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Efficacy and Safety of *Justicia gendarussa* Burm.f. Medicated Spray and Topical Diclofenac Spray for the Treatment of Mild to Moderate Soft Tissue Injury: A Randomized Double-blinded Controlled Trial

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Abstract

Kraduk Kai Dam (*Justicia gendarussa* Burm.f.) has been used in folk medicine by pounding the leaves with rice whisky and using a poultice to treat muscle pain, bruises, and joint pain. The purpose of this study was to evaluate the efficacy and safety of *J. gendarussa* medicated spray (JGS) in comparison with that of diclofenac spray (DFS) to treat patients with mild to moderate soft tissue injury. A randomized double-blinded controlled trial was performed in the Orthopedic Surgery Department of Chao Phya Abhaibhubejhr Hospital, Prachin Buri province, Thailand. Patients aged 15–70 years were randomly assigned to receive either two puffs of JGS or DFS thrice daily for seven days. The patients' decreases in rest pain and swelling were considered primary outcomes, while the use of pain medication, patients' global assessment, and reported adverse events were secondary outcomes. The results showed that there were no differences in baseline characteristics between the two groups. At the end of the study, the pain scores in the JGS and DFS groups were not different (mean difference VAS = -0.13, 95%CI -0.81, 0.56; $p = 0.68$); the reductions in swelling of the JGS and DFS groups were not differentt (risk ratio = 0.988, 95%CI 0.66, 1.48; $p = 0.951$); the amounts of paracetamol given to the patients were not different between the two groups ($p = 0.194$); and the skin irritation was not statistically different in both groups, i.e. 8.3% and 6.38% of the patients treated with JGS and DFS ($p > 0.05$), respectively. In summary, the efficacy and safety of JGS to treat mild to moderate tissue injuries were comparable to those of DFS.

Key words: *Justicia gendarussa* Burm.f., soft tissue injury, efficacy, safety, randomized controlled trial

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ประสิทธิศักย์และความปลอดภัยของสเปรย์ผสมสมุนไพรกระดูกไก่ดำเทียบกับไดโคลฟีแนกสเปรย์ในการรักษาการบาดเจ็บของเนื้อเยื่อ

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บทคัดย่อ

สมุนไพรกระดูกไก่ดำ (*Justicia gendarussa* Burm.f.) เคยมีการใช้เป็นยาพื้นบ้านโดยนำใบตำผสมสุราแล้วพอกรักษาอาการปวดกล้ามเนื้อ บวม ปวดข้อ การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบประสิทธิศักย์และความปลอดภัยของสเปรย์ผสมสมุนไพรกระดูกไก่ดำ (JGS) เปรียบเทียบกับยาสเปรย์ไดโคลฟีแนก (DFS) ในผู้ป่วยที่มีการบาดเจ็บของเนื้อเยื่อ โดยศึกษาเชิงทดลองแบบสุ่มเปรียบเทียบปกปิดทั้งสองด้าน ในผู้เข้าร่วมวิจัยที่มีการบาดเจ็บของเนื้อเยื่ออ่อนที่ระดับความรุนแรงน้อยถึงปานกลาง ที่มารับการรักษาที่โรงพยาบาลเจ้าพระยาอภัยภูเบศร อายุระหว่าง 15-70 ปี ผู้เข้าร่วมวิจัยถูกแบ่งออกเป็น 2 กลุ่มโดยวิธีการสุ่ม กลุ่มแรกได้รับยาสเปรย์ผสมสมุนไพรกระดูกไก่ดำ กลุ่มที่ 2 ได้รับยาสเปรย์ไดโคลฟีแนก พ่นบริเวณที่มีการอักเสบของเนื้อเยื่อวันละ 3 ครั้ง เช้า กลางวัน เย็น เป็นระยะเวลา 7 วัน ประเมินประสิทธิศักย์ในการบรรเทาอาการปวดด้วยคะแนนความปวดขณะพัก การบวมบริเวณที่บาดเจ็บ รวมทั้งจำนวนเม็ดยาพาราเซตมอลที่รับประทาน ประเมินระดับความรู้สึกโดยรวมของผู้เข้าร่วมวิจัย และผลข้างเคียงจากการใช้ผลิตภัณฑ์ ผลการศึกษาพบว่า ลักษณะประชากรระหว่างผู้เข้าร่วมวิจัย 2 กลุ่มไม่แตกต่างกัน เมื่อประเมิน pain score พบว่า ยา JGS มีผลลดคะแนนความปวดได้ไม่แตกต่างจากยา DFS (mean difference VAS= -0.13, 95%CI -0.81, 0.56; $p = 0.68$) ยา JGS มีผลลดการบวมได้ไม่แตกต่างกับการใช้ยา DFS (risk ratio = 0.988, 95%CI 0.66, 1.48; $p = 0.951$) เมื่อสิ้นสุดการรักษาพบว่าผู้ป่วยในทั้งสองกลุ่มใช้ยาพาราเซตามอลเพื่อแก้ปวดไม่แตกต่างกัน ($p = 0.194$) และพบผลข้างเคียงจากยาคือการระคายเคืองผิวหนังไม่แตกต่างกันทางสถิติ 8.3% และ 6.38 % ในกลุ่ม JGS และกลุ่ม DFS ตามลำดับ ($p > 0.05$) กล่าวโดยสรุป การศึกษานี้แสดงให้เห็นว่ายาสเปรย์ผสมสมุนไพรกระดูกไก่ดำมีประสิทธิศักย์และความปลอดภัยเทียบเท่ากับยาสเปรย์ไดโคลฟีแนกในการรักษาการบาดเจ็บของเนื้อเยื่อที่มีระดับความรุนแรงน้อยถึงปานกลาง

คำสำคัญ: สมุนไพรกระดูกไก่ดำ, การบาดเจ็บของเนื้อเยื่อ, ประสิทธิศักย์, ความปลอดภัย, การทดลองแบบสุ่ม

Introduction and Objectives

Soft tissue injury – commonly resulting from sprain, strain,one-off blow that forms contusion, and overuse of a particular body part – can cause pain and inflammation. Such injury limits physical mobility; and, if severe, will possibly cause short-term disability[1]. An appropriate treatment is likely to shorten the recovery time and reduce medical expenses as well as prevent permanent disability. Non-

steroidal anti-inflammatory drugs (NSAIDs), in both oral and external forms, are commonly used to reduce inflammation, pain, and swelling[1-2].

According to some systematic reviews, using external NSAIDs to treat patients for acute pain like soft tissue trauma strains and sprains could give a big advantage[3]. The relative benefit of NSAIDs was 1.7 times (95%CI 1.5-1.9) higher than a placebo and the number of required treatments was 3.9 (95%CI 3.4-4.4). The incidence of local side effects, compared to a placebo, was equal to 3.6%, while the systemic adverse effects were less than 0.5%[3]. In general, a 1%w/w diclofenac spray is a topical formulation most commonly used for acute pain in the hospitals in Thailand.

Justicia gendarussa Burm.f. (JG) or Kraduk Kai Dam in Thai, also synonym as Gendarussa vulgaris Nees in some authoritative compendia–is the native shrubby plants which grow wild and are cultivated[4] in several south and southeast Asian countries, including Thailand. In traditional Chinese medicine, 'xiao bo gu' (Pinyin transliteration 小驳骨) or dried aerial parts of JG or Gendarussae Herba in the Pharmacopoeia of the People's Republic of China are the substance used in the pharmaceutical formulations for treating injuries resulting from falls, sinew injury and fracture, bone ache caused by wind-dampness, blood-stasis amenorrhea, and postpartum abdominal pain. In addition, JG is used in Thai folk medicine to treat musculoskeletal disorders such as pain, bruising, and swelling by pounding fresh leaves with alcohol and applying the paste or poultice on the affected areas[5]. According to some current research studies, its anti-inflammatory effects is due to the inhibition of both cyclooxygenase (COX) and lipoxygenase (LOX) pathways–which prevents the secretion of various proinflammatory mediators such as prostaglandins, histamine, nitric oxide, inducible nitric oxide synthase (iNOS), and matrix metallopeptidase 9 (MMP-9). The whole plant extract acts on opioid receptors in a similar way to that of morphine, but JG juice extract is approximately 2-5 times less potent than morphine[6]. Moreover, JG–also having similar anti-inflammatory mechanisms to that of steroids–stabilizes or inhibits lysosomal membranes in white blood cells from releasing hydrolytic enzymes and suppresses immunity by reducing white blood cell mobility. In addition, JG extract has analgesic effects which are equivalent to that of aspirin[7-8]. In rat models, ethanolic extract of JG shows a significant anti-arthritic activity in a similar way to that of the aspirin[9]. JG medicated spray (JGS), recently been approved by the Thai Food and Drug Administration (TFDA) as a topical preparation for the relief of bruise, muscle ache, and joint and muscle inflammation, is wildly used in Thailand's pharmacies.

This study aimed at investigating the efficacy and safety of JGS to treat mild to moderate soft tissue injury in comparison with that of diclofenac spray (DFS).

Materials and Methods

Study design

A double-blinded controlled trial was conducted to obtain comparative data on the efficacy and safety of JGS and DFS to treat mild to moderate soft tissue injury in the Orthopedic Department of Chao Phya Abhiabhubejhr Hospital from February to March 2018. This study was registered in the Thai Clinical Trial Register (TCTR): TCTR20180523005 as well as approved (approval number: 07/2560) by the Ethics Committee for Research in Human Subjects in the Fields of Thai Traditional and Alternative Medicine, Ministry of Public Health. Subjects were fully informed about the study protocol and their rights and the participants signed the informed consent forms before taking part in this research.

Participants in this study were patients with mild to moderate soft tissue injuries predominantly recruited from the patients in the Orthopedic Department on the basis of the inclusion and exclusion criteria. They were randomly assigned into 2 groups at a 1:1 ratio: one group received JGS and the other received DFS by a computer-generated list number.

All the study participants' swelling was then assessed by a blinded physician. Meanwhile, the data on the measurement of pain by VAS scores and the participants' global assessment were collected by a blinded research assistant. Each individual participant was given a self-reported booklet to record his or her pain score, rescue medication usage and side effects daily before bedtime. After seven days of intervention, the participants were asked to bring their booklets to see a blinded physician who would assess the swelling of affected area and record the global assessment of the overall health in their booklet at the appointment time.

Participants

Inclusion and exclusion criteria of the subjects: One included as the subject of this study was the patient aged 15-70, regularly attended the Orthopedic Department, was diagnosed with mild to moderate soft tissue injuries[10], and was willing to sign the informed consent form to take part in the study. One excluded from this study was the patient who had joint dislocation, open wound on the affected areas, signs and symptoms of infections in the sprayed area, or a history of being allergic to the plants in the Acanthaceae family or diclofenac; as well as a pregnant woman, or a nursing mother.

The participants were instructed to shake a bottle of either JGS or DFS well

before applying two puffs of the solution on the affected areas thrice daily: in the morning, the afternoon, and the evening for seven consecutive days. A 10-tablet pack of 500 mg paracetamol was prescribed as the rescue medication for each participant. If the pain in the affected areas persisted, the participants were instructed to take one paracetamol tablet every 4-6 hours as a rescue medication until the pain disappeared. Five parameters evaluated the treatment outcome: pain, swelling, use of paracetamol as a rescue medication, patients' global assessment, and reported adverse events after using JGS and DFS. The data were collected before the intervention, every day of the intervention before bedtime, and at the end of the study.

Interventions

JGS spray containing 5% quercetin was manufactured by Chao Phya Abhaibhubejhr Hospital Foundation under the Pharmaceutical Inspection Co-operation Scheme (PIC/s) specified in Good Manufacturing Practice (GMP) guidelines. JGS ethanolic extract was the main ingredient of the topical spray, and its preparation is composed of cajuput oil, menthol, camphor, and peppermint oil. Quality control of raw materials, JGS extract, and finished products complied with the Thai Herbal Pharmacopeia. The analysis certification was done by the research and development unit of Chao Phya Abhaibhubejhr Hospital Foundation. The comparator medicine was the diclofenac spray (Volclonac spray®). Each 100 grams of solution DFS contains the equivalent amount of 1 g of diclofenac sodium. The DFS was purchased from the Lerd Singh Pharmaceutical Fact Ltd. (Bangkok, Thailand). Furthermore, to maintain the double-blinded condition of the study design, the DFS was repackaged within the bottle with opaqued to mask the color and smell differences. The bottle of both drugs had the same shape, size, and color.

Determination of study outcomes

The primary outcomes including pain at rest were used to assess the efficacy of the treatment. Visual analogue scale (VAS), a 10-cm line scale for the rating of pain intensity dimensioned from 0 (no pain) to 10 (unbearable pain), was used to measure the treatment outcome. The research subjects were asked to place a vertical mark on the scale to indicate the level of pain intensity every day before bed time. A blinded physician evaluated the research subjects' swelling at baseline and the end of the study as another primary outcome. The swelling that occurred was recorded in the hospital application. The swelling was assessed by expert opinion. The clinical improvement in swelling of the injured area from baseline after a treatment period of 7 days was presented as the number of patients with a

'Yes' or 'No' response.

The secondary outcomes included the number of paracetamol tablets research subjects used, the patient global assessment, and adverse events. The subjects are requested to record the number of paracetamol tablets they took each day and the adverse events (if any) in their self-report booklet. The patient global assessment was a tool for the subjects to state the level of their overall condition on a scale of 1 (very good), 2 (good), 3 (fair), 4 (poor), 5 (very poor). The patient global assessment was investigated two times at baseline and at the end of the study.

Sample size

The size of the subjects in this study was estimated upon the basis of the reduction of pain score after using *Justicia gendarussa* Burm.f. and *Sida rhombifolia* L. medicated spray cited in the pilot study of Rodfak, *et al*[11]. Based on the principle of non-inferiority trial design, the sample size, calculated using Stata version 14.2, yielded 78 subjects in both groups (39 subjects per group). However, as the loss to the follow-up rate was approximately equal to twenty percent, the number of the subjects in total was adjusted to 94. In summary, the sample size of this study was rounded up to 100 subjects.

Statistical analyses

Stata version 14.2 was used to statistically analyze the raw data. Alpha error of two-tailed analysis was set at 5%. Descriptive statistics were employed to express the baseline demographic and disease characteristics of the participants. Paired t-test was used to compare the VAS scores before and after the treatments, while repeated measure ANOVA was applied to compare the pain VAS scores day by day throughout the 7-day intervention. Survival analysis was employed to assess the onset time of clinical improvement and the hazard ratio of both treatments. Pearson's Chi-square test and Fisher's exact test were used to evaluate how different the adverse incidence of the subjects in both groups was.

Results

Figure 1 The flow diagram indicating the stages of undergoing the parallel randomized trial of the medications for treating patients in two groups for soft tissue injury, allocation, interventions, follow-up, loss to follow-up rate, and analysis

The flow diagram in Figure 1 showed the stages of undergoing the parallel randomized trial of the medications for treating patients in two groups for soft tissue injury. Two participants treated with JGS and three others

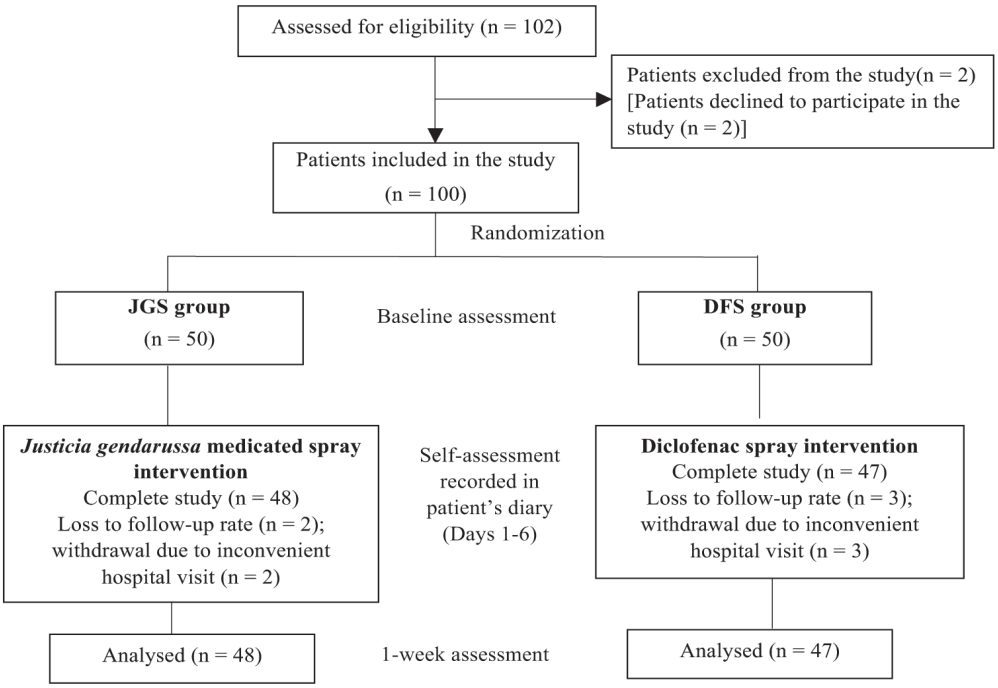

Figure 1 The flow diagram indicating the stages of undergoing the parallel randomized trial of the medications for treating patients in two groups for soft tissue injury, allocation, interventions, follow-up, loss to follow-up rate, and analysis.

treated with DFS were lost to the follow-up due to hospital visit inconvenience. The baseline demographic data and clinical characteristic of all the ninety-five participants are then analyzed as shown in Table 1.

The female participants in this study were the majority (68.09%) of the patients treated with DFS, while more males than females were treated with JGS. The difference between the number of male and female participants in both treatment groups was significant ($p = 0.023$). The average age of the participants in DFS and JGS groups were 41.36 and 40.06 years, while the average body mass indices (BMI) were 24.25 and 24.90 kg/m^2, respectively, which were not statistically significant different. The most frequently diagnosed injuries of the subjects treated with DFS were comparable to those of the subjects treated with JGS ($p = 0.965$), namely back strain (51.06% vs. 47.92%), knee sprain (19.15% vs. 25.00%), and shoulder sprain (12.77% vs. 14.58%). Swelling occurred in almost all the participants. Mean VAS pain scores and patient's global assessment values of the participants treated with DFS and JGS were 5.60 vs 5.94. and 3.19 vs 3.17, respectively, which were not significantly different as well. Hence, as shown in Table 1, baseline demographic and clinical characteristics of the two treat-

Table 1 The baseline demographic and clinical characteristics of both treatment groups

		Diclofenac spray	*Justicia gendarussa* medicated spray	*p*-value
		n = 47	n = 48	
Gender	Male	15 (31.91%)	27 (56.25%)	0.023
	Female	32 (68.09%)	21 (43.75%)	
Age (years)		41.36 (10.20)	40.06 (12.03)	0.572
	Minimum	21	16	
	Maximum	59	67	
Body Mass Index (BMI)		24.25 (4.85)	24.90 (4.51)	0.502
	Minimum	15.98	17.58	
	Maximum	41.10	38.82	
Diagnosis	Back strain	24	23	0.965
	Knee sprain	9	12	
	Shoulder sprain	6	7	
	Neck strain	4	3	
	Elbow sprain	1	2	
	Foot sprain	1	1	
	Interphalangeal joint	1	0	
	Ankle sprain	1	0	
Swelling	Yes	47	46	> 0.05
	No	0	1	
Visual Analogue Scale*		5.68 (1.63)	5.94 (1.67)	0.45
Patients' global assessment†		3.19 (0.68)	3.17 (0.60)	0.873

Values are presented as Number (Percentage) or Means (S.D.)

*VAS rating scale of 0 (No pain) to 10 (Unbearable pain)

†Patients' global assessment scale of 1 (very good) to 5 (very bad)

ment groups were not different except for the number of male and female participants.

Following a week of intervention, the VAS pain scores of both treatment groups significantly decreased ($p < 0.001$). The VAS pain score of the JGS group was a significant decrease since day 1 of the study ($p = 0.034$). In comparison, the VAS pain score of the DFS group was significantly decreased since day 2 of the study ($p < 0.001$). However, there was no significant difference of VAS pain scores between two groups on any day of the treatment period, as shown in Table 2.

Additionally, as shown in Table 3, the number of participants who still had swelling on Day 7 significantly decreased by about half in both DFS and JGS groups ($p < 0.001$). There was no difference between the two

good, and 21.28% vs. 17.39% as fair. The global assessment results of both groups were not statistically significantly different ($p = 0.875$). Similarly, the amount of paracetamol taken by

Table 2 VAS pain scores (Mean ± S.D.) at baseline and days after intervention with diclofenac and *Justicia gendarussa* Burm.f. medicated sprays.

	VAS pain scores (Mean ± S.D.)							
	Baseline	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Diclofenac spray	5.68 ± 1.63	5.33 ± 1.61	4.54 ± 1.31	3.93 ± 1.50	3.30 ± 1.49	2.78 ± 1.47	2.04 ± 1.55	2.21 ± 1.50
Justicia gendarussa spray	5.94 ± 1.67	5.17 ± 1.81	4.83 ± 1.80	4.23 ± 1.90	3.79 ± 1.88	3.02 ± 2.01	2.70 ± 2.18	2.34 ± 1.82
*p-value†	0.450	0.867	0.395	0.379	0.843	0.459	0.088	0.680

*Mixed-effects linear regression model

†Adjusted for gender

Table 3 Percentages and numbers of patients who had swelling of the affected areas and the risk ratio of swelling at a baseline and at post-treatment in the diclofenac spray and *Justicia gendarussa* Burm.f. medicated spray groups.

	Diclofenac spray (n = 46)	***Justicia gendarussa* spray (n = 45)**	***p*-value**	**Risk ratio (95%CI)†**
Swelling at baseline	100.00% (47)	97.87% (46)		
Swelling after treatment	54.35% (25)	53.33% (24)	0.951	0.988 (0.660 to 1.478)
p-value	< 0.001	< 0.001		

Values were shown as Percentage (Number)

†Adjusted for gender

groups ($p = 0.951$). While, the JGS group could reduce swelling at the risk ratio of 0.988 (95% confidence interval, 0.66-1.478) at Day 7 when compared to the DFS group.

Three days after using DFS in comparison with JGS to treat the patients for soft tissue injury, the clinical improvement of JGS group according to the hazard ratio was improved faster than that of the DFS group by 1.18 time in each day. Meanwhile, as shown in Table 4, the assessment result was not statistically different ($p = 0.447$).

The global assessment results in each group evaluated the efficacy of the medication they received on a scale of 1 to 3 (1 = fair, 2 = good, and 3 = very good). The results for DFS versus JGS, as shown in Figure 2, were 19.5% vs. 17.39% as very good, 59.57% vs. 65.22% as

Table 4 The median time to the onset of clinical improvement in the diclofenac and *Justicia gendarussa* Burm.f. spray intervention groups.

	Survival time (days)	95%CI	Hazard ratio	*p*-value
Diclofenac spray	3	3-4	1.182	0.447
Justicia gendarussa Burm.f. spray	3	2-4		

†Survival analysis

the patients in DFS and JGS groups as a rescue medication were not statistically significant either (0.85 ± 2.18 vs 1.91 ± 4.52 tablets) (p = 0.149).

Regarding the safety of the study medications, 6.38% of participants treated with DFS and 8.3% of patients treated with JGS reported skin irritation. The incidence of this adverse event was not significantly different between the two groups ($p > 0.05$). No serious adverse event was detected in either group.

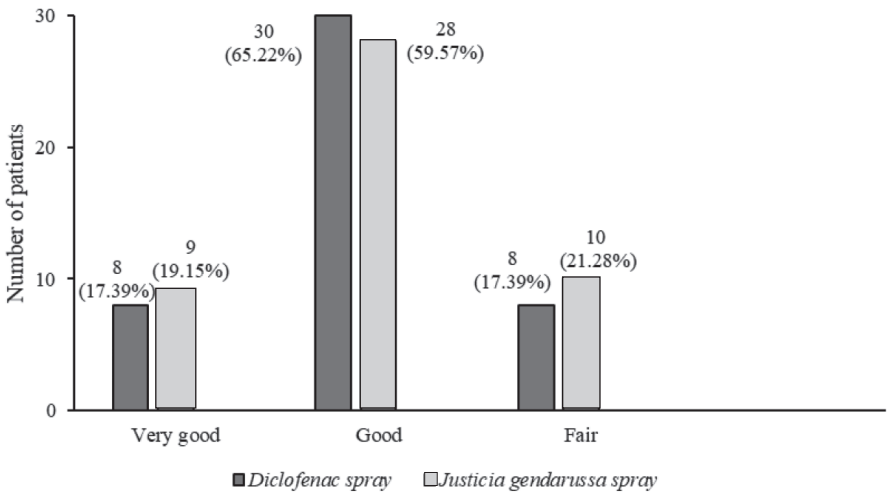

Figure 2 The patient global assessment results after a week of administering DFS and JGS.

Discussion

It was found in a previous study that topical NSAIDs, compared to a placebo, could deliver 50% relief of pain and bring about the effect of treatment in 6-14 days, according to a meta-analysis of topical NSAIDs[12]. The result of our study was consistent with the previous study, which reports that following a 7-day intervention, the VAS score of the pain of DFS provided reduced 61.05% while JGS 60.51%.

The median time JGS and DFS–both of which have COX2 inhibitory effects–reducing the swelling was 3 days. The efficacy of 4% diclofenac spray, compared to a placebo, indicated that the pain relief can become significant after 3-4 days[13-14]. Similar result was observed in our study.

Serious adverse effects on the subjects in this study were not closely observed by the subjects themselves; merely 6.38% patients in the DFS treatment group and 8.3% in the JGS treatment group reported common skin irritation. The finding of this study was consistent with the earlier study's finding; approximately 5% of the test group[15] reported the side effect of topical NSAID applied on the patients' skin. One of the limitations on verifying the efficacy of JGS in this clinical study was the small size of samples. Long-term trials of JGS will possibly provide a beneficial information about the efficacy and safety of topical therapy. Additionally, magnetic resonance imaging (MRI) scans and physical examinations are supposed to be completed before and after the intervention to confirm the anti-inflammatory effect of JG extract.

Conclusion

The efficacy and safety of using JGS to treat mild to moderate tissue injuries were not inferior to DFS. This study's findings were likely to support the traditional knowledge about the usage of JG. Further studies on JG, however, need to be done in a larger scale population, and MRI is supposed to be employed to confirm the pharmacological effect of JG.

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