Original Article

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Comparative efficacy and safety of Verbascox® – a proprietary herbal extract capable of inhibiting human cyclooxygenase-2 – and celecoxib for knee osteoarthritis

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SUMMARY The aim of this randomized, single-blind, active-controlled pilot study was to investigate the clinical efficacy of oral supplementation with Verbascox®, a proprietary herbal extract capable of inhibiting human cyclooxygenase-2 (COX-2), in patients with mild-to-moderate osteoarthritis (OA) of the knee. Patients in the control group (n = 50) did not undergo any treatment (watchful waiting). Patients in the Verbascox® group (n = 50) received oral supplementation (800 mg/day) with the herbal extract for 2 weeks. The final study group consisted of patients (n = 50) who received celecoxib, a known pharmacological inhibitor of COX-2, 200 mg/day for 2 weeks. Examining physicians and laboratory personnel were blinded to group assignment, whereas patients were unblinded. All participants were evaluated using standard measures of pain relief and improvement in functional capacity at baseline, after 1 week, and at the end of the 2-week treatment course. Moreover, serum levels of substance P (SP), a member of the tachykinin family of neuropeptides involved in pain perception, were measured at the three time points. Both Verbascox® and celecoxib reduced pain, improved functional capacity, and lowered serum SP levels at 2 weeks compared with baseline, without significant inter-arm differences. Both Verbascox® and celecoxib showed a limited number of treatment-emergent adverse events. In summary, oral supplementation with Verbascox® (800 mg/day) in patients with mild-to-moderate OA of the knee is as effective and safe as a standard therapeutic dose of celecoxib in terms of pain relief and improvement in functional capacity after a 2-week treatment course.

Keywords phytomedicine, pain, functional capacity

1. Introduction

The knee is the most common joint localization of symptomatic osteoarthritis (OA) (*1*). Knee OA, affecting more than 250 million people worldwide, has significant effects on patient function and considerable societal costs in terms of morbidity (*e.g.*, work loss and joint replacement) (*2*). The results of the OA process are cartilage degradation and synovial inflammation; these features are associated with the development of symptoms of pain, stiffness, and functional disability (*1,2*). In the current paradigm, the structural changes represent the disease, whereas the symptoms of aching, discomfort, pain, and stiffness are the reasons whereby patients seek medical care (*3*).

Current treatment of OA is based on symptom management, primarily pain control (*4*). Clinical guidelines recommend the use of oral non-steroidal anti-inflammatory drugs (NSAIDs) in patients with persistent symptoms (*5*). Although conventional NSAIDs are the most frequently prescribed medicines for OA, they are characterized by numerous potential adverse effects including gastrointestinal bleeding, cardiovascular side effects, and risk of nephrotoxicity (*6*). To overcome these issues, the use of selective cyclooxygenase-2 (COX-2) inhibitors (coxibs) – which offer the advantage of an anti-inflammatory and analgesic activity similar to that of conventional nonselective NSAIDs but with a more favorable profile in terms of adverse event – has gained momentum (*7,8*). Celecoxib is a selective NSAID indicated for the treatment of the signs and symptoms associated with OA (*9*). Its efficacy in relieving pain

and inflammation and improving physical function in patients with OA has been established (*10*), and it has a better gastrointestinal tolerability profile compared with nonselective NSAIDs (*11*).

Recent years have witnessed a growing interest in natural compounds as promising alternatives to synthetic COX-2 inhibitors (*12,13*). We have previously shown that Verbascox®, a proprietary herbal extract from *Lippia citriodora* and *Plantago lanceolata* titred in verbascoside (a natural polyphenol known for the high antioxidant power; ≥ 5%) and aucubin (a naturally occurring iridoid glycoside; ≥ 2%), inhibits LPS-stimulated expressions of COX-2 in human neutrophils in a dose-dependent fashion (*14*). Based on its *in vitro* activity, we hypothesized that Verbascox® may exert significant anti-inflammatory and analgesic effects by acting as a specific non-pharmacological COX-2 inhibitor.

Here, we sought to investigate the clinical usefulness of oral supplementation with Verbascox® in patients with mild-to-moderate OA of the knee. The primary aim of this pilot study was to compare the magnitude of pain relief and improvement in functional capacity of patients who received oral supplementation with Verbascox® compared with those who were treated with celecoxib, a known pharmacological inhibitor of COX-2 (*9*). We also measured serum levels of substance P (SP), a member of the tachykinin family of neuropeptides involved in pain perception (*15*), as a biochemical marker of treatment response in the study participants.

2. Materials and Methods

2.1. Patients

This research was a randomized, single-blind, active-controlled pilot study of 150 consecutive patients (117 women and 33 men). Patients who were 40-75 years of age with a clinical diagnosis of OA of the knee according to the American College of Rheumatology criteria (*16*) were eligible. Inclusion criteria were as follows: 1) presence of symptoms for at least one month; 2) number of leukocytes in synovial fluid < 2,000/mL; 3) pain on a visual analog scale (VAS) ≥ 2 at rest; and 4) duration of stiffness in the morning ≤ 30 min. The following exclusion criteria were applied: 1) pregnancy or breastfeeding; 2) positive history for major renal, hepatic, cardiac, gastrointestinal, or hematologic disorders; 3) presence of malignancies; 4) presence of neurologic or psychiatric disorders; 5) atopy or allergic disorders; 6) diabetes mellitus or other endocrine disorders; 7) coagulation disturbances; 8) positive history for peptic ulcer; 9) use of corticosteroids in the four weeks preceding the study; and 10) use of NSAIDs in the two weeks preceding the study. According to the Kellgren-Lawrence Grading System (*17*), 61.3% and 38.7% of the study patients had a score of 1 and 2, respectively. The patients were therefore classified as having mild-to-moderate OA of the knee. The study protocol complied with the tenets of the Declaration of Helsinki and was approved by the local ethics committee (approval number E04/18). Before the study, each patient was informed about the purpose of the study and signed informed consents were obtained.

2.2. Materials

Verbascox® was supplied by LaBiotre srl (Tavarnelle Val di Pesa, Italy). The oral supplement tested in this study was in tablet form containing 800 mg of the proprietary extract. Celecoxib 200 mg oral capsules were from Pfizer (New York, NY, USA).

2.3. Procedures

The study period was two weeks. At baseline, body mass index (BMI) and duration of pain were collected from all participants. Patients (n = 150) were randomly divided into three study groups (1:1:1 ratio). The random allocation sequence was generated by a computer program. Patients in the control group (n = 50) did not undergo any treatment (watchful waiting). Patients in the Verbascox® group (n = 50) received oral supplementation (800 mg/day; one tablet) with the herbal extract for 2 weeks. The final study group consisted of patients (n = 50) who received celecoxib (200 mg/day; one capsule) for 2 weeks. Examining physicians and laboratory personnel were blinded to group assignment, whereas the study patients were unblinded. All participants were asked to suspend other treatments during the study course. A total of three assessments were performed (baseline, at one week, and at the end of the 2-week treatment course).

2.4. Clinical endpoints

The clinical endpoints included: 1) patient's assessment of arthritis pain score (VAS) at rest (range: 0-10; where 0 is no pain and 10 is worst pain), 2) patient's assessment of arthritis pain score (VAS) upon movement (range: 0-10; where 0 is no pain and 10 is worst pain), 3) range of motion (degrees), and 4) the Western Ontario and McMaster Universities (WOMAC) index score (*18*). The WOMAC index scores consists of subscales that measure pain (range: 0-20; where 0 is no pain and 20 is worst pain), stiffness (range: 0-8, where 0 is no stiffness and 8 is worst stiffness), and physical functioning (range: 0-68, where 0 is best functioning and 68 is worst functioning). The resulting total composite score ranges from 0 to 96 (*19*).

2.5. Measurements of serum substance P levels

Venous blood samples were collected in serum separator tubes at each assessment. Blood was allowed to clot

Table 1. Baseline characteristics of the three study groups

Items	No treatment (n = 50)	Supplementation with Verbascox® 800 mg/day (n = 50)	Treatment with celecoxib 200 mg/day (n = 50)	p
Age, years	57.1 ± 5.3	56.8 ± 5.6	57.4 ± 5.5	0.31
Women/men	39/11	40/10	38/12	0.89
Body mass index, kg/m^2	25.7 ± 2.4	25.8 ± 2.2	26.0 ± 2.5	0.48
Pain duration, months	3.1 ± 0.4	2.9 ± 0.6	3.2 ± 0.5	0.51
Pain at rest, VAS (0-10)	2.6 ± 0.3	2.7 ± 0.4	2.6 ± 0.4	0.59
Pain on movement, VAS (0-10)	3.7 ± 0.6	3.9 ± 0.7	3.8 ± 0.6	0.79
Range of motion (degrees)	133 ± 10	132 ± 9	134 ± 10	0.81
WOMAC scores				
Pain	5.6 ± 0.6	5.8 ± 0.8	5.7 ± 0.8	0.73
Stiffness	1.4 ± 0.3	1.5 ± 0.4	1.4 ± 0.3	0.68
Physical function	20.1 ± 2.4	20.9 ± 3.6	20.5 ± 3.2	0.75
Total	27.1 ± 3.3	28.2 ± 4.0	27.6 ± 3.8	0.59

Data are expressed as means and standard deviations or as counts. Abbreviations: VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities.

at room temperature for 30 min and then centrifuged at 1,000 × g for 15 min. Serum was removed and aliquots were kept frozen at −80°C until measurements. Serum SP levels were assayed using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (R&D Systems, Minneapolis, MN, USA). Absorbance at 450 nm was measured on an ELISA plate reader (PerkinElmer, Waltham, MA, USA). The detection limit of this assay was 25 pg/mL, and intra- and interassay coefficients of variation were 8% and 13%, respectively. All samples were processed simultaneously at the end of the study by laboratory personnel blinded to clinical data.

2.6. Safety

Safety was assessed by recording treatment-emergent adverse events and changes from baseline in clinical laboratory tests, vital signs, and physical examinations, all of which were administered at visits on weeks 1 and 2. Clinically relevant changes in laboratory values were defined as: aspartate aminotransferase (AST) and/or alanine transaminase (ALT) ≥ 3 × upper limit of normal (ULN), creatinine ≥ 1.3 × ULN, blood urea nitrogen (BUN) ≥ 2 × ULN, hematocrit decrease ≥ 5 percentage points from baseline, and hemoglobin decrease ≥ 2 g/dL from baseline (*20*).

2.7. Statistical analysis

Categorical variables are expressed as counts and percent frequency and were compared using the chi-square test. Continuous variables are given as means ± standard deviations and were compared across the three assessment points using one-way analysis of variance (ANOVA) followed by *post-hoc* Newman-Keuls tests. All analyses were performed using GraphPad Prism, version 7.0 (GraphPad Inc., San Diego, CA, USA) and SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Two-tailed p values < 0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

The baseline characteristics of the three study groups are shown in Table 1. There were no significant intergroup differences in terms of age, sex, BMI, pain duration, pain at rest, pain upon movement, range of motion (degrees), and WOMAC index scores. Laboratory safety parameters (AST, ALT, creatinine, BUN, hematocrit, and hemoglobin) at baseline were all within the normal range (data not shown). The study sample may therefore be considered representative of a clinical population of patients with mild-to-moderate OA of the knee in need for pharmacological treatment.

3.2. Clinical endpoints

No patient withdrew from the study. The clinical endpoints in the three study groups are shown in Table 2. No significant differences over time were observed in the control arm (watchful waiting). Compared with baseline values, celecoxib significantly outperformed Verbascox® with regard to all clinical endpoints at one week. However, no significant differences were evident between the Verbascox® group and the celecoxib group at the end of the 2-week study period. Figure 1 compares the changes in the WOMAC index total score in the Verbascox® and celecoxib groups along the 2-week study course.

3.3. Serum substance P levels

Table 3 summarizes the temporal variations in serum SP levels in the three study groups. No significant differences over time were observed in the control arm (watchful waiting). At one week, celecoxib, but not Verbascox®, produced a statistically significant reduction in serum SP levels compared with baseline values. However, no significant differences were evident

Table 2. Temporal course of clinical endpoints in the three study groups

Items	No treatment (n = 50)			Supplementation with Verbascox® 800 mg/day (n = 50)			Treatment with celecoxib 200 mg/day (n = 50)		
	Baseline	One week	End of the study	Baseline	One week	End of the study	Baseline	One week	End of the study
Pain at rest, VAS (0-10)	2.8 ± 0.3	2.8 ± 0.2	2.5 ± 0.3	2.7 ± 0.4	2.5 ± 0.7	1.7 ± 0.5*	2.9 ± 0.4	2.0 ± 0.3*,†	1.5 ± 0.4*
Pain on movement, VAS (0-10)	3.7 ± 0.6	3.9 ± 0.8	3.8 ± 0.7	3.9 ± 0.7	3.6 ± 0.9	2.4 ± 0.6*	3.8 ± 0.6	2.9 ± 0.5*,†	2.2 ± 0.4*
Range of motion (degrees)	133 ± 10	135 ± 13	131 ± 12	132 ± 9	141 ± 18	168 ± 21*	134 ± 10	160 ± 15*,†	173 ± 19*
WOMAC scores									
Pain	5.6 ± 0.6	5.8 ± 0.7	5.5 ± 0.9	5.8 ± 0.8	5.2 ± 1.4	3.3 ± 0.9*	5.7 ± 0.8	4.1 ± 0.9*,†	3.0 ± 0.9*
Stiffness	1.4 ± 0.3	1.6 ± 0.3	1.5 ± 0.2	1.5 ± 0.4	1.3 ± 0.4	0.8 ± 0.3*	1.4 ± 0.3	1.0 ± 0.2*,†	0.7 ± 0.1*
Physical function	20.1 ± 2.4	22.5 ± 2.7	21.8 ± 2.6	20.9 ± 3.6	18.7 ± 2.5	11.9 ± 2.0*	20.5 ± 3.2	14.6 ± 2.1*,†	11.4 ± 1.7*
Total	27.1 ± 3.3	29.9 ± 3.8	28.8 ± 3.6	28.2 ± 4.0	25.2 ± 3.1	16.0 ± 2.7*	27.6 ± 3.8	19.7 ± 2.9*,†	15.1 ± 2.2

Abbreviations: VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities. Data are expressed as means and standard deviations. *$p < 0.001$ *versus* baseline. †$p < 0.001$ *versus* the Verbascox® arm at one week.

Table 3. Temporal course of serum substance *P* levels in the three study groups

Items	No treatment (n = 50)			Supplementation with Verbascox® 800 mg/day (n = 50)			Treatment with celecoxib 200 mg/day (n = 50)		
	Baseline	One week	End of the study	Baseline	One week	End of the study	Baseline	One week	End of the study
Serum substance P, pg/mL	423 ± 85	451 ± 96	444 ± 72	445 ± 91	423 ± 80	312 ± 77*	436 ± 95	358 ± 78*,†	299 ± 64*

Data are expressed as means and standard deviations. *$P < 0.001$ *versus* baseline. †$P < 0.001$ *versus* the Verbascox® arm at one week.

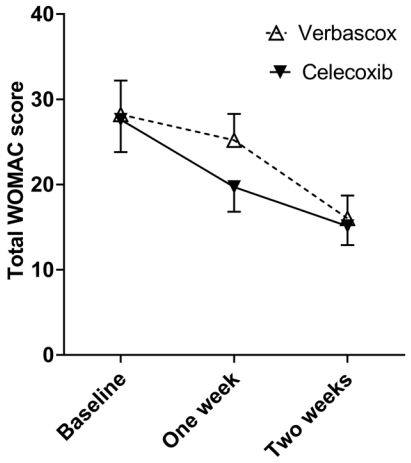

Figure 1. Comparison of changes in the WOMAC index total score in the Verbascox® and celecoxib groups along the 2-week study course. Error bars represent standard deviations.

Table 4. Treatment-emergent adverse events in the Verbascox® and celecoxib arms

Items	Supplementation with Verbascox® 800 mg/day (n = 50)	Treatment with celecoxib 200 mg/day (n = 50)
Gastrointestinal adverse events		
Dyspepsia	5 (10%)	4 (8%)
Nausea	1 (2%)	2 (4%)
Constipation	0 (0%)	2 (4%)
Diarrhea	2 (4%)	0 (0%)
Flatulence	2 (4%)	1 (2%)
Other adverse events		
Headache	2 (4%)	3 (6%)
Dizziness	1 (2%)	0 (0%)
Pruritus	0 (0%)	1 (2%)

Data are expressed as number of patients (percentages in parenthesis).

between the Verbascox® group and the celecoxib group at the end of the 2-week study period – with both treatment arms showing similar reductions compared with baseline values.

3.4. Safety

Treatment-emergent adverse events in the Verbascox® and celecoxib arms occorred sporadically and did not differ significantly in the two groups (Table 4). Clinically relevant changes in laboratory values were not observed in any of the study patients regardless of the treatment arm.

4. Discussion

In this randomized, single-blind, active-controlled pilot study, we found that supplementation with Verbascox® (800 mg/day) in patients with mild-to-moderate OA of the knee is as effective and safe as a standard therapeutic dose of celecoxib in terms of pain relief and improvement in functional capacity after a 2-week

treatment course, although celecoxib was more rapidly effective. Interestingly, the temporal course of serum SP reduction followed a similar pattern, suggesting that the clinical effects of both Verbascox® and celecoxib could at least in part mediated by a decrease in serum levels of this biochemical mediator of pain and inflammation. Supplementation with Verbascox® for two weeks was safe, with treatment-emergent adverse events being sporadic and similar to those observed in the celecoxib arm and none of them leading to withdrawal. Notably, there were no changes from baseline in clinical laboratory safety tests in both the Verbascox® and celecoxib groups.

Although several herbal inhibitors of COX-2 have been developed (*12,13*), to date there has been little direct evidence comparing such extracts with celecoxib, one of the most commonly used coxibs. The efficient analgesic effect of celecoxib in knee OA begins within two days of treatment initiation, and taking 200 mg/day is known to ensure an efficient control of pain (*21*). We therefore utilized this dosage for the active-controlled treatment arm.

Using *in vitro* experiments, we have previously shown that Verbascox® is capable of inhibiting COX-2 in a dose-dependent fashion (*14*). In the current study, the comparable clinical efficacy of Verbascox® and celecoxib was shown by similar scores in VAS at rest, VAS upon movement, range of motion, and WOMAC index total score at 2 weeks, although celecoxib acted more rapidly – with several improvements being already evident at one week. We believe that two potential explanations can be offered for the more rapid efficacy of celecoxib. First, our *in vitro* experiments indicated that celecoxib is a more potent inhibitor of COX-2 than Verbascox® (*14*). Second, it is possible that Verbascox® and celecoxib may differ in terms of pharmacokinetic properties – with steady state plasma concentrations possibly being reached more rapidly by the former. Additional studies are required to shed more light on the pharmacokinetics of Verbascox®.

Verbascox® may be clinically effective against mild-to-moderate OA not only in light of its capacity to inhibit COX-2, but also because of other active properties of its components. Verbascoside is indeed characterized by a high antioxidant power (*22*), whereas aucubin can attenuate tumor necrosis factor-α-induced inflammatory responses (*23*). The possibility that Verbascox® may exert additional COX-2-independent antioxidant and anti-inflammatory effects would explain why its clinical effectiveness was found to be similar to that of celecoxib at 2 weeks, despite being a less potent inhibitor of COX-2 (*14*). Importantly, the amount of point reductions in the WOMAC index total score at the end of the study (from 28.2 ± 4.0 to 16.0 ± 2.7 in the Verbascox® group and from 27.6 ± 3.8 to 15.1 ± 2.2 in the celecoxib group) should be considered as a clinically relevant improvement. Accordingly, the minimum clinically important differences in the WOMAC index total score have been reported to be 9.5-10.1 for OA of the knee and hip (*21*). In our study, improvements in clinical symptoms in both study arms were paralleled by a significant reduction in serum levels of SP, a neuropeptide released from sensory nerves that exerts different pro-inflammatory effects (*15*). It is notable that SP signaling is not only involved in pain perception but is also capable of upregulating COX-2 expression (*24,25*). In recent years, growing evidence has shown a role of SP in human joint disease including OA (*26,27*). It is therefore feasible that the reduction in SP levels can contribute to the anti-inflammatory and pain-mitigating effects of both Verbascox® and celecoxib.

There are several limitations to this study which need to be mentioned. First, the relatively small sample size may give rise to overestimation of treatment effects (*28*), Moreover, continuous endpoints and self-reported outcomes can also lead to potential bias. To circumvent these issues, we also used objectively measured clinical (range of motion) and biochemical (SP) endpoints. Finally, our study should be considered as an exploratory analysis; the follow-up time was short and independent replication is needed to extend and confirm our results. Patients with OA of the knee patients should be treated on an individual basis according to each patient's disease characteristics, based on clinical trial data. Larger studies that include a placebo group or a cross-over design will be required to further elucidate the clinical usefulness of Verbascox® in patients with inflammatory joint disorders.

These caveats notwithstanding, our results suggest that supplementation with Verbascox® and treatment with celecoxib for 2 weeks have similar effects in reducing symptoms of mild-to-moderate OA of the knee, although celecoxib was more rapidly effective. Reduction in serum SP levels followed a similar temporal pattern and could be at least in part responsible for the observed clinical effects.

Conflict of interest:

This study was partly funded by LaBiotre srl (Italy). The sponsor had no influence in the performance, analysis, and interpretation of the study.

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