SUPPLEMENTARY MATERIAL

The effect and safety of highly standardized Ginger (*Zingiber officinale*) and Echinacea (*Echinacea angustifolia*) extract supplementation on inflammation and chronic pain in NSAIDs poor responders. A pilot study in subjects with knee arthrosis.

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ABSTRACT

The study aimed to evaluate the effect of *Zingiber officinale*, and *Echinacea angustifolia* extract supplementation (25 mg of ginger and 5 mg of Echinacea) for 30 days on inflammation and chronic pain in knee osteoarthritis (OA).

Consecutive non-steroidal-anti-inflammatory-drugs (NSAIDs) poor responders with chronic inflammation and pain due to knee arthrosis were assessed (15 subjects, age:67.2±7.9, BMI:30.6±7.1, men/women:2/13). The primary endpoint was to determine pain improvement from baseline to day 30 by Tegner Lysholm Knee Scoring. The secondary endpoints were the assessment of Visual Analog Scale for Pain, health-related quality of life, by the ShortForm36 (SF-36), anthropometric parameters, hydration.

After supplementation, a significant improvement of 12.27 points were observed for Lysholm scale score ($p<0.05$), SF-36 ($p<0.05$), and a decrease of -0.52 cm in knee circumference (left) ($p<0.01$).

This pilot study provides feasibility and safety data for the use of highly standardized Ginger and Echinacea extract supplementation in people with knee OA.

KEYWORDS: Chronic pain; dietary supplement; *Echinacea angustifolia*; inflammation; knee osteoarthritis; *Zingiber officinale*; ginger; arthritis

3. Experimental

3.1. Subjects

Consecutive patients with chronic inflammation and pain due to knee arthrosis occurred to our physical medicine and rehabilitation division (Santa Margherita Institute, Pavia, Italy), during the period of May 2015 to November 2015, were invited to participate in the study after a diagnosis made by the senior Physiatrist.

All the patients had received previous treatment with analgesic agents, but they are poor responders. The patients had to fulfill the following four inclusion criteria: (I) age between 18 and 70 years, (II) chronic pain with Tegner Lysholm Knee Scoring (Lysholm & Gillquist 1982) with score <65 (poor) or a score within 65-83 points (fair), and (III) previous treatment with conventional nonsteroidal anti-inflammatory drugs (NSAID) in adequate doses, drugs to which the patients respond poorly (IV) Body Mass Index (BMI) between 30 and 40.

Exclusion criteria were as follows: current signs or symptoms of severe, progressive, or uncontrolled hepatic, hematological, pulmonary, cardiac, neurological, or cerebral disease; ongoing or past serious infection; pregnancy or breast feeding; current malignancy or history of malignancy within the past five years; congestive heart failure; allergy to *Zingiber officinale* and/or *Echinacea*

This study was approved by the Ethics Committee of University of Pavia and informed consent was obtained from all patients.

3.2. Study design.

The efficacy of supplementation was determined by evaluating changes in Tegner Lysholm Knee Scoring (Lysholm & Gillquist 1982) and Visual Analog Scale for Pain (VAS) and by judging alterations in clinical and functional assessments.

The primary study endpoint was to determine the proportion of pain improvement from baseline of the Tegner Lysholm Knee Scoring.

The secondary endpoints of the study were to determine the improvement from baseline to day 30 in the following parameter: the Visual Analog Scale for Pain (VAS), health-related quality of life assessed using the Short Form- (SF-) 36 questionnaire, body composition by anthropometric parameters, hydration by bioimpedence evaluation.

3.3. Dietary supplement

Soft gelatin capsules, each containing 25 mg of ginger and 5 mg of Echinacea standardized extracts, were used: MITIDOL[®], WO WO2012/013551 and WO2010/083967 (Indena SpA, Milan, I). The ginger extract was characterized by a high content of total gingerols (25.0 – 33.0% total gingerols);

the *Echinacea angustifolia* lipophilic extract is standardized to contain total alkylamides (22.5-27.5%) and alkylamide 8 ($\geq 12.0\%$);

All subjects were instructed to take 1 capsule immediately before starting breakfast and lunch for 4 weeks. Compliance to the supplementation regimen was defined as the number of cps actually taken by each subject, divided by the number of cps that should have been taken over the course of the treatment.

3.4. Safety

The participants were asked to report any adverse event occurring during the procedures or during the interval between them.

3.5. Procedures

Each participant underwent three evaluations, at baseline, at 15-day interval and after 30 days (Table 1).

3.6. Measurements

Bioimpedance analysis: Impedance measurements were conducted in all participants with the same Akern – BIA 101 body composition analyser (Akern S.r.l, Pontassieve – Florence, Italy). The level of the following components were assessed: fat mass (FM), fat free mass (FFM), total body water (TBW), and extra-cellular water (ECW). Water with its dissolved ions, which is a good electricity conductor, is an indicator of the level of fat and fat free content in the body. Mutual relations between resistance and reactance are established on the basis of phase dependence equations. The reliability of the BIA method has been confirmed in many studies (Lukaski 1997; Sun et al. 2003). In the current study the percentage content of components in total body mass was used.

Body composition and swelling assessment: Body weight was measured to the nearest 0.1 kg on a precision scale with the participants wearing light clothing, without shoes, with the use of a standardized technique (Frisancho 1984). Waist measurements were taken at the midpoint between the lowest rib and the top of the hip bone (iliac crest), with the use of a standardized technique (Frisancho 1984). Also other anthropometric measurements (Arm Circumference, Calf circumference, Hips circumference, knee circumference were performed by a standardized technique (Frisancho 1984) and waist to hip ratio (WHR), arm muscle area (AMA), arm fat area (AFA), mid-arm circumference (MAC) were calculated.

Assessment of quality of life: The participants were tested with the Short-Form 36-Item Health Survey (SF-36) (Ware & Sherbourne 1992) to assess their quality of life. This questionnaire is a valid generic measure that is used for rating health-related quality of life in several research fields because of its validity, high internal consistency, and high test–retest reliability. The SF-36 scales were summarized in 2 dimensions. The first 5 make up the “physical health” dimension, and the last 5 the “mental health” dimension (MCS, mental component summary). The vitality and general health scales are parts of both dimensions. Thus, each dimension includes 3 specific and 2 overlapping scales. The standardized summary scores for physical and mental components were calculated and used separately as outcome measures. The quality of- life SF-36 was administered before and after the treatment period.

Visual Analogue Scale (VAS) of pain: A Visual Analogue Scale (VAS) is a measurement instrument that tries to measure a characteristic or attitude that is believed to range across a continuum of values and cannot easily be directly measured. For example, the amount of pain that a patient feels ranges across a continuum from none to an extreme amount of pain. The VAS score is determined by measuring in millimetres from the left hand end of the line to the point that the patient marks.

Assessment of knee symptoms and function: Each patient completed a self-report questionnaire, Tegner Lysholm Knee Scoring Scale (Lysholm & Gillquist 1982), related to knee symptoms and function.

Statistical Analysis: Data were analyzed by descriptive statistics. Intra-group comparisons was conducted using the Student’s t-test, with a significant p-value ($p < 0.05$). All analyses was performed using SPSS version 21.

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Table S1. Clinical characteristics of patients at baseline and following treatment (time points at 15 days and 30 days).

Variables	Baseline	After 15 days	After 30 days	P- Value
Age (yrs)	67.27 ± 7.92	-	-	-
Body Mass Index (Kg/m ²)	30.64 ±7.15	NR	30.55 ±7.19	0.400
Body composition assessment				
Wrist circumference (cm)	17.05 ±1.47	NR	17.05 ±1.78	0.553
Arm Circumference (cm)	30.318 ±3.20	NR	30.545 ±3.26	1.000
Calf circumference (cm)	35.55 ±4.10	NR	35.36 ±4.03	0.603
Waist circumference (cm)	101.32 ±16.78	NR	99.95 ±16.99	0.492
Hips circumference (cm)	105.45 ±13.45	NR	105.50 ±12.59	0.102
WHR	0.94 ±0.87	NR	0.94 ±0.08	0.928
AMA (cm ²)	31.30 ±11.73	NR	33.30± 14.21	0.320
AFA (cm ²)	3.6427 ±1.26	NR	3.86 ±1.10	0.525
MAC (cm)	21.69 ±3.65	NR	22.19 ±4.23	0.443
knee circumference (right) (cm)	37.14 ±2.94	NR	38.40 ±3.49	0.601
knee circumference (left) (cm)	38.20 ±3.37	NR	37.68 ±3.29	0.004
Bioimpedance analysis				
Total Body Water (L)	37.13 ±8.52	NR	36.18 ±7.77	0.340
Extra Cellular Water (L)	16.90 ±3.59	NR	16.50 ±3.81	0.297
Intra Cellular Water (L)	20.24 ±5.26	NR	19.70 ±4.35	0.363
Free Fat Mass (Kg)	46.77 ±10.47	NR	45.56 ±9.54	0.164
Fat Mass (Kg)	28.03 ±12.13	NR	28.84 ±11.07	0.188
LYSHOLM scale	64.09 ±17.9	79.50 ± 14.10	76.36 ±18.63	0.020
SF-36 MCS score	145±52	NR	161±99	0.46
SF-36 PCS score	136±46	NR	186±84	0.03
VAS L score	3.36 ±2.73	2.73 ±2.39	2.18 ±2.40	0,71
VAS R score	3.36 ±2.11	2.64 ±	2.64 ±2.28	0,17

Bold values or figures; Significant difference as compared to baseline (p <0.05)

-; no change, NR; not recorded, MCS; mental component summary, PCS; physical component summary, SF-36; Short-Form 36-Item Health Survey