acute oral toxicity study was to determine the toxicity of the BioSOLVE Curcumin® following a single oral dosage to rats. The study findings are believed to be useful in forecasting any potential toxicity in humans of the test product when taken orally, as well as providing information for hazard classification.

The goal of the study was to gather information on potential health risks that may result from acute oral exposure. Based on the study results, it was observed that the acute oral LD50 of BioSOLVE Curcumin® was determined as 5000 mg/kg bw. The test item, BioSOLVE Curcumin® Powder falls under category '5' with an LD50 cut-off value of 5000 mg/kg bw according to the Globally Harmonized System Hours (GHS) classification system.

Toxicity studies

The acute oral toxicity study of BioSOLVE Curcumin® was conducted in accordance with OECD principles of Good Laboratory Practice (GLP).

BioSOLVE Curcumin® 20% powder was used as a test product for safety evaluation. For the identification method at the commencement of acclimatization, the animals were marked with temporary numbers at the tip of their tails. Before the test product was administered, they were marked with permanent animal numbers near the base of the tail.

Based on the results of a preliminary solubility test, distilled water was chosen as the vehicle. For the solubility of pharmaceuticals or compounds, distilled water is a typical polar solvent. The rat model was chosen as the test system because it is routinely employed for

acute oral toxicity studies and complies with the regulatory requirements of the majority of regulatory authorities. A veterinarian performed a physical health assessment on all animals prior to acclimatization. During the acclimatization and treatment periods, animals were kept in polypropylene rat cages (approximate interior dimensions of 365 mm × 202 mm × 180 mm) with corn cob bedding (1–2 rats per cage).

Test procedure

A starting dosage of 5000mg/kg bw was used. In Group-I, one female rat (overnight fasting) was given the test product via oral intubation at a dosage of 5000 mg/kg bw. To corroborate the results of Group-I, two female rats (overnight fasting) were also administered BioSOLVE Curcumin® Powder at a dosage level of 5000 mg/kg bw by oral intubation in Group-I (one animal) and (two animals).

The dosage was administered once daily during the first 30 minutes of the acclimatization period and at roughly 1, 2, 3 and 4 hours following administration on test day, then once daily for 14 days. Modifications in skin, fur, eyes and mucous membranes, respiratory, circulatory, autonomic, and central nervous system, somato-motor activity, and behavioural patterns were all observed. All the animals were examined for clinical evidence of toxicity and death.

According to the classification criteria correspond to the obtained LD50 value, the test item was classified according to the Globally Harmonized System of Classification and Labelling of Chemicals.

Oral LD50 mg/kg bw	Hazard category
≥5000	5

Table 1 GHS classification system*
* Globally Harmonized System of Classification and Labelling of Chemicals (GHS) Eighth Revised Edition, United Nations (2019). ST/SG/AC.10/30/Rev.8.

Results

Throughout the trial, all the animals were checked for mortality/viability twice daily. Throughout the monitoring period, none of the animals exhibited clinical symptoms of harm or death and clinical symptoms of toxicity were also not observed in any of the animals.

Group/ Step	Dose mg/kg bw	No. of animals treated	No. of Animal deaths	Percent mortality (up to 14 days)
Group-I (one animal)	5000	1	0	0.00
Group-I (two animals)	5000	2	0	0.00

Key: mg/kg=milligram/kilogram; bw=body weight; No.=number

Table 2 Percentage mortality calculations

Clinical signs

Group & Dose mg/kg bw	A.No.	Sex	Day of Observation																	
			1*					2	3	4	5	6	7	8	9	10	11	12	13	14	15
			30 min	1hr	2hr	3hr	4hr														
Group-I & 5000	01	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Group-I & 5000	02	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	03	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Key: A.No.=Animal Number; mg/kg=milligram/kilogram; bw=body weight; F=Female;1=Normal; min=minutes; hr=hours
Table 3 Clinical observations

*Examinations were performed within the first 30 minutes and at approximately 1, 2, 3 and 4 hours after treatment on test day 1.

Human clinical study objectives

A randomized double-blind placebo-controlled study was carried out in severe arthritis individuals to understand the clinical efficacy and safety of BioSOLVE Curcumin® at a dose of 250 mg twice a day for 90 days. The major objectives of the study were to compare the efficacy of BioSOLVE Curcumin® and placebo in active RA patients using the ACR20, VAS, and WOMAC scales and to understand the safety of the trial product.

Method of investigative process

Research plan and design

The research was registered with the Clinical Trials Registry-India (CTRI) with registration number CTRI/2019/08/020535. The study was initiated on 16 July, 2019, once the ACE Independent Ethics Committee reviewed and approved the study protocol. The objective of the study was to compare the efficacy of the Investigational Products (IP) BioSOLVE Curcumin® with a placebo in the management of symptoms in RA subjects. A total of 24 eligible individuals satisfied the study inclusion/exclusion criteria and were randomly assigned to either active or placebo for 12 weeks in a 1:1 ratio (*n*=12 each group) (90 days). All participants were routinely warned against taking any illegal drugs during the trial period. Throughout the trial, any adverse events were tracked. There were no participant dropouts or withdrawals in either group. All enrolled participants in each group completed the study.

Study design

The Declaration of Helsinki and local regulations were used to guide the ethics review process for this study. Before taking part in the study, all participants signed a written informed permission form. The study lasted nearly 12 weeks, during which all individuals were extensively observed for protocol compliance and efficacy and safety parameters were assessed. Participants were assessed for predetermined efficacy variables over four visits, including a screening visit.

A total of 24 study individuals were enrolled in the study and were randomly assigned to one of two groups (*n*=12 in each) to obtain BioSOLVE Curcumin® or placebo capsules for 90 days. On Day -7 to 0 (Screening/Baseline visit), Day 30±2 (visit 2), Day 60±2 (visit 3), and Day 90±2 (visit 4), individuals were examined for preset efficacy factors (end of study visit 4). Adverse Events were monitored throughout the study period. **Fig. 1** depicts the flow diagram of a general study participant as a CONSORT Diagram. **Table 5** in this section contains information about various evaluations performed during specific visits.

Accountability for investigational products

All individuals in both study groups consumed the products in accordance with protocol requirements on IPs. IP consumption was greater than 95% throughout the study period, as evaluated at the scheduled study visits.

IP Accountability (Number of used IP) [Mean ±SD]			
Groups	**Day 30**	**Day 60**	**Day 90**
A (Test)	58.50 ± 1.00	59.33 ± 1.23	60.00 ± 0.00
B (Placebo)	58.25 ± 1.06	59.83 ± 0.39	60.00 ± 0.00

Table 4 Investigational Product accountability

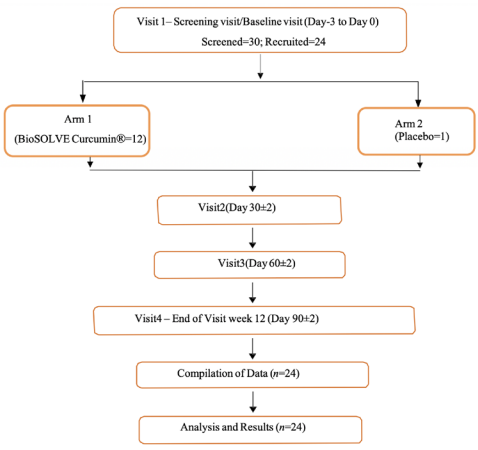

Figure 1 Flow diagram of the study

Visits	Screening visit/ Baseline Visit V1 (Day -3 to Day1)	Visit 2 V2 (Day 30)	Visit 3 V3 (Day 60)	End of Study Visit V4 (Day 90)
Informed Consent Process	X			
Demography	X			
Physical Examination	X	X	X	X
Vital Signs	X	X	X	X
Medical History	X			
Concomitant Medication	X	X	X	X
Adverse Event/Serious Adverse Event assessment		X	X	X
ACR20	X	X	X	X
VAS Scale	X	X	X	X
Western Ontario and McMaster Osteoarthritis Index	X	X	X	X
Inclusion/Exclusion Criteria	X			
Randomization	X			
IP Dispensing	X	X	X	
Issue of Diary Card	X	X	X	
Subject Diary Compliance		X	X	X
IP Accountability/ Compliance		X	X	X

Table 5 Visit specific schedule

Primary and secondary end points

The ACR20 scores at baseline and at follow-up visits (Day 30, Day 60, and Day 90) are evidence of improvement in RA following supplementation with BioSOLVE Curcumin®. ACR20 is considered to be the primary efficacy variable when evaluating new treatments for RA. ACR20 measures an improvement of at least 20% in a patient' painful and swollen joints and an improvement of at least 20% in three of five other measures: the patient's overall score, physician's overall score, HAQ, intensive reagent, and the patient's pain score (PN), as defined by ACR.

Secondary efficacy endpoints include comparing improvements in the Western Ontario and McMaster Osteoarthritis Index (WOMAC) at baseline and each visit (Baseline, Day 30, Day 60, and Day 90), as well as improvements in the VAS (Visual Analogue Scale) at baseline and each visit (Day 1, Day 30, Day 60, and Day 90).

With respect to safety endpoints, BioSOLVE Curcumin® was evaluated for safety in terms of vital signs (blood pressure, heart rate, and respiratory rate) and any adverse events during the supplementation were monitored.

Study population

At the chosen study site, study investigators screened potential individuals. Inclusion criteria to determine subject eligibility were: the revised 2010 ACR criteria (with a RA functional class of II); at least eight swollen joint counts (SJC) and tender joint counts (TJC); secondary Sjogren's syndrome; recurring pain; limited cutaneous vasculitis. Exclusion criteria included the use of any DMARD or Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) within 30 days of enrolment; any consequent disease; systemic involvement of secondary RA; pregnant or lactating women; other systemic immune disorders; history of alcohol abuse; history of hyperglycaemia and motor coordination disorders; any surgical procedure related to bone, joint, or synovectomy within 12 weeks of screening.

Withdrawal criteria

The study withdrawal criteria stipulated that individuals could opt out of the study at any time and could be withdrawn from the study at the discretion of the investigator due to any situation or circumstance that rendered the subject ineligible for further participation in the study.

Intervention

Zeus Hygia Lifesciences manufactured and supplied BioSOLVE Curcumin® water dis-

persible and bioavailable curcumin powder from turmeric extract (*Curcuma longa*) for this study. BioSOLVE Curcumin® 250 mg powder was supplied as Hydroxypropyl Methylcellulose (HPMC) capsules. A placebo capsule containing microcrystalline cellulose (MCC) 250 mg served as the control. Both the treatment and placebo capsules lacked flavour and were identical in colour and shape. Participants were randomly assigned to receive one capsule of 250 mg placebo or IP twice daily after food with a glass of water for 90 days. At each visit, each participant received a calculated amount of IP. A subsequent visit was used to account for consumed versus remaining IP from the previous visit. For each visit, the percentage of treatment compliance was calculated. The protocol-defined treatment regulatory criteria were considered to be 90% met.

Statistical analyses

Considering a minimum 20% improvement in the test product compared to the placebo, and an expected standard deviation of 5 in type I and type II errors of 5% and 20% respectively, the sample size was calculated as *n*=24. Hence, for study purposes, a sufficient number of individuals were screened, but only 24 eligible individuals were recruited for further evaluations. The sample size can be further justified based on the effect size of earlier clinical studies conducted on various formulations containing curcumin. The nominal effect size obtained in these studies ranges from moderate to high (0.6 to 1). Considering the effect of a perfect '1', α (type I error rate) = 0.05, β (type II error rate) = 0.3, the proportion of individuals in each group = 0.5, and a standard deviation of '1', the total sample size equals 25 with N_0=13 and N_1=12. Hence, the chosen sample size was sufficient enough to detect the change from baseline in efficacy variables to reject the null hypothesis.

Demographics and baseline

Summary statistics were used to present continuous data by treatment group (number of observations, mean and standard deviation). Frequencies and percentages were used to summarize categorical variables per treatment group.

Results

Table 6 shows descriptive statistics comparing demographic factors (age and weight), baseline efficacy indicators (ACR20, WOMAC Index total and WOMAC sub-scales evaluations), and safety measures (heart rate). There were no significant differences between the treatment and control groups in demographic or baseline outcome measures.

	Group A (BioSOLVE Curcumin®)	Group B (Placebo)	*P*-value
Age (years)	41.27 ± 13.27	43.89 ± 11.05	0.636
Weight (kg)	68.42 ± 9.67	69.92 ± 7.65	0.678
HR (beats/minute)	76.50 ± 5.14	77.42 ± 4.56	0.649
SBP (mmHg)	123.17 ± 8.38	122.67 ± 4.77	0.859
DBP (mmHg)	81.33 ± 6.68	80.33 ± 4.42	0.670
Respiratory rate (breaths per minute)	14.83 ± 1.75	15.08 ± 1.93	0.743
ACR20 Assessment (number and percentage)			
Physician's Global Assessment Score	15.33 ± 2.96 (27 ±5%)	12.58 ± 3.26 (22 ± 6%)	0.042
Total Painful Joint Counts (PJC)	11.42 ± 2.54 (41 ±9%)	10.17 ± 2.33 (36 ± 8%)	0.222
Total Swollen Joint Counts (SJC)	3.92 ± 1.93 (14 ±7%)	2.42 ± 1.51 (9 ± 5%)	0.046
Function Index (FN)	19.83 ± 2.79 (67 ±12%)	19.42 ± 2.57 (65 ± 8%)	0.708
Subject's Pain Score (PN)	5.83 ± 1.47 (65 ±16%)	5.42 ± 1.31 (60 ± 15%)	0.471
Patient's Global Assessment Score	25.67 ± 2.93 (66 ±8%)	24.83 ± 3.66 (64 ± 9%)	0.545
WOMAC Scale Assessment			
WOMAC-Pain	11.33 ± 1.72	10.42 ± 1.78	0.214
WOMAC-Stiffness	4.83 ± 0.83	4.08 ± 0.79	0.034
WOMAC-Physical Function	39.08 ± 4.78	34.58 ± 5.98	0.054
WOMAC Index Total	54.50 ± 6.16	50.33 ± 6.60	0.124
Pain VAS (Percentage & Number)	Moderate: 16.67% (2) Severe: 83.33% (10)	Moderate: 33.32% (4) Severe: 66.64% (8)	-

Table 6 Demographic and baseline characteristics [Mean ±SD]

The demographic and baseline characteristics of both study groups were observed to be similar. At the beginning of the study, the study groups did not differ in terms of efficacy or safety, such as heart rate, blood pressure, and respiratory rate.

Study efficacy analysis

In this study, both objective and subjective efficacy assessments were used. Three efficacy data points (ACR20, WOMAC, and Pain VAS) and three safety data points (Vitals-HR, BP, and RR) – a total of six data points – were collected four times for each participant. A total of 576 data points were collected from 24 individuals over the period of the research. These were examined by a biostatistician to determine the nature of the findings. All participants who took any masked investigational product were evaluated for their safety. The efficacy analysis of outcome variables in the ITT (Intention To Treat) population was based on mean changes from baseline to endpoint. The same

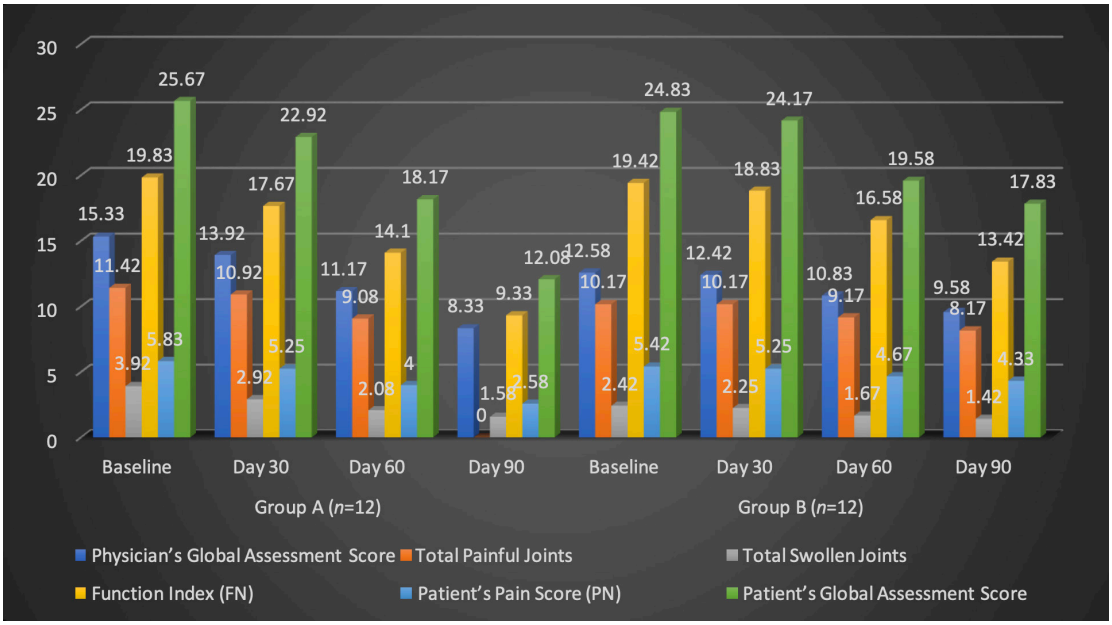

Figure 2 Effect of IP on ACR20 criteria

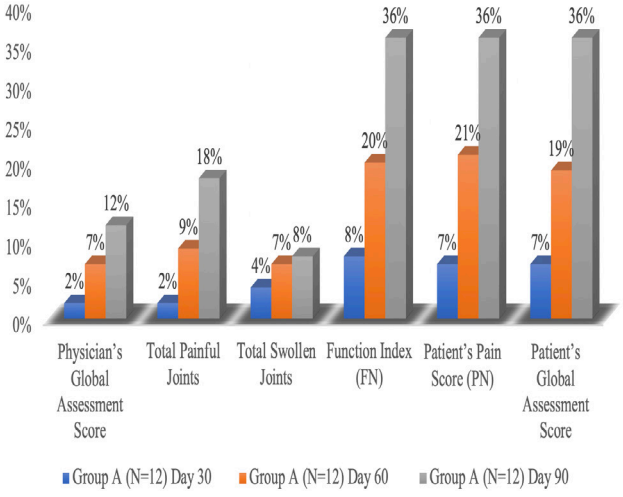

Figure 3a
Improvements in ACR20 criteria in BioSOLVE Curcumin® group

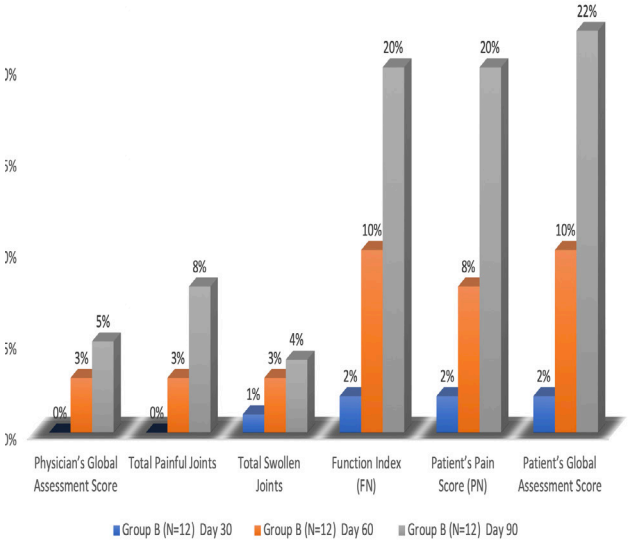

Figure 3b
Improvement in ACR-20 Criteria in Placebo group

statistical software products were used for this analysis: IBM SPSS-20 and Microsoft Excel (version 13), both of which included additional data analysis features. To exclude the null hypothesis, only results with p=0.05 were considered statistically significant. To ensure that these studies were parametric, i.e., normally distributed, they were subjected to paired and unpaired t-test analyses.

The WOMAC index and its sub-scales

When compared to the baseline, the interventional group had a progressive decrease in WOMAC subscale scores. On Day 30 (p=0.05), Day 60 (p=0.05), and Day 90 (p=0.05), there was a statistically significant decrease in pain score and stiffness intensity within the group (see **Figs 4, 5**). On Day 60 (p=0.05) and Day 90 (p=0.05), there was a significant decrease in the difficulty of functional ability within the group (see **Fig. 6**). On Day 90, there was a considerable reduction in stiffness intensity (p=0.017) and difficulty regarding physical function (p=0.05) in the placebo group.

On days 60 (p=0.05) and day 90 (p=0.05), there was a considerable reduction in the total WOMAC index in both the interference and placebo groups (**Fig. 4**). The reduction in pain score in the placebo group was insignificant at all visits. The p-values of intergroup comparisons for the sub-scales are presented in **Table 7**.

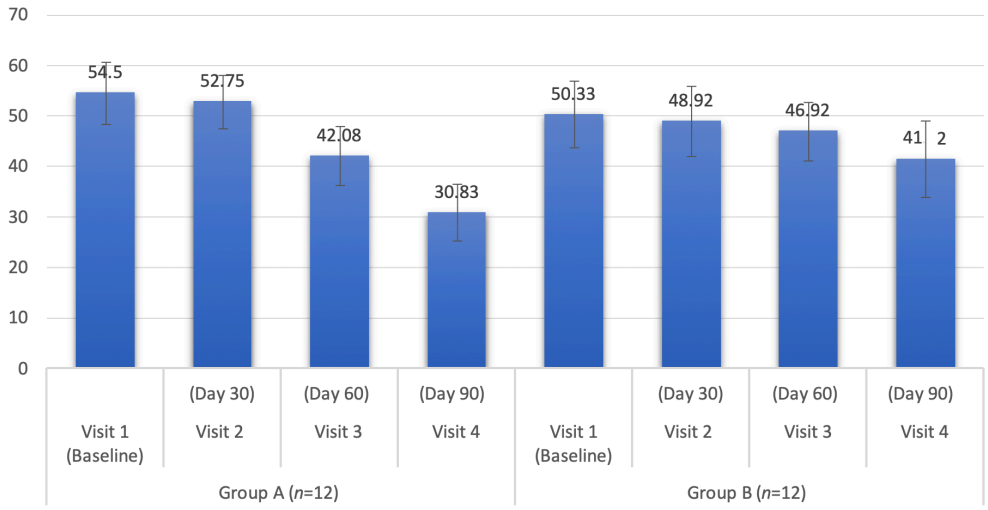

Figure 4
Effect of IP on the WOMAC index

Parameters	Group A (N=12) Visit 1 (Baseline)	Visit 2 (Day 30)	Visit 3 (Day 60)	Visit 4 (Day 90)	Group B (N=12) Visit 1 (Baseline)	Visit 2 (Day 30)	Visit 3 (Day 60)	Visit 4 (Day 90)	Intergroup p at EOS
WOMAC Pain	11.33 ± 1.72	10.00 ± 1.48	7.50 ± 1.00	5.00 ± 1.54	10.42 ± 1.78	10.42 ± 1.78	9.67 ± 1.30	8.75 ± 2.26	**0.0001**
Change from baseline	-	1.33 ± 0.49	3.83 ± 1.53	6.33 ± 1.67	-	0.00 ± 1.28	0.75 ± 1.96	1.67 ± 3.11	**0.0002**
Within-group p	-	**< 0.001**	**< 0.001**	**< 0.001**	-	1	0.211	0.09	-
WOMAC Stiffness	4.83 ± 0.83	4.33 ± 0.98	3.50 ± 0.67	2.50 ± 0.52	4.08 ± 0.79	4.00 ± 0.85	3.92 ± 0.90	3.25 ± 0.45	**0.0011**
Change from baseline	-	0.50 ± 0.52	1.33 ± 0.65	2.33 ± 0.98	-	0.08 ± 0.29	0.17 ± 0.39	0.83 ± 1.03	**0.0014**
Within-group p	-	**0.007**	**< 0.001**	**< 0.001**	-	0.338	0.166	**0.017**	-
WOMAC Physical Function	39.08 ± 4.78	38.08 ± 4.76	31.17 ± 5.37	23.75 ± 4.59	34.58 ± 5.98	34.92 ± 4.74	33.42 ± 4.87	28.92 ± 5.79	**0.0246**
Change from baseline	-	1.00 ± 2.92	7.92 ± 4.12	15.33 ± 5.18	-	-0.33 ± 4.36	1.17 ± 5.04	5.67 ± 4.42	**< 0.001**
Within-group p	-	0.260	**< 0.001**	**< 0.001**	-	0.795	0.439	**0.001**	-
WOMAC Index	54.50 ± 6.16	52.75 ± 5.29	42.08 ± 5.87	30.83 ± 5.62	50.33 ± 6.60	48.92 ± 6.95	46.92 ± 5.81	41.42 ± 7.56	**0.0008**
Change from baseline	-	1.75 ± 4.20	12.42 ± 5.28	23.67 ± 7.20	-	1.42 ± 2.31	3.42 ± 1.78	8.92 ± 6.67	**< 0.001**
Within-group p	-	0.176	**< 0.001**	**< 0.001**	-	0.057	**< 0.001**	**0.0007**	-

Table 7 Effect of IP on WOMAC index [Mean ± SD]

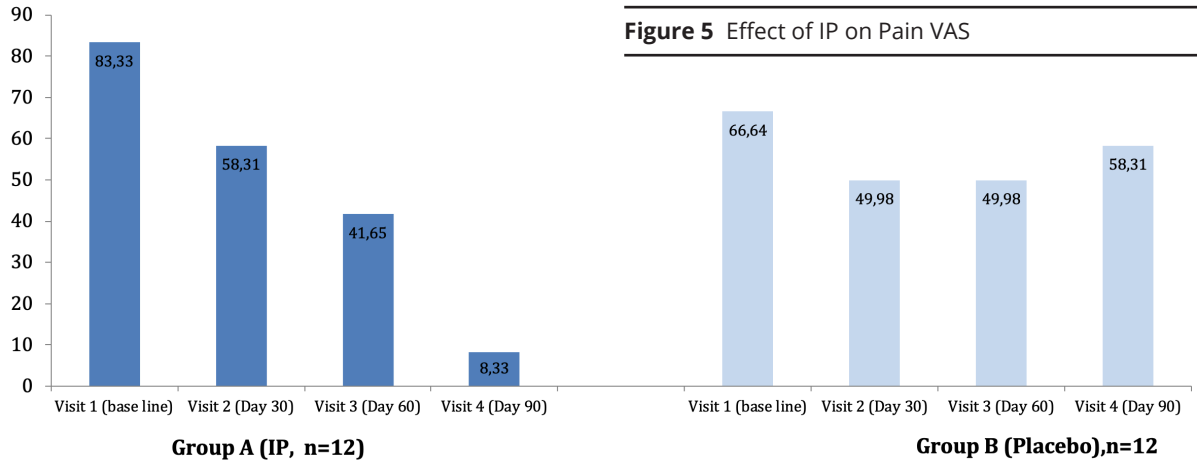

Figure 5 Effect of IP on Pain VAS

Pain VAS score difference

Groups	Group A (N=12)								Group B (N=12)							
Visit / Parameters	Visit 1 (Baseline)		Visit 2 (Day 30)		Visit 3 (Day 60)		Visit 4 (Day 90)		Visit 1 (Baseline)		Visit 2 (Day 30)		Visit 3 (Day 60)		Visit 4 (Day 90)	
M=Moderate S= Severe	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S
Number	2	10	5	7	7	5	11	1	4	8	6	6	6	6	5	7
%	16.67	83.33	41.65	58.31	58.31	41.65	91.63	8.33	33.32	66.64	49.98	49.98	49.98	49.98	41.65	58.31
% Change from baseline	-	-	24.98	-25.02	41.64	-41.68	74.96	-75	-	-	16.66	-16.66	16.66	-16.66	8.33	-8.33

Table 8
Effect of IP on pain VAS [Mean ± SD]

The pain VAS score indicates that, at the end of 30 days of supplement, Group A showed a significant reduction (25%) in participants moving from the severe pain category to the medium pain category, indicating a significant reduction in pain associated with RA in enrolled study individuals and suggesting a rapid onset of action. Interestingly, only one individual remained in the severe pain category at the completion of the 90-day treatment in the BioSOLVE Curcumin® group (**Fig. 7**).

Overall, about 75% of participants benefited from BioSOLVE Curcumin® supplement, according to the statistics. However, in the case of the placebo group (Group B), there was a minimal improvement in pain score as measured on Day 30 (16.66%), Day 60 (16.66%), and Day 90 (8.33%). Compared to Group A, this improvement was negligible, and Day 90 data shows nearly identical numbers of participants in the moderate and severe categories at baseline for the placebo group.

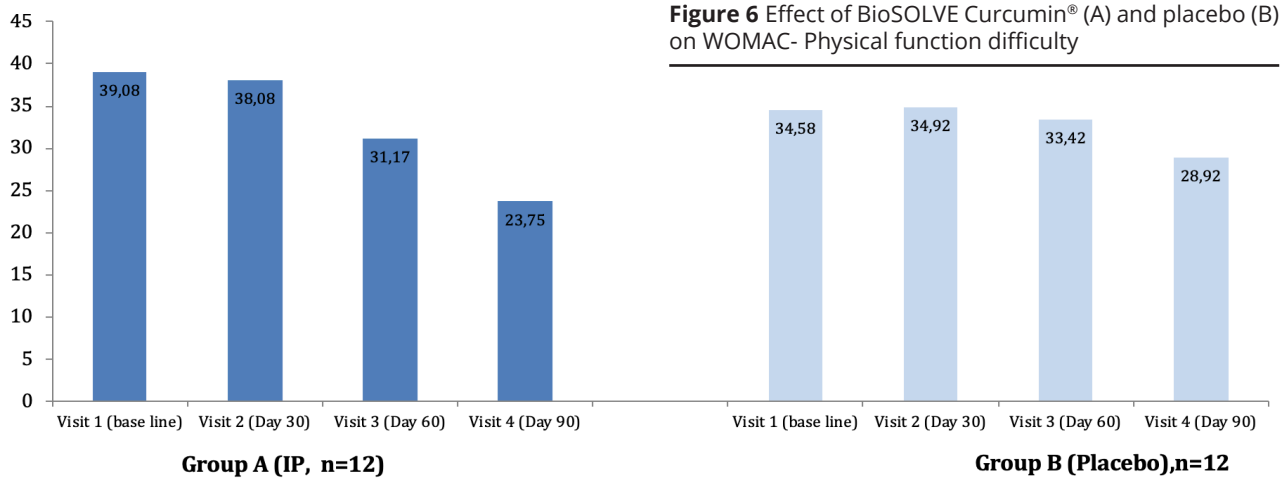

Figure 6 Effect of BioSOLVE Curcumin® (A) and placebo (B) on WOMAC- Physical function difficulty

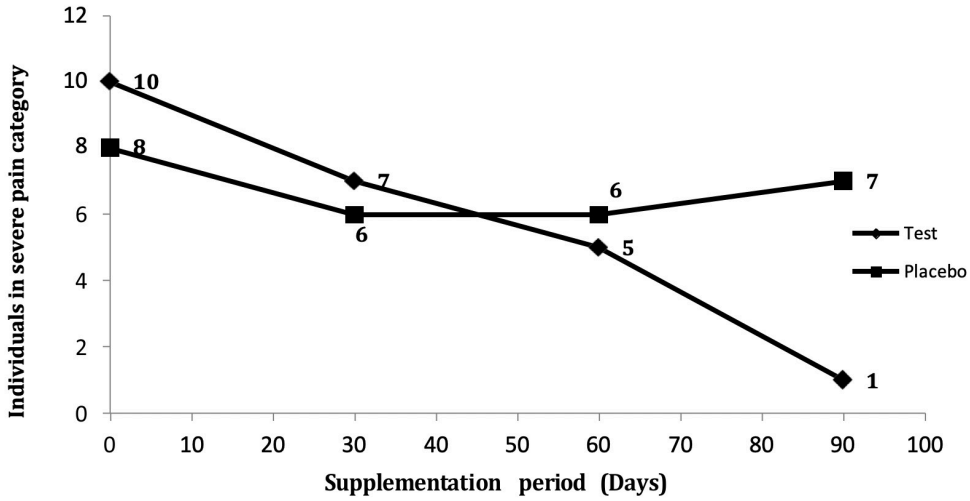

Figure 7
Effect of test (BioSOLVE Curcumin®) and placebo on pain VAS score

Parameters	Group A (N=12)		Group B (N=12)	
	Baseline	End of Study	Baseline	End of Study
Systolic BP (mm Hg)	123.17 ± 8.38	120.17 ± 7.36	122.67 ± 4.77	119.08 ± 2.81
Diastolic BP (mm Hg)	81.33 ± 6.68	78.92 ± 7.79	80.33 ± 4.42	74.67 ± 3.73
Heart rate (Beats per minute)	76.50 ± 5.14	84.17 ± 6.58	77.42 ± 4.56	86.75 ± 0.97
Respiratory rate (Breaths per minute)	14.83 ± 1.75	14.67 ± 0.65	15.08 ± 1.93	14.58 ± 0.67

Table 9
Effect of IP on vital signs [Mean ± SD]

Evaluation of safety

Curcumin is a traditional natural herb with a well-established safety record. The current research did not anticipate any safety concerns for BioSOLVE Curcumin® throughout the studied period of 90 days. Blood pressure, heart rate and respiratory rate were evaluated and compared at the start of study and at the end of study. Throughout the trial period, both therapies had vital parameter values that were well within the clinically normal range.

Adverse events

Participants were evaluated for adverse events and serious adverse events throughout the research. None occurred during the study period of 90 days.

Discussion

While the specific mechanisms are still unknown, curcumin inhibits inflammation by interacting with a variety of cellular targets [11]. Curcumin should first be oxidatively activated before it can exert its antioxidant and anti-inflammatory properties [12]. Curcumin appears to work via binding to the proteins COX-2, lipoxygenase, and GSK3b, according to the majority of research. Reduced expression of these bioactive chemical mediators contributes to curcumin's anti-inflammatory properties. Mechanism of action of curcumin is similar to that of NSAIDs, a frequent treatment for osteoarthritis. The inducible COX-2 isoenzyme, which controls inflammation, is inhibited by NSAIDs like celecoxib. Nevertheless, as research reveals that COX-2 inhibitors diminish prostacyclin synthesis, an antithrombotic substance,

the cardiovascular safety of COX-2 inhibitors and non-specific characteristics of NSAIDs has grown more worrying [13]. Curcumin inhibits the expression of matrix metalloproteinase-3 (MMP3) in osteoarthritis synovial cells, according to other investigations. MMP3 is a protein that breaks down extracellular matrix proteins in both normal and diseased processes, such as arthritis and tumour development. In osteoarthritis, decreasing MMP3 should reduce inflammation and cartilage deterioration [14].

Curcumin also inhibits the NF-KB pathway, which reduces inflammation [15]. It has antioxidant properties in addition to its anti-inflammatory properties [16]. Curcumin's therapeutic benefits in targeting various disease processes could be explained by the involvement of these pathways in normal rheumatic disease progression [17].

Curcumin is insoluble in water, unreactive, and has poor pharmacokinetic properties. The bioavailability in humans is influenced by these qualities, which carries a direct impact on its therapeutic benefits [18]. The most significant limitation of curcumin is a high daily dose and poor bioavailability [19]. Curcumin's low bioavailability is primarily due to pharmacokinetic issues such as low absorption in the small intestine, extensive phase I and II conjugative and reductive biotransformation in the liver, and immediate elimination via the gall bladder. Aside from such pharmacodynamics, enterocyte complexation affects curcumin transformation, resulting in changes [20]. In this study, major improvements in ACR20 criteria, WOMAC index and sub-scales, and VAS pain were observed in the intervention group at a relatively lower daily dose of 500 mg, which could be attributed to enhanced bioavailability and *in vivo* properties of BioSOLVE Curcumin®.

The use of varied curcumin formulations and technologies used in different studies, makes it difficult to directly compare dosage and therapeutic results across all studies.

However, the beneficial role of curcumin in disease progression is supported by numerous positive outcomes of clinical trials in osteoarthritis. Furthermore, studies have revealed that curcumin has the same efficacy as common NSAIDs such as ibuprofen, which cause serious gastrointestinal side-effects. Curcumin, by contrast, has been shown to have few or no adverse events. Follow-up assessments in additional research should be conducted to evaluate long-term effects, and additional research in RA, lupus, and other autoimmune diseases is warranted.

Conclusion

Based on the toxicology study findings, the acute oral LD50 of test product was calculated to be 5000 mg/kg bw. According to the GHS classification system, the test item, BioSOLVE Curcumin® powder, falls into category '5' with an LD50 cut-off value of 5000 mg/kg body weight.

The effect of BioSOLVE Curcumin®, a specialized formulation containing highly bioavailable water dispersible curcumin, on the symptoms of RA in selected individuals were investigated in this clinical study. The efficacy and safety of BioSOLVE Curcumin®, was evaluated at a dose of 250 mg capsule supplemented twice daily for 12 weeks for RA symptoms. The primary outcome was ACR20 modification; secondary outcome measures included the WOMAC Index and Pain VAS. Compared to the placebo group, the active group experienced a significant improvement in RA symptom relief, a significant increase in ACR20, and a very promising decrease in WOMAC and VAS pain score. BioSOLVE Curcumin® significantly reduced the painful symptoms associated with RA and improved physical function in otherwise healthy middle-aged and older RA participants.

In conclusion, supplementation for 12 weeks with BioSOLVE Curcumin® was safe, effective and well-tolerated in all individuals in

the current study. Despite the current data being very promising, this could be considered as a starting point for future long-term studies with the product alone or in combination with existing standard therapies in RA, which will provide a comprehensive picture of the complete functionality of BioSOLVE Curcumin®.

Conflict of Interest

Shankaranarayanan Jeyakodi and *Arunkanth Krishnakumar* work for Zeus Hygia LifeSciences, a nutraceutical research company.

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