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An Open label, phase II clinical study to evaluate the efficacy and safety of DPOR/JR2007 in osteoarthritis of knee

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ABSTRACT

Aim: To evaluate the effect of DPOR/JR2007 in patients with moderate to severe knee osteoarthritis.
Methods: Forty-two subjects received DPOR/2007 (1400 mg) tablets twice a day over a 12-week period. Each tablet contained 7 standardised botanical ingredients i.e. *Aristolochia indica*, *Balsamodendron mukul*, *Cassia tora*, *Hemidesmus indicus*, *Withania somnifera*, *Pterospermum acerifolium*, and *Vitex negundo*. Primary efficacy variable was assessment of change in Q.1 (Pain while walking on flat surface) of The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)-Pain. Secondary efficacy variables consisted, change in Q.2 (Pain while going up and down the stairs) of WOMAC-Pain, WOMAC domains, Short Form-8 (SF-8) Quality of life (QoL) questionnaire, Global assessment of OA and use of rescue medication. Tumour Necrosis Factor - α (TNF-α) and Interleukin-6 (IL-6) serum levels were also measured in the study.
Results: The mean (SD) Q.1 baseline scores of 3.5 (0.73) were significantly reduced to 2.5 (0.66) at the end of study period; $p < 0.001$. Similar mean reduction was reported for Q.2 and each of the WOMAC dimensions; $p < 0.001$ and the SF-8 scores also demonstrated significant improvement in subjects QoL; $p < 0.001$. For biomarker assessment, TNF-α was reduced in both sexes with significant decrease being observed in female subjects; $p < 0.05$. Analysis showed that serum IL-6 levels were increased in subjects but was not of clinical significance. Further, DPOR/JR2007 was well-tolerated with fewer adverse events, increased global assessment scores and minimum rescue medication usage.
Conclusion: DPOR/JR2007 supplementation was effective in relieving the pain and stiffness in patients with chronic knee OA and improvement was evident within 8–12 weeks. Future studies are warranted for understanding the exact mechanism of DPOR/JR2007 with respect to inflammatory cytokines.

1. Introduction

Knee osteoarthritis (OA), also known as a degenerative joint disorder, is normally due to the result of wear and tear, progressive deterioration and loss of articular cartilage (Berenbaum, 2013). One of the most common forms of arthritis, its major symptoms include chronic joint pain and instability, stiffness, altered biomechanics and radiographic joint space narrowing (Chen et al., 2017). Knee OA is associated with significant physical disability and an equal deterioration of health-related quality of life (HRQoL) ensuing a considerable socio-economic burden on patients and societies (Hiligsmann et al., 2013). Being a complex multifactorial disease, the root cause of OA is not completely understood. However, it is safe to say that its development is an amalgamation of multiple contributory mechanisms. One such mechanism that has been extensively studied in relation to pathogenesis of knee OA is the cytokine mediated inflammatory cascade (Nees et al., 2019).

The relationship between inflammatory and degenerative disorders is now being highlighted with the recognition of ongoing immune processes within OA joints and synovium. (Sokolove and Lepus, 2013) Researchers today consider inflammation to be the most prominent feature in patients' diagnosed with knee OA (Wang, 2017).

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Inflammation is now said to be prevalent in OA joints long before the development of significant radiographic change (Mathiessen and Conaghan, 2017). Inflammatory components, such as cytokines and chemokines, are produced by chondrocytes and synoviocytes in the OA joints (Wojdasiewicz et al., 2014). Further, activated neutrophils and macrophages also secrete cytokines such as Tumour Necrosis Factor - α (TNF-α) and Interleukin (IL)-1β that amplify the inflammatory process. Chondrocyte and synoviocyte culture inflammation triggered by TNF-α and IL-1βhave also been shown to release TNF-α, IL-6 and IL-8 (Chow and Chin, 2020). Besides the aforementioned cytokines, the trans-signaling IL-6 activates the immune system by recruiting monocytes to the already inflamed joint area (Arango Duque and Descoteaux, 2014). This induces upregulation of the macrophage colony-stimulating factor (M-CSF) receptor that skews the differentiation of monocytes to macrophages (Hamilton, 2019). All these molecules and pathways are intertwined for regulating the joint anabolism and catabolism process in OA. Therefore, the alteration of cytokines such as TNF-α & IL-6 could have a prominent role to play in designing anti-inflammatory interventions for treatment of OA (Livshits et al., 2009).

Drugs addressing the underlying biological causes of OA are currently not available on the market. Current therapies for OA are limited to symptom-relieving drugs and total knee arthroplasty for severe cases (Wang et al., 2018). In the last few decades, herbal dietary supplements have gained significance in the area of clinical practice and research of rheumatology. Hence, it is vital that the patients and medical practitioners are aware of the evidence for or against these approaches. Several herbal medicines and dietary supplements have the ability to reduce the pain and symptoms associated with OA (Mobasheri, 2012). Unfortunately, the scientific rationale for their application is not thoroughly established as very few have been shown demonstrably in clinical studies (Parveen et al., 2015; Petchi et al., 2015). With individuals seeking safe, effective and natural OA therapies, DeltasPharma India Pvt. Ltd. Developed DPOR/JR2007, a standardised polyherbal formulation containing several herbs of documented anti-arthritic potential (Mathew et al., 2011; Tandon and Gupta, 2006). Therefore, the present study was aimed at investigating the efficacy and safety of DPOR/JR2007 on degenerative changes associated with knee OA.

2. Materials & methods

2.1. Ethical considerations

The study was approved by the ethics committee - Inter System Biomedica Ethics Committee (ISBEC) - Mumbai, India, registered with the Drug Controller General of India (ECR/108/Indt/MH/2013). Written informed consents were voluntarily obtained from all subjects and the study was registered on Clinical trial registry - India (http://ctri.nic.in/Clinicaltrials; CTRI/2017/07/009,054). The study was performed in compliance with the Helsinki Declaration and ICH-GCP guidelines.

2.2. Subjects

Men and non-pregnant women aged between 45–76 years were recruited through a single outpatient clinic. Study subjects without any history of any autoimmune disorder, psoriatic arthritis, and severe systemic disease were enrolled and individuals undergone or planning to undergo knee surgery were excluded. Also, subjects undertaking or having previous history of any intra-articular treatments or dietary supplements 90 days prior to screening were not included for study purpose. A urine pregnancy test was mandatory for fertile females and women planning to conceive or lactating were not included. Characterization of joint dysfunction was as per the ACR (American College of Rheumatology; Altman et al., 1986) criteria for OA.

2.3. Intervention

The present study was designed to investigate the potential of an Ayurvedic formulation DPOR/JR2007 in moderate to severe cases of knee OA. DPOR/JR2007 is formulated with extracts of herbs such as *Aristolochia indica, Balsamodendron mukul, Cassia tora, Hemidesmus indicus, Withania somnifera, Pterospermum acerifolium,* and *Vitex negundo*. These herbs were identified as per the standards of Ayurvedic Pharmacopoeia of India (API) and procured from state of Andhra Pradesh, India and a voucher specimen (#DP2013/02−09) was retained. Further, the aqueous extracts of the above mentioned medicinal herbs were obtained using spray drying technique. The investigational products (IP) were manufactured in the form of 1400 mg tablets, and packed in duly labelled high-density polyethylene bottles. Subjects were instructed to consume 2 tablets per day in divided dosage after meals for the 12-week study duration. Use of Paracetamol and Ibuprofen as rescue medication was permitted in case of severe pain but prohibited 48 h prior to assessment visits. Composition of the IP is provided in Table 1.

2.4. Study conduct

This was an 'open label', non-comparative, phase II clinical trial in individuals with moderate to severe complications of knee OA. The first research subject was enrolled in July 2016 and the final subject completed the protocol designated last visit in August 2016. Prior to subject selection (baseline visit), a 7-day placebo run-in period was completed by all subjects for identifying placebo responders and ascertaining treatment compliance. Subjects with no placebo response as per visual analogue scores were provided the IP. Post enrolment, subjects consumed 'DPOR/JR2007′ tablets twice every day after lunch and biological serum samples collection was performed on baseline (day 0) and at the final study visit (day 84). The study subjects were provided with a study diary for recording the use of non-study and rescue medication use during the course of the study. Furthermore, the subjects were informed to maintain record in the diary provided of any discomfort experienced throughout the study period. All unused tablets were returned and analysed for percent treatment compliance and the study coordinator also checked the subject diary on scheduled visits to further verify IP compliance. Subjects visited the clinical site on baseline (day 0), 1st follow-up visit (week 4), 2nd follow-up visit (week 8) and end of study visit (week 12). Subjects were instructed to consume IP for the entirety of the study period that was 84 days and the rationale for the same was based on previous research findings consisting of similar herbal ingredients used for OA treatment (Nipanikar et al., 2013; Kulkarni et al., 2011).

2.5. Outcome measures

2.5.1. Primary outcome measure

Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) used in this study have been previously validated for Indian (Asian) usage (Chopra and Saluja, 2012). WOMAC sub-scales comprise of 24 questions: Pain - 5, stiffness - 2, and physical function – 17 and

Table 1
Composition of Investigational Product, i.e., 'DPOR/JR.2007′

Ingredient	Botanical name	Quantity (milligrams)
Eshwarmool	*Aristolochia indica*	75 mg
Mahishakshi Guggulu	*Balsamodendron mukul*	40 mg
Pawar	*Cassia tora*	75 mg
Anantmool	*Hemidesmus indicus*	115 mg
Ashwagandha	*Withania somnifera*	70 mg
Muchkund	*Pterospermum acerifolium*	50 mg
Nirgundi	*Vitex negundo*	75 mg
Excipients	———————	900 mg
Total		**1400 mg**

each question is assessed on a Likert based response rated from 0 to 4 points (0 indicates 'no pain' and 4 'extreme pain'). Lower scores indicate improvement. WOMAC questionnaire was self-administered by the subject on baseline visit (day 0), visit 1 (week 4), visit 2 (week 8) and visit 3 (week 12). The primary outcome measure of the study was to evaluate the change in Q.1 of WOMAC pain subscale i.e. Pain while walking on flat surface. It was selected as per the recommended criteria for assessing pain by Dworkin et al., 2011.

2.5.2. Secondary outcome measures

The Secondary outcome measure in the study evaluated subject response to Q.2 of WOMAC pain subscale i.e. Pain experienced while walking up and down stairs. Complete WOMAC questionnaire was also evaluated for WOMAC domains of pain, stiffness, physical difficulty and an optional question in WOMAC scale. Further, SF-8 was selected for HRQoL assessment. SF-8 contains questions on following attributes [Physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), and mental health (MH)]. Each item has a 5 or 6 point response range. Reverse scoring of the scale was done to achieve a better linear fit with the participant general health evaluation. Accordingly, lower scores indicated a better HRQoL of study subjects. (Diane et al., 2003). All the subjects responded to WOMAC and SF-8 questionnaires on baseline visit (day 0), visit 1 (week 4), visit 2 (week 8) and visit 3 (week 12). Additionally, 'Investigator' and 'Subject' global assessment of treatment response were evaluated at baseline and end of study visit. Use of rescue medication was analysed in the clinical study for understanding the visit wise frequency of rescue medication.

2.5.3. Biomarker analysis

Freshly drawn fasting blood samples were used for analyses of Tumour necrosis factor and Interleukin-6. High sensitivity biomarkers were determined using Enzyme-linked immunosorbent assay kits by DIAsource ImmunoAssays S.A. (Rue du Bosquet, Belgium) as per the manufacturer's protocol on Cobas E Immunoassay Analyzer (Roche Diagnostics).

2.6. Safety measures

Vitals (pulse rate and blood pressure), clinical examination, occurrence of AE/SAEs were monitored throughout the study duration. Complete blood count with serum creatinine and uric acid analysis was performed on baseline (day 0) and end of study visit (day 84) respectively. Blood samples were collected and analysis was performed as per standard operating procedures by Metropolis Healthcare, accredited by the College of American Pathologists' (CAP) certification.

2.7. Sample size calculation

Sample size calculation was based on assumption for proportions of severity of symptoms for the group at the beginning of the study in respect to the end of the study. We assumed that proportions of the severity at the baseline as 0.7; Group 1 and the proportions of severity at the end of study as 0.4; Group 2. The difference selected in the proportions were based on the "Standard Effect Size" described in several randomized controlled trials (Carroll et al., 1993; McQuay et al., 1992; Barden et al., 2002) for "percent reduction in pain" as ≥ 30 and that the item one of the WOMAC pain subscale (pain walking on a flat surface) was selected based on the recommended criteria for assessing pain as per Dworkin et al., 2011.

Considering significance level as 0.05 at power of 0.82 (1-β), the minimum subjects required for establishing efficacy of the DPOR/JR2007 was 32 subjects. However, considering a screen failure rate ~ 30 % and post enrolment drop-out rate of ~ 20 %, it was decided to screen ~ 56 subjects in the study for having an adequate study sample size. Post study completion, 45 patients were included in the study out of which 3 patients were lost to follow-up. The power procedure of Pearson Chi-square Test having equal weight of 1 for each group was used; the data of 42 subjects was expressed at the power 0.913. Sample size was calculated using SAS package 9.4 (SAS® Institute Inc., USA)

2.8. Statistical analysis

The type II error probability associated with the test of the null hypothesis was set at 0.05. Per-protocol analysis for conducting comparison was used. Only subjects completing each of their assessment visits with more than 90 % medication compliance and without any major protocol deviations were analysed. A descriptive and exploratory analysis of the variables was conducted, where their distribution, outliers and missing data was evaluated. The data normality was evaluated using the Shapiro-Wilk test. The summary (mean, standard deviation, minimum and maximum) and analysis of change (mean difference, standard error) of efficacy and safety parameters was compiled using Mann-Whitney U test, Kolmogorov-Smirnov test, Wilcoxon-Mann-Whitney and Kruskal-Wallis test. Baseline characteristics and use of rescue medication was characterized by frequency percentage distribution. All statistical analyses were performed using SAS package 9.4 (SAS® Institute Inc., USA) and analysis was displayed as mean ± SD with statistical tests being interpreted at 5% level of significance.

3. Results

A total of 56 subjects was screened for eligibility and 45 subjects were enrolled in the present study. Eleven subjects were adjudged to be screening failures as per the set inclusion/exclusion criteria (Existing conditions and concurrent use of prohibited medications). The PP population consisted of 42 subjects with 3 subjects dropping out (lost to follow-up) during the course of the study (Fig. 1).

3.1. Baseline characteristics

The mean ± SD age of subjects was 56.73 ± 8.96 years consisting of 19 males (45.23 %) and 23 females (54.76 %). The mean ± SD score of WOMAC pain subscale Q.1 was 3.05 ± 0.73 and mean ± SD scores of WOMAC domains pain, stiffness, physical difficulty & optional scale were 14.10 ± 2.13, 6.43 ± 0.99, 43.67 ± 6.39 & 10.14 ± 1.37 respectively (Table 2).

3.2. Efficacy analysis

3.2.1. Primary efficacy analysis

3.2.1.1. WOMAC Pain – Q.1 – Pain when walking on a flat surface. At baseline, mean ± SD score of WOMAC pain subscale Q.1 was 3.05 ± 0.73 that reduced to 2.05 ± 0.66 at the end of week 12; $p < 0.001$. This indicates that study subjects experienced clinically significant relief in pain experienced while 'walking on a flat surface' (Table 3).

3.2.2. Secondary efficacy analysis

3.2.2.1. WOMAC Pain – Q.2 – Pain while going up or down the stairs. At baseline, mean ± SD score of WOMAC pain subscale Q.2 was 3.60 ± 0.54 that reduced to 1.60 ± 0.63 at the end of week 12; $p < 0.001$. This indicates that study subjects experienced clinically significant relief in pain experienced while 'going up or down stairs' (Table 3).

3.2.2.2. WOMAC domain - pain. At baseline, the mean ± SD pain score was 14.10 ± 02.13 and results demonstrated that pain scores were significantly reduced at the end of 12-weeks to 7.10 ± 2.53; $p < 0.001$. The difference between the two time points clearly displays the superiority of DPOR/JR007 in reducing knee OA pain in study volunteers

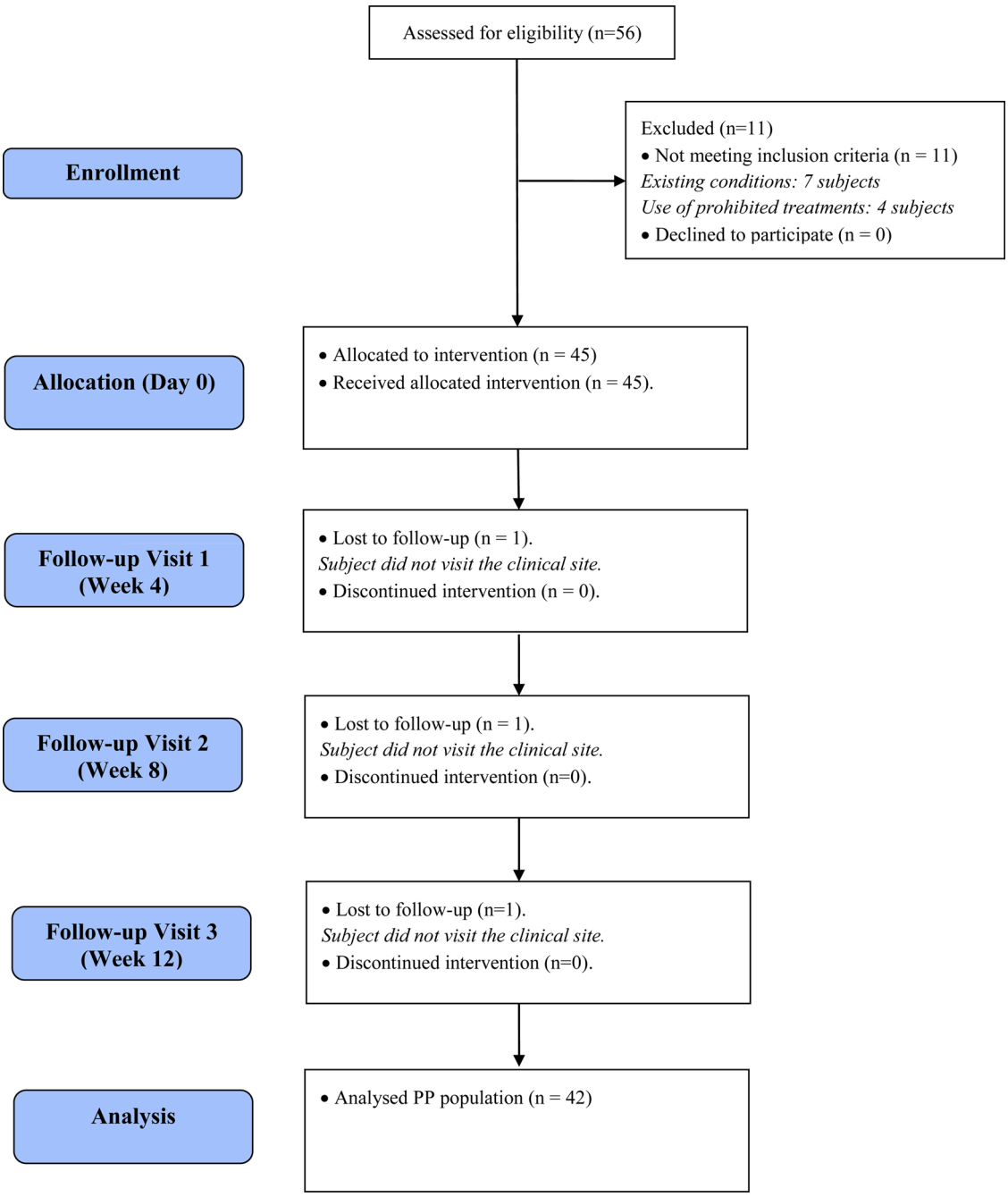

Fig. 1. n = number of patients, PP = per protocal.
Caption: Flow diagram of patient disposition in DPOR/JR2007 study

(Table 4 & Fig. 2).

3.2.2.3. WOMAC domain – physical function. At baseline, the mean ± SD physical function score was 43.67 ± 6.39 and at the end of 12-weeks the reduction in scores to 7.10 ± 2.53 achieved statistical significance; $p < 0.001$. The difference between the two scores clearly shows that administration of DPOR/JR2007 improved knee joint functioning in study volunteers (Table 4 & Fig. 2).

3.2.2.4. WOMAC domain - stiffness. The mean ± SD stiffness sub-scores at baseline that was 6.43 ± 0.99 was also observed to be decreased to 3.21 ± 1.22; $p < 0.001$. This significant reduction demonstrates that DPOR/JR2007 can relieve knee joint stiffness of individuals when consumed for a period of 12-weeks (Table 4 & Fig. 2).

3.2.2.5. WOMAC domain – optional. The optional domain of Asian WOMAC questionnaire includes questions such as physical difficulties faced when - Sitting cross legged on the floor; Arising from cross legged position; and Squatting. The questions are an extension of the physical difficulty domain especially for the aforementioned activities seen predominantly in the Indian population. Results demonstrate that a statistically significant improvement was observed in mean ± SD score for optional domain that was 10.14 ± 1.37 at baseline and reduced to 4.55 ± 1.74 at the end of 12-weeks; $p < 0.001$. This clearly implies that individuals demonstrated improvement in knee joint functions with reference to above mentioned physical activities (Table 4 & Fig. 2).

3.2.2.6. SF-8 HRQoL. At the end of 12 weeks the subjects experienced considerable reduction in each of the generic SF-8 health concepts

Table 2
Subject Demographics and Baseline Characteristics.

DPOR/JR2007 (n = 42)	
Gender	
Male	19 (45.23 %)
Female	23 (54.76 %)
Age (Years)	
Mean	56.73
Std. Dev.	8.96
Median	58
(Min, Max)	(4172)
Height (Cm)	
(Min, Max)	(126,171)
Weight (Kg)	
(Min, Max)	(5290)
Mean scores Q.1 of subscale pain	
Mean	3.05
Std. Dev	0.73
WOMAC Domains Mean ± SD score	
WOMAC Pain	14.10 ± 2.13
WOMAC Stiffness	6.43 ± 0.99
WOMAC Physical function	43.67 ± 6.39
WOMAC Optional scale	10.14 ± 1.37

n (%) – number / percentage of subjects.

Table 3
Effect of DPOR/JR2007 on Q. I & II of WOMAC Pain Domain.

Q.1 – Absolute change from Baseline to Week 12				
Visit	n	Mean	SD	p value
Baseline	42	3.05	0.73	<0.001
Week 12	42	2.05	0.66	
Q.2 – Absolute change from Baseline to Week 12				
Visit	n	Mean	SD	p value
Baseline	42	3.6	0.54	<0.001
Week 12	42	1.6	0.63	

n- number of subjects.
SD - standard deviation.

Table 4
Effect of DPOR/JR2007 on WOMAC Domains of Pain, Stiffness, Physical function and Optional scale.

Pain (n = 42)			
Visit	Mean	SD	p value
Baseline	14.10	2.13	<0.001
Week 12	7.10	2.53	
Physical function (n = 42)			
Visit	Mean	SD	p value
Baseline	43.67	6.39	<0.001
Week 12	20.71	7.26	
Stiffness (n = 42)			
Visit	Mean	SD	p value
Baseline	6.43	0.99	<0.001
Week 12	3.21	1.22	
Optional scale (n = 42)			
Visit	Mean	SD	p value
Baseline	10.14	1.37	<0.001
Week 12	4.55	1.74	
Total			
Visit	Mean	SD	p value
Baseline	74.33	10.07	<0.001
Week 12	35.57	11.41	

n - number of subjects.
SD - standard deviation.

(Table 5). The mean ± SD SF- 8 total score at baseline was 18.36 ± 2.17 which reduced significantly 7.24 ± 3.32 at the end of 12-weeks; $p < 0.001$ (Table 6). This implies that DPOR/JR2007 improved the QoL of study subjects that parallels and further validates reduction in pain and stiffness with improving physical function of study subjects.

3.2.2.7. Use of rescue medication. In the present study, none of the subjects used Ibuprofen as rescue medication. Analysis showed that 8 (19.05 %) out of 42 subjects used Paracetamol for alleviating joint pain during day 0–28 and 7 (16.67 %) out of 42 subjects during the day 28–56 study period. It is worth mentioning that from day 56 until the end of the treatment period, none of the subjects used Paracetamol for pain management. This not only depicts the analgesic potency of DPOR/JR2007 as an effective alternative to presently used relief medications but also shows that a period of 8 - 12 weeks is necessary for experiencing optimum and sustained therapeutic effect.

3.2.2.8. Biomarker analysis. At the end of 12 weeks, TNF-α levels were reduced in both male and female subjects. At baseline, the mean ± SD TNF-α value in male subjects was 8.52 ± 4.15 pg/mL that was reduced to 6.78 ± 3.45 pg/mL at the end of the study. Whereas, in female subjects, TNF-α levels were significantly reduced from being 7.48 ± 2.72 pg/mL at baseline to 5.25 ± 2.97 pg/mL at the end of 12 weeks; $p = 0.011$.

The mean ± SD serum IL-6 values at the beginning of the trial in male subjects was 9.88 ± 12.80 pg/mL, increased to 14.67 ± 23.95 pg/mL at end of the study. Even in the female subjects, mean ± SD value of IL-6 increased from being 6.80 ± 4.28 pg/mL at baseline to 9.06 ± 2.98 pg/mL at the end of 12-weeks. Though there was a mild increase in IL-6 levels post treatment however, this increase was not of clinical significance (Refer Table 7).

3.2.2.9. Uric acid and creatinine analysis. The Uric acid levels were reduced in both male and female subjects at the end of 12 weeks. At baseline, the mean ± SD value uric acid value in male subjects was 5.80 ± 1.58 umol/L that was reduced to 5.33 ± 1.72 umol/L at the end of the study. Whereas, in female subjects, uric acid level was reduced to 1.37 ± 1.06 umol/L as compared with baseline value of 5.69 ± 1.57 umol/L. Creatinine levels did not change over the period of 12-weeks in both male and female subjects.

3.3. Safety analysis

As a part of the safety evaluation, clinical examination, vital signs and adverse events were monitored throughout the study duration. Vital signs and clinical examination did not reveal any clinically significant findings in the present study. Similarly, laboratory tests performed on baseline (week 0) and final visit (week 12) were not indicative of any clinically abnormal change in the parameters. Out of 42 subjects, 6 (14.28 %) reported adverse events which were mild to moderate in nature; not requiring any form of medical intervention or stoppage of study medication. The causal relationship of AEs to the drug could not be determined and none of the subjects experienced any serious AEs (Refer Table 8).

4. Discussion

Therapeutic approaches for OA have largely been symptom based and tried to modify/improve joint structural features. However, to date there have been no effective therapies demonstrating serious potential for delaying or halting OA progression (Grässel, and Muschter, 2020). Traditional treatments (e.g. NSAIDS, analgesics etc.) are limited in controlling OA symptoms and do not provide a sense of long lasting relief (Yu and Hunter, 2015). Also, none of them can reverse the prevalent joint damage and have an unreasonable degree of adverse effects (Zhang et al., 2016). Consequently, treatments with proven efficacy and safety profile are currently needed, especially for patients that do not respond to traditional therapies. Ergo, present day researchers are extensively exploring the benefits of herbal products in degenerative musculoskeletal disorders. Nonetheless, many complementary and alternative treatments lack substantial research for endorsing their relevant clinical benefits. In the present study, we tried to explore the

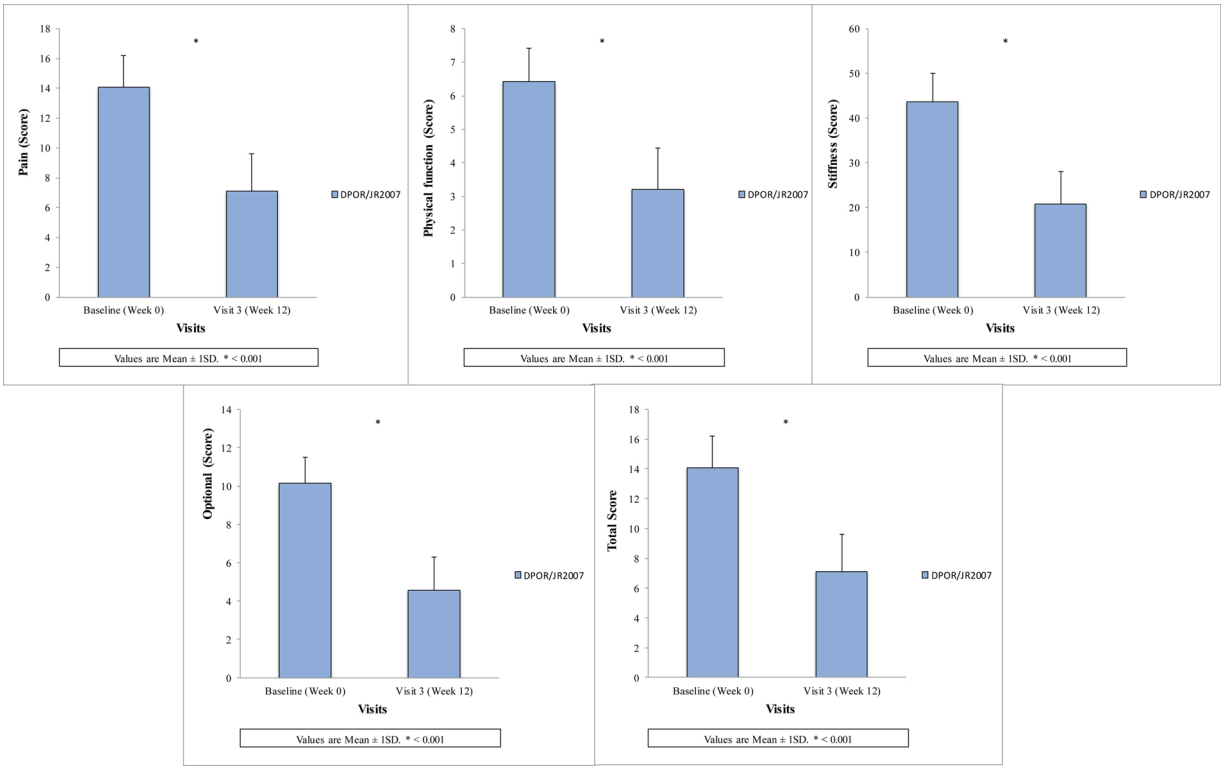

Fig. 2. Effect of DPOR/JR2007 on WOMAC Domains of Pain, Stiffness, Physical function, Optional scale and Total score.
Caption: Effect of DPOR/JR2007 on each WOMAC domains (a: pain; b: stiffness; c: physical difficulty; d: optional) and mean WOMAC score (e: total WOMAC score). Bars indicate 1 SD. *p<0.001 between baseline (week 0) and visit 3 (week 12).

Table 5
Effect of DPOR/JR2007 on SF – 8 scores.

Visit	Overall Health change	Physical functioning	Physical health	Pain	Energy /Fatigue	Social functioning	Emotional problem	Emotional Well being	Total score
Baseline	4.11	2.49	2.38	3.67	1.98	1.44	0.98	1.22	18.26
Week 4	3.05	1.89	1.80	2.64	1.55	0.91	0.75	0.98	13.54
Week 8	2.18	1.61	1.34	2.00	1.41	0.66	0.50	0.80	10.49
Week 12	1.55	1.02	0.88	1.24	1.05	0.06	0.24	0.62	6.65

n – number of subjects.
Note – Only mean values are presented.

Table 6
Effect of DPOR/JR2007 on Total SF – 8 scores.

Visit	n	Mean	SD	Mean	SD	p value	p value
Baseline	42	18.36	2.17	5.17	0.60	<0.001	<0.001
Week 4	42	13.19	2.77				
Baseline	42	18.36	2.17	7.79	0.68	<0.001	<0.001
Week 8	42	10.57	2.86				
Baseline	42	18.36	2.17	11.12	1.15	<0.001	<0.001
Week 12	42	7.24	3.32				

n – number of subjects.
SD - Standard deviation.

benefits of a proprietary formulation DPOR/JR2007 comprising 7 standardised botanical ingredients: *Aristolochia indica* (Mathew et al., 2011), *Balsamodendron mukul* (Gujral et al., 1960; Kimura et al., 2001), *Pterospermum acerifolium* (Sannigrahi et al., 2010), *Cassia tora* (Kumar et al., 2017), *Withania somnifera* (Sumantran et al., 2008, 2007), *Vitex. negundo* (Zheng et al., 2014; Chattopadhyay et al., 2012) and *Hemidesmus indicus* (Mehta et al., 2012) in individuals with manifestations of knee OA.

Hip and knee OA are the greatest contributors to walking difficulty in patients (Kendzerska et al., 2017; King et al., 2018) especially, from a resting position. Therefore, we evaluated the subject response to 'Pain experienced while walking on a flat surface' as the primary study measure. Results demonstrated that DPOR/JR2007 supplementation was associated with significant improvement in Q.I and Q.II of WOMAC pain subscale. At the end of 12 weeks, marked and sustained improvement was demonstrated in pain reduction and enhanced mobility as per Q.I & II; $p < 0.001$. This result is further validated by similar improvement being visible in all of the WOMAC domains in the present study. A significant clinical difference of 49.65 %, 50.08 % and 52.52 % was observed for pain, stiffness and physical function and the responder outcome of our study significantly surpasses placebo effect mentioned in various studies and meta-analysis (Wandel et al., 2010; da Costa et al., 2017). Additionally, our findings are in line with results of studies having ingredients similar to DPOR/JR2007 (Nipanikar et al., 2013; Chopra et al., 2000, 2004).

In OA patients, most common complaints interfering with daily living and recreational activities are walking difficulties, stair negotiation and ability to exercise and squatting. These physical manifestations of knee OA have a direct impact on other aspects of patients' lives such as social interactions, mental functioning, and sleep quality (Törmälehto et al., 2018). Hence, HRQoL measures are relevant and important

Table 7
Effect of DPOR/JR2007 on Biomarkers and Haematological parameters.

Uric acid							
	Males			Females			
	n	Mean	SD		n	Mean	SD
Baseline	19	5.80	1.58	Baseline	23	5.69	1.57
Week 12	19	5.33	1.72	Week 12	23	1.37	1.06
P value -	0.5895			P value -	0.5453		
Creatinine							
	Males			Females			
	n	Mean	SD		n	Mean	SD
Baseline	19	0.99	0.25	Baseline	23	0.78	0.17
Week 12	19	0.95	0.19	Week 12	23	0.78	0.13
P value -	0.6169			P value -	0.9476		
TNF – α							
	Males			Females			
	n	Mean	SD		n	Mean	SD
Baseline	19	8.52	4.15	Baseline	23	7.48	2.72
Week 12	19	6.78	3.45	Week 12	23	5.25	2.97
P value -	0.1681			P value -	0.0110		
Interleukin – 6							
	Males			Females			
	n	Mean	SD		n	Mean	SD
Baseline	19	9.88	12.80	Baseline	23	6.80	4.28
Week 12	19	14.67	23.95	Week 12	23	9.06	2.98
P value -	0.4475			P value -	0.0437		

n – number of subjects.
SD - standard deviation.

Table 8
Summary of Adverse Events.

		DPOR/JR2007 (n = 42)	
System organ class	Preferred term	n	%
Vestibular disorders	Dizziness	3	6.6
Gastrointestinal disorders	Constipation	1	2.2
General disorders and administration site Conditions	Fever	1	2.2
Gastrointestinal disorders	Diarrhea	1	2.2

n/% - number/percentage of subjects reporting at least one specified symptom during treatment.

adjunct outcomes that help quantify the physical, social, and emotional impact of knee OA and of various OA therapies. In the present study, significant improvement was evidenced in each of the SF-8 parameters. These findings can be further correlated to outcome of 'Investigator' and 'Subject Global' assessments that displayed significantly higher scores at the end of the treatment period (Supplementary material I). As the study progressed, none of the subjects required rescue medication. This clearly infers that study subjects were satisfied with the quantum of pain relief and ease of mobility experienced after DPOR/JR2007 consumption. The study results are similar to a study published in 2013 (Nipanikar et al., 2013) that also reported a similar decrease in use of rescue medications. Furthermore, AEs reported were mild to moderate in nature and resolved without any sort of medical intervention with none of the subjects dropping out due to abrupt discontinuation of study product. The analysis of complete blood count, vitals (heart rate and blood pressure) and clinical examination did not reveal any findings of significant nature and none of the subjects reported worsening of knee OA symptoms over the study period. This further advocates the excellent safety and tolerability profile exhibited by DPOR/JR2007 over the course of the study.

As per literature, one of the major factors involved in the OA progression are cytokine alterations resulting in enhanced activation of the inflammatory pathways (Gallelli et al., 2013; Imamura et al., 2015). Localised inflammation due to the overproduction of TNF-α, IL-6 and IL-8 is reckoned as a major cause of OA pain (Gallelli et al., 2013; Imamura et al., 2015; Scanzello et al., 2009). Several studies have compared the presence of TNF-α with other pro-inflammatory biomarkers in knee OA. Results suggest that TNF-α is significantly elevated in comparison to the other proinflammatory biomarkers in patients with knee OA (Hochberg et al., 2012; Pearle et al., 2007). A 2017 study reported that TNF-α is predominantly responsible for escalating the development and severity of inflammation (Wang, 2017). It is worth mentioning that TNF-α is also important for host defence mechanism and has protective functions against several stressors during intracellular infection (Monaco et al., 2014). With regards to IL-6, it is a pleiotropic cytokine that has both pro- and anti-inflammatory activities (Balto et al., 2001). Various tissues and cell types such as T cells, B cells, monocytes, fibroblasts, osteoblasts, mesangial cells and also a few tumour cells are known to produce IL-6 (Srirangan and Choy, 2010). Its constant dysregulation has been shown to have a pathological role in multiple chronic and autoimmune inflammatory disorders. However, in conditions that are environmentally stressful, IL-6 has been reported for contributing to hosts' defence mechanism (Tanaka and Kishimoto, 2012). A study by Tsuchida et.al demonstrated that high concentrations of IL-6 are produced by chondrocytes (especially by osteoarthritic chondrocytes) during the regeneration process. With regards to the results presented in our study, TNF-α values were decreased in study volunteers. This can be interpreted as reduction in the local inflammatory activity whereas, the IL-6 levels although increased could plausibly represent the onset of neo-cartilage regeneration as elucidated by the Tsuchida study. Hence, this increase in IL-6 combined with reduction in TNF-α levels may represent mitigation of connective tissue degeneration that can have a cellular repair effect in individuals with chronic knee OA. Although a significant amount of literature can correlate our present findings, further research should be carried out to ascertain if physiological concentrations of inflammatory cytokines earlier thought to promote cartilage degradation may also have an important role to play in cartilage regeneration.

The ingredients of our polyherbal formulation 'DPOR/JR2007' possess potent anti-inflammatory, antioxidant, chondroprotective, antinociceptive and anti-arthritic properties (Mathew et al., 2011; Gujral et al., 1960; Kimura et al., 2001; Sannigrahi et al., 2010; Kumar et al., 2017; Sumantran et al., 2008; Zheng et al., 2010 and Mehta et al., 2012). It is evident from the results that the synergistic action of herbs present in DPOR/JR2007 not only alleviates pain in individuals but also increases their mobility by reducing knee tightness or stiffness. Moreover, TNF-α and IL-6 findings give credence to the anti-inflammatory potential of DPOR/JR2007 in OA induced chronic inflammation. But it is understood there are limitations to this research study. The sample size though sufficiently powered is small and the open-label uncontrolled trial design limits the overall conclusion that can be drawn presently. However, the responder outcome in the study significantly surpassed the placebo effect reported in various studies and meta-analysis (Wandel et al., 2010; da Costa et al., 2017). Also, the ingredients used in the present study have significant amounts of research advocating its use in knee OA. Our findings thus provide an exciting insight for future clinical studies and a natural progression of this work would be to understand the exact mechanism of DPOR/JR2007 with respect to cytokines in larger randomized controlled trials.

5. Conclusion

DPOR/JR2007 supplementation was effective in relieving the pain and stiffness in patients with chronic knee OA and improvement was evident within 8–12 weeks. Future studies are warranted for understanding the exact mechanism of DPOR/JR2007 with respect to inflammatory cytokines.

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CRediT authorship contribution statement

Aasin Maurya: Conceptualization, Supervision, Writing - review & editing. **Ankul Suresh Kokate:** Writing - original draft, Writing - review & editing. **Kumaraswamy Dussa:** Investigation. **Anirudh Tripathi:** Investigation.

Declaration of Competing Interest

Dr. Aasin Maurya is affiliated with Integrity Healthcare Services. The authors report no other conflicts of interest in this work.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.hermed.2021.100422.

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