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Original Article

Mangifera indica L. extract tablets supplementation in patients with knee osteoarthritis pain. A controlled pilot study

[Suplementación con tabletas de extracto de *Mangifera indica* L. en pacientes con dolor por osteoartritis de rodilla. Un estudio piloto controlado]

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Abstract

Context: Several experimental results and clinical reports using *Mangifera indica* L extract (MSBE) suggest its potential utility in osteoarthritis (OA) mixed pain.

Aims: To examine the possible therapeutic effects and safety of supplementation on osteoarthritis (OA) pain.

Methods: Fifty patients with painful knee OA who had undergone a year of conventional treatment that included paracetamol and non-pharmacological therapies were randomly allocated to the experimental group (n = 21), which received a daily dose of 900 mg of extract supplementation or preceding usual treatment and placebo in the same form (n = 17) for a period of 120 days. The primary measure outcome was the change in the average daily pain diary score (ADPS) using the Likert scale. Also, a multidimensional measure of pain, stiffness and functional disability on The Western Ontario and Mc Master Universities (WOMAC) index for knee OA and ultrasonographic chronic signs of synovitis such as effusion and synovial thickness were evaluated.

Results: Change from baseline in ADPS of the MSBE supplemented group showed a significant reduction after two weeks that lasted for 120 days with respect to the placebo group. Significant improvements in pain and functional disability WOMAC sub-scores, number of joints with synovial thickness and effusion after MSBE supplementation vs. placebo were observed. Non-adverse effects were reported in the experimental group.

Conclusions: These results suggest that MSBE supplementation has a beneficial effect on OA pain and disability.

Keywords: inflammation; *Mangifera indica* extract; mangiferin; osteoarthritis; pain.

Resumen

Contexto: Varios resultados experimentales e informes clínicos que utilizan extracto de *Mangifera indica* L (ECAM) sugieren su utilidad potencial en el dolor mixto de la osteoartritis (OA).

Objetivos: Examinar los posibles efectos terapéuticos y la seguridad de la suplementación con MSBE sobre el dolor de la osteoartritis (OA).

Métodos: Cincuenta pacientes con artrosis de rodilla dolorosa que habían recibido un año de tratamiento convencional que incluía paracetamol y terapias no farmacológicas fueron asignados aleatoriamente al grupo experimental (n = 21), que recibió además una dosis diaria de 900 mg de suplemento con el extracto o el tratamiento precedente habitual y placebo en la misma forma (n = 17) durante un período de 120 días. El resultado primario de medida fue el cambio en el puntaje diario promedio del dolor (PDMD) usando la escala Likert. Además, se evaluó una medida multidimensional del dolor, la rigidez y la discapacidad funcional en el índice *The Western Ontario and Mc Master Universities* (WOMAC) para la artrosis de rodilla y los signos ecográficos crónicos de sinovitis, como el derrame y el grosor sinovial.

Resultados: El cambio desde el inicio en PDMD del grupo suplementado con ECAM mostró una reducción significativa después de dos semanas que duró 120 días con respecto al grupo placebo. Se observaron mejoras significativas en las subpuntuaciones WOMAC de dolor y discapacidad funcional, número de articulaciones con engrosamiento sinovial y derrame después de la suplementación con ECAM frente a placebo. No se informaron efectos adversos en el grupo experimental.

Conclusiones: Estos resultados sugieren que la suplementación con ECAM tiene un efecto beneficioso sobre el dolor y la discapacidad por OA.

Palabras Clave: dolor; inflamación; *Mangifera indica* extracto; mangiferin; osteoartritis.

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INTRODUCTION

Osteoarthritis (OA) is the most frequent musculoskeletal disorder, with a significant influence on the quality of life of the population (GBD 2015 DALYs and HALE Collaborators, 2016; Kolasinski et al., 2020). Currently, the therapeutic approaches to treating OA are limited because no drugs are available, which control disease progression, and treatment with analgesic compounds has reduced efficacy and significant side effects (Kolasinski et al., 2020). Therefore, the development of effective disease-modifying drugs and new therapeutic strategies according to OA phenotypes is urgently required (Poulet and Staines, 2016; Van Spil et al., 2019). Several studies have revealed the role of nitroxidative stress and inflammation in the progression of this disease (Salvemini et al., 2011; Robinson et al., 2019). Likewise, more of these molecular mechanisms are implicated in the peripheral and central sensitization underlying chronic pain, which is its main clinical manifestation (Neogi et al., 2016; Arendt-Nielsen, 2017). Subsequently, neuroimmune activation, mitochondrial dysfuction and oxidative stress have been accepted as new targets for therapeutic intervention in OA pain (Salvemini et al., 2011; Bjurström et al., 2020). In the early stages of OA, pain is usually restricted to the affected joint, but persistent pain may result in a progression to more regional or even widespread symptoms. Therefore, clinically, OA patients suffer secondary hyperalgesia as a consequence of pain hypersensitivity by central neural plasticity (Arendt-Nielsen, 2017). In addition, several studies using the mono-iodoacetate (MIA) induced-rat knee OA demonstrated that neuronal damage occurs in OA, probably in relation to the neuroinflammatory environment suggesting the involvement of a real neuropathic component (Ferreira-Gomes et al., 2010; Nascimento et al., 2011). The contribution of the spinal glial cell to distal allodynia has also been reported in this OA model (Sagar et al., 2011).

Polyphenols derived from natural products are able to modulate inflammation-related gene expression by means of their regulation (Shen et al., 2012). The *Mangifera indica* L extract (MSBE), developed in Cuba, contains a definite mixture of components, including polyphenols, triterpenes, flavonoids, phytosterols, fatty acids, and microelements (Núñez-Sellés et al., 2002). Previously, several properties including antioxidant (Martínez Sánchez et al., 2003; Pardo-Andreu et al., 2008), anti-inflammatory, analgesic (Garrido et al., 2001; 2004a; 2004b), immunomodulatory (García et al., 2002) and neuroprotective (Lemus-Molina et al., 2009) were recognized. This extract and mangiferin, its major component (about 15–20% in the extract), prevent tumor necrosis factor *alpha* (TNF-α)-induced IκB degradation and the binding of nuclear factor *kappa* B (NF-κB) to the DNA (Garrido et al., 2005). Our previous studies advised that MSBE could be used to treat chronic pain with neuropathic or inflammatory components supported in preclinical data (Garrido-Suárez et al., 2014a). Moreover, some clinical reports using MSBE (Vimang®) pharmacological formulations in chronic pain conditions have been published (Garrido-Suárez et al., 2011; Valverde et al., 2009; López-Mantecón et al., 2014). In addition, several experimental results suggest that mangiferin could be an attractive multitarget molecule with potential utility in OA mixed pain (Garrido-Suárez et al., 2018; 2020) without toxicity (Prado et al., 2015). MSBE formulation could modulate specific targets of OA and other molecular targets involved in peripheral and central pain mechanisms, and even it could prevent neuronal injury in OA joints that are linked to disease severity and progression. This study aimed to evaluate the therapeutic effects of MSBE supplementation on pain control and functional improvement in relation to chronic OA synovitis. Besides, it has shown some preliminary evidence about tolerance and safety in knee OA patients treated with simple analgesics in the context of multimodal pharmacotherapy and domiciliary physical exercise program.

MATERIAL AND METHODS

Study design and setting

This was a randomised, double blind, placebo-controlled parallel group pilot study that took place in the Centro de Referencia de Enfermedades Reumatológicas at "10 de Octubre" Hospital (Havana, Cuba) between June and November 2014. The protocol (number: SC00509) was approved by the Ethic Committee from this hospital and the Centro de Investigación y Desarrollo de Medicamentos (CIDEM) in accordance with ICH Good Clinical Practice Guidelines and the Helsinki Declaration. Written informed consent was obtained from each patient after being fully informed of the study details. This study was not registered as a clinical trial as it did not meet applicable clinical trial guidelines under the Cuban regulatory authority, the Centro para el Control Estatal de Medicamentos, Equipos y Dispositivos Médicos (CECMED) Act.

Study participants

The study was conducted in patients with primary knee OA of the medial tibiofemoral compartment diagnosed according to the clinical and radiographic

criteria of the American College of Rheumatology (ACR) (Altman et al., 1986). Grade I-IV radiographic OA cases, according to the Kellgren and Lawrence grading system, were accepted (Kellgren and Lawrence, 1957). Adult patients (between 45 and 80 years) of either sex with symptomatic knee [pain score ≥ 4 on an 11-point numeric scale (Likert scale) for at least a month before enrolment] were included. Patients that received treatment with non-pharmacological therapies (education therapies and domiciliary physical exercise program) and paracetamol as a symptomatic treatment for episodic use during a year were accepted. Patients with other concomitant connective tissue diseases, active infections or malignancy, treatment with symptomatic slow-acting or disease-modifying OA drugs in the three months preceding enrolment, corticosteroid administration in the month prior to enrolment, and invasive therapies to the knee were excluded. Patients with psychiatric disorders were also excluded.

Interventions

Eligible patients who had signed the informed consent form were randomised sequentially into two groups by means of a computer-generated randomisation list, which was held securely, and released only after study completion. Group I (MSBE; Exercise and paracetamol-MSBE): Treated with paracetamol (1000-3500 mg/days) plus a daily dose of 900 mg of extract (coated MSBE tablets, 300 mg each, three times daily before meals); and Group II (placebo; Exercise and paracetamol-placebo): Treated with paracetamol (1000-3500 mg/days) plus placebo tablets three times daily before meals. The MSBE doses were selected according to previous reports (Garrido-Suárez et al., 2011; López-Mantecón et al., 2014) and on early case series study in knee OA patients (Valverde et al., 2009). The treatments were performed for 120 days. Concomitant medication with paracetamol, constituted a control outcome in this study and could be progressively withdrawn by the patient according to necessity. The administration of dipyrone (300 mg tablets) as an analgesic rescue dosage was also controlled. All patients maintained the same individualised home-based exercise program, which was previously recommended and supervised by its physical therapist through the year before its enrolment in this study. This comprises an isometric training and progressive isotonic exercises for muscle strengthening (quadriceps, ischiotibial, gluteus medius and maximus, adductors, and sural triceps) starting with five repetitions and increasing its number to a maximum of 25 repetitions in each series at least 3 times a week (Friol-González et al., 2002).

Preparation of drugs

Vimang® (tablets 300 mg) and placebo tablets having similar physic characteristics and compositions, except for active MSBE, were purchased from Laboratorios Oriente (Santiago, Cuba). The active pharmaceutical ingredient of Vimang® tablets was a standardised aqueous extract obtained from the stem bark of *Mangifera indica* (MSBE). MSBE is prepared by decoction for 1 h and concentrated by evaporation and spray dried in a Niro Atomizer Standard Spray Drying (Soeborg, Denmark) to obtain a fine homogeneous brown powder with a particle size of 30-60 µm, which melts at 210-215°C, with decomposition (Acosta-Esquijarosa et al., 2009). Vimang® tablets weighing 300 mg were prepared using a tablet press machine with the MSBE powder equivalent to 115 mg of total polyphenols expressed as tannins (Lemus-Rodriguez et al., 2006). The chemical composition of this extract has been characterised by chromatographic (planar, liquid and gas) methods, mass spectrometry, UV/VIS spectrophotometry and NMR spectroscopic methods. As shown in Table 1, these investigations have led to the isolation of seven phenolic constituents: gallic acid, 3,4-dihydroxy benzoic acid, gallic acid methyl ester, gallic acid propyl ester, (+)-catechin, (-)-epicatechin, and mangiferin, which was found to be the predominant component (Núñez-Sellés et al., 2002). Other studies have been carried out for the identification of terpenoids (β-elemene, aromandrene, α-guaiene, β-selinene, hinesol, β-eudesmol, and cycloartanols), phytosterols (β-sitosterol and β-campesterol), fatty and dicarboxylic acids (myristic, palmitic, linoleic, oleic, stearic, eicosatrienoic, succinic, and malonic), among others (Núñez-Sellés et al., 2002; Curiel Hernández et al., 2007). The capillary electrophoresis (CE) method for the quantitative analysis of mangiferin, which was selected as a marker in the quality control of active pharmaceutical ingredient and its formulations, was validated (Lemus-Rodriguez et al., 2006; Curiel Hernández et al., 2007; 2011).

Paracetamol tablets 500 mg and dipyrone tablets 300 mg (Laboratorios Medsol, La Habana, Cuba) were also utilised in this study.

Study procedures and outcome measures

The primary measure outcome was the change in average daily pain score (ADPS) using the Likert scale, where 0 indicates the absence of pain and 10 the worst pain imaginable (baseline week versus final week of the study) evaluated from the daily pain diary (Garrido-Suárez et al., 2011). Secondary outcomes included: multidimensional measure of pain, stiffness, and functional disability on The Western Ontario and Mc Master Universities (WOMAC) index for

Table 1. Chemical composition of MSBE raw material for pharmaceutical formulations.

Component	Content (%)	Component	Content (%)
Polyphenols	**40-60**	**Polyalcohols**	**3-5**
Mangiferin		**Sorbitol**	
(+) Catechin		Myoinositol	
(-) Epicatechin		Xylitol	
Gallic acid, propyl ester		**Fatty acids**	**1-3**
Gallic acid, methyl ester		Myristic	
Benzoic acid, propyl ester		Palmitic	
3,4-Dihydroxibenzoic acid		Linoleic	
Benzoic acid		Oleic	
Gallic acid		Estearic	
Lignans		Eicosatrienoic	
Terpenoids	**10-20**	**Phytosterols**	**1-3**
β-Elemene		β-Sitosterol	
Aromandrene		β-Campesterol	
α-Guaiene		**Elements**	**1**
β-Selinene		Calcium	
Hinesol		Potassium	
β-Eudesmol		Magnesium	
Cycloartanols		Iron	
Free Sugars	**5-8**	Selenium	
Glucose		Copper	
Galactose		Zinc	
Arabinose			

(Núñez-Sellés et al., 2002; Martínez Sánchez et al., 2003; Lemus-Rodriguez et al., 2006)

knee OA (Bellamy et al., 1988). The clinical outcomes were evaluated during five visits, before treatment and at 4, 8, 12, and 16 weeks (final visit). Information from patients' pain diaries were collected at each clinic visit. In addition, ultrasonographic (US) outcomes of chronic inflammatory synovitis as the amount of synovial effusion and thickness were determined using an ALOKA 1100 device with a 7.5 mHz transducer of the soft tissue of both knees. Synovitis was defined as hypoechoic synovial hypertrophy with thickness ≥4 mm with the knee semi-flexed at 45% on the medial median longitudinal plane crossing the quadriceps tendon. Joint effusion was defined as an anechoic area and was measured in the supra-patella recess with the leg in full extension and measured at the maximal depth observed with a longitudinal scan. It was recorded as absent if the effusion depth was <4 mm and present if ≥4 mm (D'Agostino et al., 2005). Other inflammatory lesions in knee joints, such as chondropathy by synovial plica, perimeniscitis, tendonitis or bursitis were also informed. US outcomes were assessed at baseline and at the end of the treatment (120 days).

Information on adverse events, dosage and administration frequency of the accepted analgesic concomitant (paracetamol) and rescue (dipyrone) drugs were also collected in the patient diary. In addition to adverse events, safety endpoints included the collection of laboratory safety data containing the haematological profile and a serum-chemistry profile of aspartate transaminase (AST), alanine transaminase (ALT) and serum creatinine level, as well as electrocardiogram and vital signs (sitting/standing blood pressure, pulse rate).

Statistical analysis

Data were analysed using the statistical program Graph Pad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). A comparison of the mean of each clinical outcome before and after treatment was carried out using one-way ANOVA followed by Bonferroni's or

Turkey's multiple comparison tests. Continuous variable data was expressed as the mean ± standard error of the mean (S.E.M). Categorical variables were expressed as percentages and compared using the χ^2 test or Fisher exact test, as appropriate. Values of $p<0.05$ were considered statistically significant.

RESULTS

Characteristics of study participants

In this study, 50 patients were screened, and 44 were randomised. Of the six patients withdrawn prior to randomisation, in all cases, they did not meet the inclusion criteria. In total, 22 patients were randomised to MSBE supplemented to conventional treatment with exercise and paracetamol and 22 to its conventional treatment and placebo. During the first weeks of the study, 6 (2.6%) patients withdrew from the trial (Fig. 1). The most common reasons for withdrawal were nausea, vomiting and rash observed in three patients treated with dipyrone and paracetamol, and other reasons like non-attendance at scheduled visits and travelling to other provinces, among others.

Compliance with the study treatment was 96% for the MSBE group and 95.3% for the placebo group, with no significant differences between them. The treatment groups were well matched at baseline demographic characteristics, radiographic grade OA, clinic parameters and conventional treatment (Table 2). No statistically significant differences in ADPS and sub-scores (pain, stiffness and functional disability) on The Western Ontario and Mc Master Universities (WOMAC) were noted between groups, and the same was true for the concomitant medication received during the study. The baseline ADPS was similar for the MSBE and placebo groups: 5.6 ± 0.3 and 5.5 ± 0.3, respectively. Baseline individual patient characteristics and concomitant analgesic treatment (% patients) before and at the end of the study (120 days) are shown in Table 3.

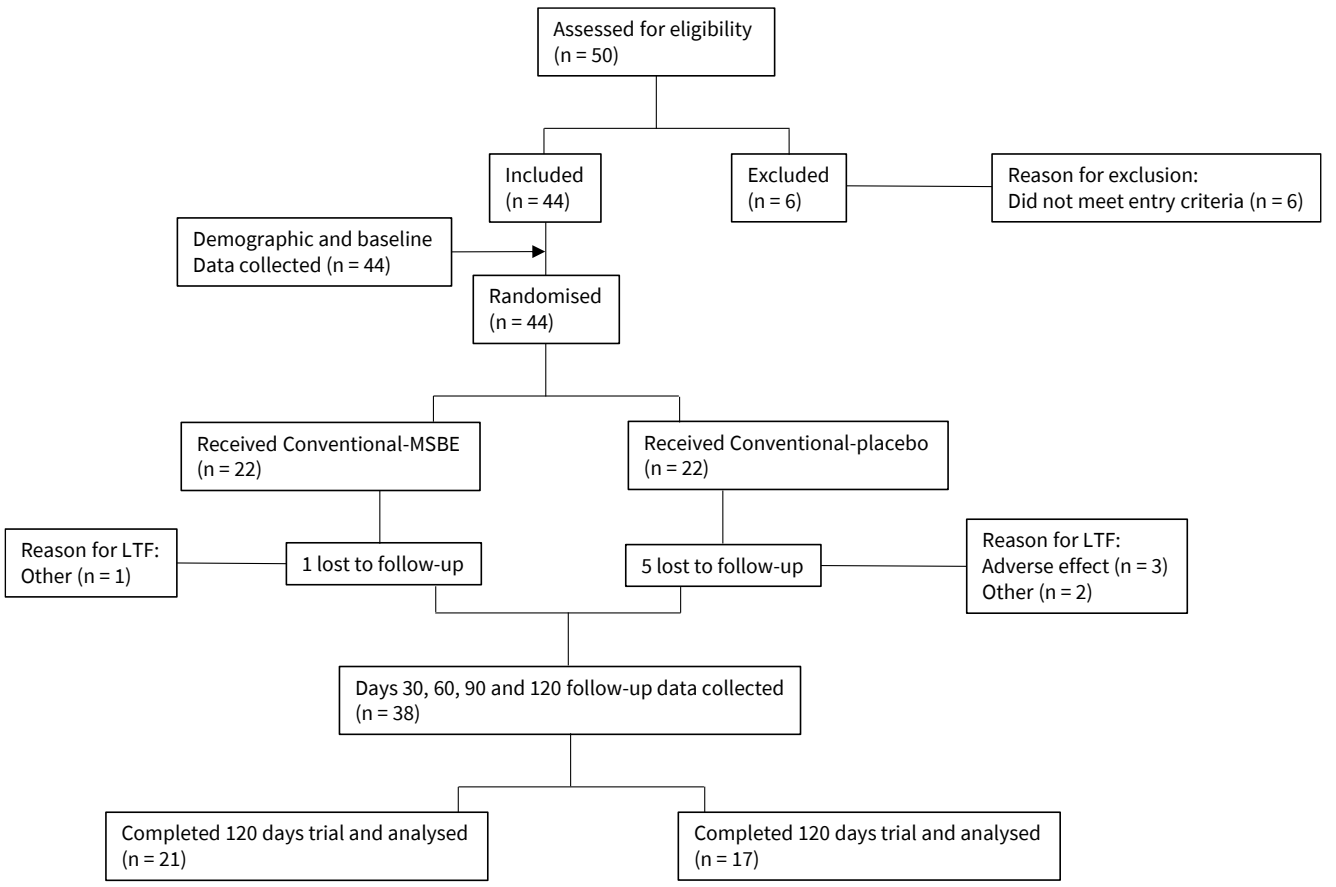

Figure 1. Flow of the patients through the trial.

Table 2. Demographic and clinical characteristics of patients with painful knee OA at baseline.

Parameter	MSBE-group	Placebo-group	P-value
Age (years)	69 ± 2.5	68 ± 2.5	NS
Women, n (%)	14 (66.7)	11 (64.7)	NS
Men, n (%)	7 (33.3)	6 (35.3)	NS
Weigh (kg)	72.8 ± 2.0	70.2 ± 2.0	NS
Height (cm)	158 ± 0.0	161 ± 2.0	NS
Body mass index (kg/cm^2)	29 ± 0.8	27 ± 0.9	NS
Grade radiographic OA			
I – II, n (%)	7 (33.3)	6 (35.3)	NS
III – IV, n (%)	14 (67.0)	11 (65.0)	NS
ADPS	5.6 ± 0.3	5.5 ± 0.3	NS
WOMAC pain (0 – 10)	9.1 ± 0.6	9.2 ± 0.9	NS
WOMAC stiffness	3.7 ± 0.3	3.9 ± 0.3	NS
WOMAC functional disability	30.8 ± 2.2	31.1 ± 3.3	NS
Concomitant medication			
Paracetamol, n (%)	16 (76.2)	13 (76.5)	NS
Dipyrone (rescues), n (%)	7 (33.3)	5 (29.4)	NS

MSBE: *Mangifera indica* stem bark extract; NS: no significance; OA: osteoarthritis; ADPS: average daily pain diary score; WOMAC: The Western Ontario and Mc Master Universities (WOMAC) index.

Table 3. Clinical characteristics of patients after 120 days of treatment.

Parameter	MSBE-group			Placebo-group			P-value**
	Baseline	120 days	P-value*	Baseline	120 days	P-value*	
ADPS	5.6 ± 0.3	0.1 ± 0.1	<0.0001	5.5 ± 0.3	4 ± 0.2	<0.0001	<0.0001
Pain	9.1 ± 0.6	1.4 ± 0.2	<0.0001	9.2 ± 0.9	6.8 ± 0.6	NS	<0.0001
Stiffness	3.7 ± 0.3	0.7 ± 0.2	<0.0001	3.9 ± 0.3	2.2 ± 0.3	<0.0001	<0.0001
Functional disability	30.8 ± 2.2	4.4 ± 1.0	<0.0001	31.1 ± 3.3	29.2 ± 2.4	NS	<0.0001
Mild analgesics	21 (100%)	1 (4.76%)	<0.0001	17 (100%)	17 (100%)	NS	<0.0001

MSBE: *Mangifera indica* stem bark extract; ADPS: average daily pain score. The results are presented as mean ± SEM. p<0.001 indicates significant differences with respect to *baseline value of their respective group or **between groups as shows the column at right. One-way analysis of variance test followed by Bonferroni's or Turkey's multiple comparison tests. The Fisher's exact test was used to check the discrete variables, presented in proportion. Mild analgesics: Include paracetamol and dipyrone (rescues). NS: no statistically significant differences.

Therapeutic effects and efficacy measures

Change in the average daily pain diary score (ADPS): ADPS showed a significant reduction in MSBE supplementation treatment after two weeks (p<0.001), with respect to baseline data and compared with ADPS data of the placebo group (Table 3 and Fig. 2). These effects were maintained and increased until the last evaluation at 120 days (p<0.001). The average score for the placebo group was 5.5 vs. 4.0 (a reduction of 27.2%), while for MSBE it was 5.6 vs. 0.1 (a reduction of 98.2%). The difference between placebo and MSBE was 71.0% (p<0.0001).

The Western Ontario and Mc Master Universities (WOMAC) index for knee OA: As shown in Table 3 and Fig. 3, only the patients of the MSBE group showed a statistically significant improvement in pain and functional disability WOMAC sub-scores regarding baseline data (p<0.0001). In relation to WOMAC stiffness, both groups decreased the mean sub-score with respect to baseline data. However, the stiffness improvement was superior in the MSBE-supplemented group (p<0.0001).

Ultrasonographic outcomes of chronic inflammatory synovitis: At the beginning of the study, 22 joints

in 17 (80.9%) patients of the MSBE-supplemented group presented synovial thickness (6.4 ± 0.7 mm), as did 13 joints (7.2 ± 1.1 mm) in 8 (47%) patients of the control group. Despite these differences in the proportion of affected patients, the analysis was performed according to the number of joints with synovial hypertrophy (Table 4). At the end of the study, as shown in Table 5, the number of affected joints decreased exclusively in MSBE-supplemented patients (p=0.0011). In addition, joint effusion was analysed similarly: 17 joints presented deep effusion (5.5 ± 0.3 mm) in 14 (66.6%) patients of the experimental group and 13 joints (4.9 ± 0.2 mm) in 10 (58.8%) patients of the control group. After 120 days of treatment, the effusion was absent in all joints of the MSBE patient group; however, 11 joints of 9 (52.9%) patients maintained the presence of joint effusion (p<0.0001) in the non-supplemented group. In addition, other inflammatory lesions (chondropathy by synovial plica, perimeniscitis, popliteal tendonitis/synovitis, prepatellar bursitis, anserine bursitis/tendonitis), which play a role in knee pain, were observed in patients during the trial. As shown in Table 6, all patients presented one or two of these types of lesions. In total, 23 inflammatory lesions in the MSBE group, and 18 in the placebo group were observed at baseline. These improved in both groups at the end of the study; however, the total percentage of these lesions was significantly decreased (p=0.0002) in the MSBE-supplemented group.

Concomitant pharmacological treatment and adverse effects

At the beginning of the study, 13 patients (76.5%) in the placebo group and 16 (76.2%) in the MSBE group received treatment with paracetamol. Fig. 3A and 3B show the change from baseline in concomitant paracetamol and dipyrone rescues doses in both groups, respectively. The MSBE-supplemented patients significantly decreased their doses of paracetamol from week 4. For dipyrone doses, the changes only were significant at this time point. The average of patients who suspended the concomitant treatment and analgesic rescues with paracetamol and dipyrone, respectively, at 120 days is illustrated in Fig. 3C. In the MSBE group, all patients at least decreased doses (1 decreased dose and 20 suspended), and there were no patients with an analgesic rescue, in contrast to the placebo group, where all consumed paracetamol (15 patients) or were rescued with dipyrone (2 patients), and only 5 patients reported decreased doses of paracetamol, none suspended this treatment (p<0.001).

Adverse events were reported within the first two weeks in the control group, requiring withdrawal from the trial. Nausea, vomiting and rash were observed in three patients treated with dipyrone and paracetamol. No adverse effects were observed in the MSBE-supplemented patients.

Table 4. Ultrasonographic knee synovitis at baseline.

Parameter	MSBE-group, n = 21 (42 joints)			Placebo-group, n = 17 (34 joints)			P-value**
	Present	Absent	P-value*	Present	Absent	P-value*	
Synovial thickness (mm)	6.4 ± 0.7	1.1 ± 0.3	<0.0001	7.2 ± 1.1	1.7 ± 0.3	<0.0001	NS
Nº patients (%)	17 (80.9)	4 (19.0)		8 (47.0)	9 (52.9)		0.0420
Nº joints (%)	22 (52.4)	20 (47.6)		13 (38.2)	21 (61.8)		NS
Effusion depth (mm)	5.5 ± 0.3	1.2 ± 0.1	<0.0001	4.9 ± 0.2	1.3 ± 0.1	<0.0001	NS
Nº patients (%)	14 (66.6)	7 (33.3)		10 (58.8)	7 (41.2)		NS
Nº joints (%)	17 (40.5)	25 (59.5)		13 (38.2)	21 (61.8)		NS

MSBE: *Mangifera indica* stem bark extract. Data are presented as mean ± SEM. P <0.05 indicates *significant differences between their respective group or **with respect placebo-group through the trial as shown in the column at right. One-way analysis of variance test followed by Bonferroni's multiple comparison tests. The Fisher's exact test was used to check the discrete variables presented in proportion. NS: no statistically significant differences.

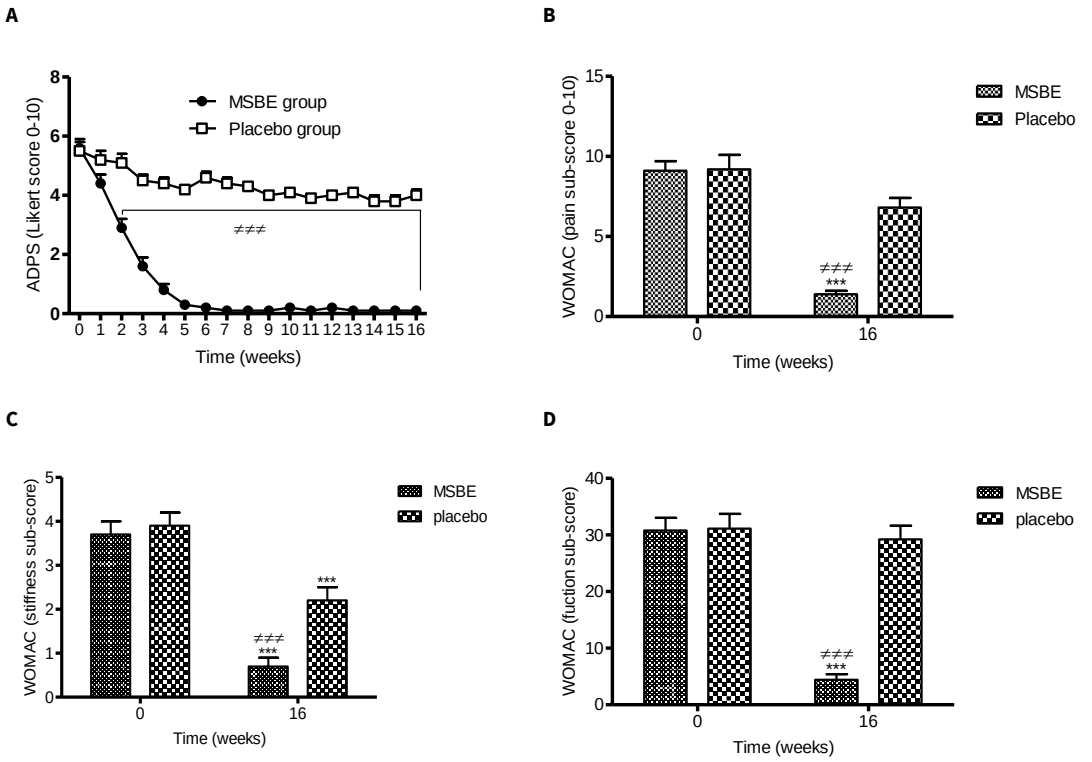

Figure 2. Change from baseline in average daily pain score (ADPS). (**A**), the Western Ontario and Mc Master Universities (WOMAC) index pain sub-score (**B**), WOMAC stiffness sub-score (**C**), WOMAC function sub-score (**D**) in painful knee osteoarthritis (OA) patients treated with exercises, paracetamol and daily dose of 900 mg of *Mangifera indica* stem bark extract (MSBE) supplementation or the same conventional treatment and placebo during 120 days.

The results are presented as mean ± S.E.M. ###p<0.001 indicates significant differences with respect to placebo, ***p<0.001 indicates significant differences with respect baseline data.

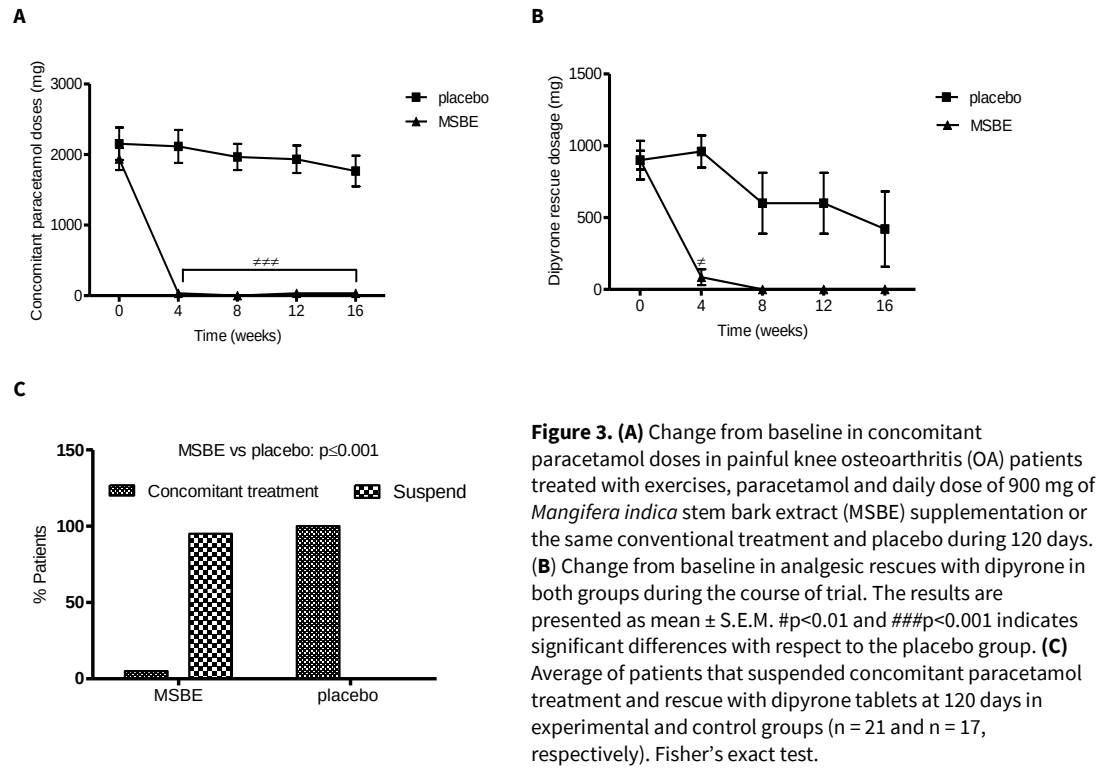

Figure 3. (A) Change from baseline in concomitant paracetamol doses in painful knee osteoarthritis (OA) patients treated with exercises, paracetamol and daily dose of 900 mg of *Mangifera indica* stem bark extract (MSBE) supplementation or the same conventional treatment and placebo during 120 days. (**B**) Change from baseline in analgesic rescues with dipyrone in both groups during the course of trial. The results are presented as mean ± S.E.M. #p<0.01 and ###p<0.001 indicates significant differences with respect to the placebo group. **(C)** Average of patients that suspended concomitant paracetamol treatment and rescue with dipyrone tablets at 120 days in experimental and control groups (n = 21 and n = 17, respectively). Fisher's exact test.

Table 5. Ultrasonographic knee synovitis after 120 days of treatment.

Parameter	MSBE-group (42 joints)			Placebo-group (34 joints)			P-value**
	Present	Absent	P-value *	Present	Absent	P-value*	
Synovial thickness (mm)	4 ± 0.8	0.2 ± 0.2	<0.05	7.4 ± 1.0	1.7 ± 0.3	<0.0001	<0.01
Nº patients (%)	5 (23.8)	16 (76.2)		8 (47.0)	9 (52.9)		NS
Nº joints (%)	7 (16.6)	35 (83.3)	0.0011	12 (35.3)	22 (64.7)	NS	NS
Effusion depth (mm)	1.2 ± 0.1	0.8 ± 0.1	NS	4.3 ± 0.4	1.6 ± 0.3	<0.0001	<0.0001
Nº patients (%)	0 (0)	21 (100)		9 (52.9)	8 (47.0)		<0.0001
Nº joints (%)	0 (0)	42 (100)	<0.0001	11 (32.4)	23 (67.6)	NS	<0.0001

MSBE: *Mangifera indica* stem bark extract. Data are presented as mean ± SEM or percentages. p<0.05 indicates *significant differences between their respective group or **with respect placebo-group through the trial as shows the column at right. One-way analysis of variance test followed by Bonferroni's multiple comparison tests. The Fisher's exact test was used to check the discrete variables presented in percentages. NS: no statistically significant differences.

Table 6. Evolution of other inflammatory lesions in knee joints through the trial.

Other inflammatory lesions	MSBE-group (n = 21)				Placebo-group (n = 17)			
	Baseline		120 days		Baseline		120 days	
	Nº	%	Nº	%	Nº	%	Nº	%
Chondropathy by synovial plica	5	21.7	2	8.7	5	27.7	4	22.2
Perimeniscitis	8	4.7	0	0.0	6	33.3	5	27.7
Popliteal tendonitis/synovitis	6	26.0	0	0.0	2	11.1	1	5.5
Prepatellar bursitis	2	8.7	0	0.0	3	16.6	2	11.1
Anserine bursitis/tendonitis	2	8.7	0	0.0	2	11.1	0	0.0
Total	23	100	2	8.7≠	18	100	12	66.6

MSBE: *Mangifera indica* stem bark extract. ≠p=0.0002 indicates significant differences with respect placebo at 120 days. Fisher's exact test.

DISCUSSION

The findings of this study provide some clinical evidence on the analgesic effect of MSBE supplementation according to changes from baseline in ADPS and significant improvements in pain and functional disability WOMAC sub-scores. In addition, it provides some elements to support the safety of MSBE tablets and its interactions with other non-opioid analgesic drugs, such as paracetamol and dipyrone, during the first four weeks of the study. Currently, the combination of pharmacotherapy remains an important useful strategy in the context of multifactorial mechanisms of chronic pain. In a clinical setting, some results suggest that non-steroidal anti-inflammatory drugs (NSAIDs) plus gabapentin or duloxetine, which shows efficacy in neuropathic pain, might be a promising option for OA patients (Enteshari-Moghaddam et al., 2019; Kolasinski et al., 2020). Likewise, natural product derivatives as curcumin display synergistic interactions with other standard drugs (Leksiri et al., 2020). Here, all patients at least decreased the doses (one patient decreased the dose, and 20 of them suspended paracetamol), and there were no patients with analgesic rescues in contrast to the placebo group, also suggesting the analgesic efficacy of MSBE. The significant analgesic effect of MSBE supplementation in the present study could be, at least in part, related to its anti-inflammatory and antioxidant abilities that have been previously associated with the presence of polyphenols in this extract (between 40% and 60%) (Garrido et al., 2001; 2004a; 2004b; Núñez-Sellés et al., 2002; Martínez Sánchez et al., 2003; Pardo-Andreu et al., 2008).

However, metabolism-based pharmacokinetic interactions between natural products rich in polyphenolic compounds and standard drugs with severe effects on the toxicity of pharmaceuticals have also been recognised (Liu and Hu, 2007; Rodeiro et al., 2009). Although paracetamol is conditionally recommended for patients with knee OA, it is frequently used in clinical settings as an alternative in patients with intolerance or contraindications to the use of NSAIDs (Kolasinski et al., 2020). Ninety percent of paracetamol is metabolised by glucuroconjugation and sulphoconjugation, while only 4% of this drug is catabolised by cytochrome P450 2E1 (CYP2E1) and

others such as CYP1A2, CYP2A6, CYP3A4 and CYP2D6 into N-acetyl-para-benzoquinone imine (NAPQI); this is rapidly reduced by glutathione and excreted in the urine (Bessems and Vermeulen, 2001). Similarly, the major metabolic pathway for polyphenols is by means of phase II conjugation (Liu and Hu, 2007). Some of the compounds found in MSBE also modulate CYP enzymatic expression or/and activity *in vitro*. Although MSBE did not modify CYP2E1, it induced the activity of CYP2B1 and reduced that of CYP1A1/2 and CYP3A4 (Rodeiro et al., 2009). Subsequently, the modulation of drug metabolic pathways by MSBE could induce potential herbal–drug interactions that could be relevant in paracetamol overdoses when the conjugation systems are saturated, shifting into the CYP pathway and producing the massive accumulation of NAPQI. Nevertheless, clinical adverse effects were not observed in the MSBE supplemented group. In addition, mitochondrial dysfunction by covalent binding to mitochondrial proteins and the depletion of hepatocellular glutathione via NAPQI have been involved in the hepatotoxic mechanisms of paracetamol (Mitchell et al., 1973). The utility of MSBE for its preventive effect on oxidative damage during hepatic injury associated with free radical generation has been also reported (Martínez Sánchez et al., 2003).

On the other hand, an interesting result was the link between the subjective and behavioural outcomes of this study, with chronic ultrasonographic signs of synovitis, such as effusion and synovial thickness. We selected the dose used in the present study from early case series because the inhibition of synovial membrane proliferation compared with initial data was only significant in patients treated with 900 mg/day, an effect that was corroborated in the present study using a major sample (Valverde et al., 2009). To date, the pivotal role of the synovial inflammatory process, nitroxidative stress, and its relation to cartilage/subchondral bone interactions in the progression of OA have been recognised (Poulet and Staines, 2016; Robinson et al., 2016). Synovitis is a source of catabolic and pro-inflammatory mediators in OA disease, which causes a disruption in the balance between cartilage matrix degradation and repair, in turn amplifying inflammatory processes. The result is a vicious cycle that promotes progressive joint degeneration (Robinson et al., 2016). Several of these molecular pathways have also been implicated in peripheral and central chronic pain mechanisms, its principal symptom (Salvemini et al., 2011; Neogi et al., 2016; Arendt-Nielsen, 2017; Vincent, 2020). The ability of dietary polyphenols to decrease OA progression and its associated symptoms has been previously reported in early studies. In particular, the anti-osteoarthritic effects of curcumin, epigallocatechin gallate and green tea extracts, resveratrol and nobiletin, have been linked to the down-regulation of inflammatory cytokines, antioxidant or anti-inflammatory pathways, and their signalling mechanisms. In addition, its anti-catabolic activity has been reported by reducing matrix degradation and chondrocyte apoptosis (Huang et al., 2010; Shen et al., 2012). Mangiferin, the main polyphenol of MSBE, has chondroprotective effects by stimulating the induction of anabolic genes such as bone morphogenetic proteins and transforming growth factor-β, as well as inhibiting the expression of catabolic genes such as matrix metalloproteinases (Huh et al., 2014). Another element that could support this suggestion is the significant involution of other inflammatory lesions in knee joints through the trial comparing MSBE-treated patients with a placebo group, which could contribute to the improvement in pain due to the possible role of soft tissues of the joint in pain in OA (Vincent, 2020).

Nevertheless, other analgesic mechanisms may be involved in the MSBE effect, as patients without ultrasonographic signs of inflammatory synovitis also showed improved ADPS. This is a fact that may be favourable in light of the current knowledge about OA pain as a mixed pain state, showing that some factors are superimposed upon the more traditional peripheral factors in the central nervous system of some patients (Arendt-Nielsen, 2017). As such, the magnitude of damage or inflammation to the synovium, cartilage and bone may fail to predict the symptoms as pain (Vincent, 2020). Previously, in a series of patients treated with MSBE formulations with a clinical-radiological diagnosis of knee OA, the analgesic effect was not proportional to the changes in the synovial effusion and synovial thickness assessed by ultrasonography, suggesting other peripheral or central analgesic mechanisms unrelated to its primary anti-inflammatory effect (Valverde et al., 2009).

Patients with OA pain display a facilitated degree of temporal summation, indicating an enhanced central integrative mechanism (central sensitisation), as well as a reduced efficacy of endogenous pain modulation (Arendt-Nielsen, 2017; Petersen et al., 2019; Barroso et al., 2020). MSBE, has a homogeneous inhibitory effect on both nociceptive behaviours (flinching and licking/biting) in the tonic phase of the formalin test, suggesting that the important fraction in this extract, possibly polyphenols, modulate spinal and supraspinal circuits (Garrido-Suárez et al., 2014a; 2014b). The critical contribution of mangiferin to MSBE antinociceptive effects was also corroborated in this model, which was related with the activity of this xanthone on spinal α_2 adrenergic receptors in cooperation with the opioid system, both involved in descending inhibitory pathways (Lopes et al., 2013; Gar-

rido-Suárez et al., 2020). MSBE also decreased the long-term injury in the formalin test, which is attributed to glial cell activation (Garrido-Suárez et al., 2014a). In particular, pro-inflammatory cytokines can activate glial cells resulting in a neuroinflammatory response that is important for generating and maintaining central sensitisation in OA pain (Sagar et al., 2011). Pronociceptive reactive oxygen and nitrogen species may contribute to developing this process, activating neuroimmune responses via the redox-sensitive transcription factor NF-κB and several mitogen-activated protein kinases, including p38 and ERK1/2, as well as inducing alterations in glutamatergic neurotransmission by nitration (Salvemini et al., 2011). By decreasing TNF-α, interleukin-1β and peroxynitrite formation, MSBE may improve the function and expression of glutamate transporters (Martínez Sánchez et al., 2003; Garrido et al., 2005).

A limitation of this pilot study is the small sample size. In future studies, it will be necessary for the inclusion of more patients. However, concomitant medication with mild analgesics and non-pharmacological therapies as exercise should be accepted, despite the recognised symptom-modifying effects and possible OA disease-modifying effects (Davis et al., 2020). This is an exploratory study, and it aimed to evaluate the possible therapeutic effects, tolerance, and safety of this natural product in a multimodal approach that is currently recommended for OA mixed pain. The major symptom of this disease is pain, which may lead to stiffness and a loss of mobility, as well as swelling around the joints (Arendt-Nielsen, 2017; Kolasinski et al., 2020); therefore, the Ethics Committee of the hospital does not accept the exposure of patients to a unique therapy that could be ineffective. Today, the treatment tendency of OA pain combines several drugs at low doses, even more, if neuroplasticity or real neuropathic component are corroborated. As a result, several phase II clinical trials have used more flexible and less experimental designs, which are more similar to clinical practice.

CONCLUSION

The results of the present pilot study suggest that MSBE supplementation could be beneficial and safe for reducing pain and disability in OA patients. Also, other specific feasibility objectives, such as the capacity and willingness of the institution, the sufficiency of eligibility criteria, and the quality of collected data, were determined. These provide the basis for the feasibility of a large expensive full-scale study to confirm this hypothesis.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Garrido-Suárez BB	Garrido G	López-Mantecón AM	Piñeros O	Castro-Lopes JM	Delgado-Hernández R
Concepts or ideas	x	x		x	x	x
Design	x					
Definition of intellectual content	x					
Literature search	x	x				
Experimental studies	x		x			
Data acquisition	x					
Data analysis	x					
Statistical analysis	x					
Manuscript preparation	x	x				
Manuscript editing	x	x				
Manuscript review	x	x	x	x	x	x

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