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Original Research

Effect of Traditional Chinese Formula Dingkun Pill on Primary Dysmenorrhea: A Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Background: Primary dysmenorrhea (PD) afflicts many childbearing-age women, with a high prevalence ranging from 17% to 90%. The Dingkun pill (DKP), a traditional Chinese medicine formula, has been prescribed for managing menstrual disorders empirically in clinical practice for a long time, but there are very few high-quality studies supporting this practice. Therefore, this trial aimed to assess the efficacy and safety of DKP in patients with PD. **Methods**: Our study was a multicenter, prospective, randomized, double-blind, placebo-controlled study. DKP or placebo was prescribed to participants from the 5th to 14th day of each menstrual cycle for 12 weeks. Changes in pain intensity were measured by a visual analog scale (VAS) and were compared between groups using repeated measures analysis. The pain mediators and sex hormones were also assessed before and after the treatment, and their intergroup changes from the baseline were analysed by student *t*-test. The hemodynamic indices and safety profile of DKP were also investigated. **Results**: A total of 156 women were recruited and randomly allocated to receive either DKP or placebo, of whom 142 (73 in DKP and 69 in sham control) completed the study. A more distinctive reduction in VAS scores was observed in the DKP group, compared with placebo (-2.68 ± 0.21 *vs.* -1.29 ± 0.14, $p < 0.001$). Compared to placebo, DKP treatment resulted in a pronounced suppression of serum PGF2α, oxytocin and vasopressin, along with a significant increase in beta-endorphin level ($p < 0.001$). Moreover, uterine artery flow measured by ultrasonography indicated increased blood perfusion after DKP treatment ($p < 0.01$), while no change was detected in the placebo group. Additionally, except for an inhibited serum follicular stimulating hormone (FSH) ($p = 0.037$), no statistical difference in hormonal status and safety indicators was detected before and after the treatment. **Conclusions**: DKP treatment attenuated pain severity in patients with primary dysmenorrhea, and no harmful side effect was observed during 12 weeks of treatment. **Clinical Trial Registration**: ClinicalTrials.gov, NCT03953716. Registered 17 May 2019. https://clinicaltrials.gov/ct2/show/NCT03953716.

Keywords: primary dysmenorrhea; Dingkun pill; traditional Chinese medicine; alternative therapy; pain management

1. Introduction

Dysmenorrhea, the painful menstruation of uterine origin, is the most common complaint in adolescent girls and women of reproductive age during their gynecological visits [1]. Primary dysmenorrhea (PD) is characterized by a spasmodic, cramping sensation at the lower abdomen without any identifiable pelvic pathology [2,3]. Typically, painful sensation usually occurs after a few months when ovulatory cycles are attained and are accompanied by systemic symptoms such as fatigue, nausea, vomiting, diarrhea, and sleep disturbance [3]. The prevalence rate varies based on different assessment methods. Generally, PD has been estimated to affect 50% to 95% of females around the world [4,5]. As the leading cause of absenteeism in school, PD is associated with loss of productivity at work; menstrual cramp also increases the risk of developing depressive symptoms and chronic pain disorders [6,7]. Given the high prevalence and negative consequences, PD has drastically affected the social relationships of young females by interfering with their daily activities and has become a public health concern [8].

The pathomechanism of PD remains complex and has not been fully elucidated. The over-production of uterine prostaglandins (PG) appears to be the main cause of the hypercontractility of smooth muscles in vessels and uterine myometrium [9]. Based on the PG-dominated etiology, non-steroid anti-inflammatory drugs have been prescribed as the first-line treatment, followed by combined oral contraceptives [2]. However, both pharmacological agents are not suitable for long-term administration and have adverse effects on the digestive tract and central nervous system [10]. Additionally, conventional use of hormone contracep-

tives is associated with an increased risk of venous thromboembolism [11]. These concerns show an urgent need for alternative approaches to managing menstrual pain.

There's mounting evidence supporting the application of complementary and alternative medicine in the pain management field as it contains a wide range of medical practices that have been greatly developed and evolved in recent years. A systematic review found the pulsed electromagnetic fields had a significant inhibitory effect on the pain scores in patients with osteoarthritis when compared with the sham group (standardized mean differences [SMD] = 0.71, 95% confidence interval [CI]: 0.08–1.34, p = 0.03) [12]. Moreover, the association between acupuncture analgesia and the endogenous opioid system has been corroborated by many studies [13]. Results from a randomized trial also showed rubbing oil could provide additional benefits to people suffering from low back pain [14]. In China, it has been reported that over half of the patients with painful periods use traditional Chinese medicine (TCM) to treat menstruation-associated discomforts and TCM shows significant efficacy [15]. Based on the theory of conventional Chinese medicine, PD is the clinical manifestation of Qi stagnation and blood stasis syndrome [16]. TCM acts on pain perception in a holistic way with multiple components and targets, in contrast to western painkillers with a single targeted pathway [17]. The Dingkun pill (DKP), one of the well-known TCM, was developed during the Qing dynasty (A.D. 1730) and has been used as a treatment strategy for various gynecological diseases over the past three centuries in East Asia [18]. DKP, comprising thirty herbs and animal-orient ingredients, has been used in the clinical setting for its Qi-nourishing and blood-activating features [19]. In the Chinese literature, there is strong evidence that DKP has an analgesic effect on dysmenorrhea [18,20]. However, most of them are empirical reports and do not strictly comply with the standard of the CONSORT statement, which makes it difficult to support the conclusion that DKP is therapeutic in dysmenorrhea. Here, we hypothesized that DKP could alleviate PD compared with placebo and aimed to investigate the clinical efficacy, safety, and mechanism of action of DKP in PD using high-level evidence.

2. Methods

2.1 Trial Design

This was a prospective, multicenter, randomized controlled trial to evaluate the safety and efficacy of DKP on PD management, and the study was designed in a double-blinded, placebo-controlled manner to minimize the potential placebo effect on pain management. The protocol of this study was evaluated and authorized by the Institutional Review Board of eight participating hospitals (No.ZS-1913), and the trial was registered on ClinicalTrials.gov with identify the number (NCT03953716). All patients recruited were fully informed, and the informed consent was signed by each participant.

2.2 Participants

Patients diagnosed with PD aged between 18 to 35 years with regular menstrual cycles were eligible for the recruitment. The exclusion criteria were described following: (1) had been diagnosed with pelvic pathologies related to secondary dysmenorrhea including but not limited to endometriosis, previous pelvic inflammatory disease, uterine fibromatosis, adenomyosis, intrauterine devices, and ovarian cysts; (2) administration of any hormonal or analgesic agents within 12 weeks before enrollment; (3) diagnosed with concurrent basic diseases including neuropathologic pain, anemia, immunodeficiency or liver and kidney malfunction; (4) addiction for drugs, alcohol, and cigarette.

2.3 Sample Size

We performed a t-sample t-test power analysis via PASS software (version:15.0.13 NCSS LLC, Kaysville, UT, USA), the analysis showed that with a sample population of 132 women, the analysis would be able to detect a 2-point pain reduction weighed by the visual analog scale (α = 0.05 and 1-β = 0.90). a total of 156 patients with PD were required with a dropout rate of 15% (78 in each group).

2.4 Interventions

Generally, the DKP (Shanxi Guangyuyuan Traditional Chinese Medicine Co., Ltd., Taiyuan, Shanxi, China) is a solid pellet developed by the water-honeyed protocol. The placebo pills, provided by Guangyuyuan Co., Ltd., were made of 45% caramel and 55% starch with identical appearance, smell and taste to the DKP. Both participants and investigators were blinded to the allocation and treatment. To ensure the blinding process, both the placebo and DKP were encapsulated in identical bottles and boxes. The randomization list was kept by an independent investigator who didn't participate in any process of intervention assignment, data collection, or analysis.

2.5 Randomization and Blinding

Eligible patients were randomly allocated to receive either DKP or placebo for 12 weeks in a 1:1 ratio, according to a computerized random number table which was generated by SAS (version 9.2, Cary, NC, USA). The daily doses of both pills were filled into small plastic bottles, each of them containing 7 grams (g) of interventional medicine. Participants were instructed to orally take 3.5 g of DKP or placebo twice daily from the 5th to 14th day of the menstrual cycle for 12 weeks. Adherence was assessed by bottle counts at each visit. The usage of rescue painkillers (Ibuprofen, batch number: 3200600, Sino-GlaxoSmithKline, Tianjin, China) was allowed when unbearable pain occurred. The specific dosage and time of painkillers that participants requested were recorded in the case report form.

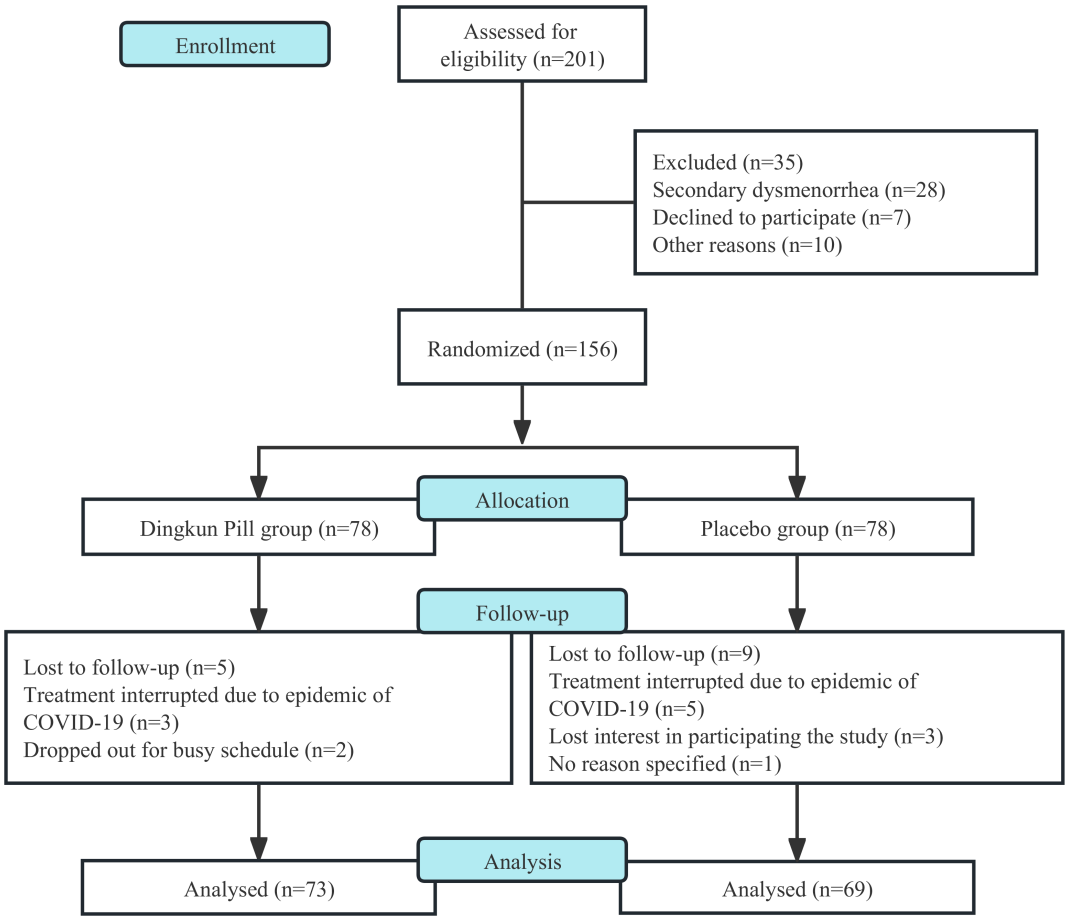

Fig. 1. The process of study from screening to completion during the 12 weeks.

2.6 Outcomes

The pain intensity of dysmenorrhea was described using a 10-point visual analog scale (VAS) ranging from 0 points (no pain) to 10 points (worst). The measure was taken on the first or second day of menstruation at baseline and every 4 weeks for 3 continuous cycles as the primary outcome. Additionally, based on the VAS rating, pain intensity was further stratified into mild pain (1–3 scores), moderate pain (4–6 scores), and severe pain (7–10 scores).

As the secondary endpoints, the levels of serum beta-endorphin, prostaglandin F2alpha (PGF2α), oxytocin, and vasopressin were quantified by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (RENJIE Biotech Inc., Shanghai, China). Blood samples were collected on the 2nd day of the menstrual cycle at baseline and week 12 of the treatment. The samples were centrifuged at 3000 revolutions per minute for 20 min, after which the supernatants were stored at –80 °C. Serums were thawed at room temperature. The color reaction was measured at 450 nm using a colorimetric microplate reader (Infinite® F50, Tecan, Switzerland). The sensitivity of the assay for beta-endorphin and PGF2α was both 0.1 pg/mL, while for oxytocin and vasopressin was 1.0 pg/mL and 10 pg/mL, respectively. Likewise, the levels of endogenous hormone parameters in the early follicular phase, namely follicular stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and testosterone were measured by chemiluminescence immunoassay-based technique using Unicel DxI 800 (Beckman Coulter Inc., Brea, CA, USA).

We conducted sonographic assessments at 10:00–12:00 AM on the 7th to 12th day of the menstrual cycle in all the patients. The color Doppler ultrasound used in the study was Logiq 500 (General Electric, Boston, MA, USA) with a 5 MHz transvaginal probe. All the scanning techniques were performed by the same examiner in each participating center, who was instructed with standardized procedures for ultrasonographic measurements before the recruitment. At first, the conventional ultrasound was used to eliminate the pathology in the uterine and adnexa. Then, using the color Doppler ultrasound imaging, the lateral level of the uterine cervico-coopereal was visualized in a longitudinal plane. The values of resistance index (RI = maximal systolic flow – minimal diastolic flow/maximal systolic flow), pulsatility index (PI = maximal systolic flow

– minimal diastolic flow/mean flow), and the ratio of flow velocity between peak-systolic and end-diastolic (S/D) in the bilateral uterine ascending arteries were calculated. All measurements were performed at the study entry and after the treatment.

2.7 Statistical Analysis

Data from patients who completed the full treatment course stuck to the instruction and finished follow-ups in time were included for final analysis. and the results were demonstrated as mean ± standard error of the mean (SEM), the inter-group comparisons of demographic characteristics, changes in serial tests, and uterine artery flow indices were performed with an independent student *t*-test, while the paired *t*-test was used to compare intra-group changes longitudinally. Statistical analyses were performed with SPSS (v26.0, IBM, Armonk, NY, USA), and the threshold of significance was set at $p < 0.05$.

3. Results

3.1 Demographic Characteristics

In this research, 201 patients at the base were screened for eligibility, in which 156 of them were eligible to participate (Fig. 1). With full consent, they were randomly assigned to either the DKP group (n = 78) or the placebo group (n = 78). During the treatment course, 14 (9.0%) of them failed and were lost to follow-up, and 142 participants completed the 12-week study and subsequent follow-up (73 in DKP and 69 in placebo). Data from these patients were included in final analyses, and the well-matched demographic characteristics at study entry were listed in Table 1.

3.2 Pain Scores

The absolute changes in VAS scores of all participants were calculated and demonstrated in Fig. 2. A significant reduction of VAS score was found in the DKP group, compared to the placebo group ($p < 0.001$). For all participants, the mean change in pain scores from baseline was –2.69 ± 0.18 in the experimental group *vs.* –1.29 ± 0.19 in the placebo-control group. From the data, the pain alleviation effect of DKP occurred during the first menstruation cycle of treatment, and this analgesic effect persisted during the whole course. Subgroup analysis revealed that DKP significantly improved pain symptoms, especially for patients with moderate and severe dysmenorrhea (scored equal to or above 4 at baseline). The reduction in pain scores of the DKP group and placebo group was –2.30 ± 0.17 *vs.* –1.15 ± 0.22 ($p < 0.001$) for moderate pain (scored 4–6) and –4.54 ± 0.21 *vs.* –2.13 ± 0.17 ($p < 0.001$) in severe cases (scored 7–10). However, the analgesic effect of DKP did not differ from the placebo for mild dysmenorrhea since the difference in pain scores was non-significant compared with the placebo group ($p = 0.058$).

Additionally, rescue painkillers were used in eleven cases (15.9%) in the placebo group and four cases (5.4%) in the DKP group. In the second month of the treatment, the number of patients taking additional analgesics decreased to seven (10.1%) in the placebo group and two (2.7%) in the DKP group. Four participants (5.8%) in the placebo group continued to take painkillers at the 12th week.

3.3 Biochemical Parameters

The absolute changes in the concentration of pain mediators are summarized in Fig. 3. Compared with baseline values, both control and DKP groups demonstrated significant modulation of beta-endorphin. The decline in concentrations of PGF2α, oxytocin, and vasopressin was much more robust in the DKP group compared with the placebo group (–6.67 ± 0.36 *vs.* –0.86 ± 0.45, –34.46 ± 1.31 *vs.* –3.14 ± 3.01, –275.04 ± 9.00 *vs.* –38.29 ± 20.46, $p < 0.001$). The mean elevation of serum beta-endorphin was significantly more pronounced in the DKP group than in the placebo group (5.35 ± 0.15 *vs.* 2.97 ± 0.42, $p < 0.001$).

On the other hand, the reproductive hormones in the early follicular phase maintained a normal profile during the medical intervention (Table 2). Patients treated with DKP showed a significantly lower level of FSH compared with patients on placebo pills (6.33 ± 0.23 *vs.* 7.11 ± 0.29, $p = 0.03$) in the 3rd month of treatment. Serum estradiol level was significantly increased from baseline levels in both groups, however, these changes did not differ between groups.

3.4 Blood Flow Indices of Uterine Artery

Bilateral uterine arteries were visualized in all participants. The mean values of PI, RI, and S/D of uterine ascending artery flow during pain-free follicular phrases are presented in Table 3. All indices responded to DKP with significantly reduced resistance of uterine blood flow, which indicated improved perfusion. No significant alteration was detected in the control group.

3.5 Safety

No patient reported serious adverse effects during the study process. Changes in blood pressure, heart rate, blood cell count, and functional indicators of the liver and kidney are shown in Table 4. No meaningful abnormality was identified after the intervention.

4. Discussion

It was the first randomized, double-blinded, placebo-controlled study that assessed the clinical effectiveness and safety of DKP in the treatment of PD. Comprehensive indicators including pain scores, pain biochemical mediators, ovarian hormonal profiles, and uterine artery flow indices were evaluated. We found that DKP produced significantly more relief of menstrual pain compared with the placebo. The levels of pain mediators in the DKP group were significantly improved in comparison with those in the placebo group, which further validated the subjective

Table 1. Characteristics at baseline between DKP group and placebo group.

Characteristics	DKP group (n = 73)	Placebo group (n = 69)	*p* value
Age, yr	24.86 ± 0.47	25.58 ± 0.50	0.292
Height, cm	162.73 ± 0.67	161.31 ± 0.69	0.141
Weight, kg	53.15 ± 0.86	53.54 ± 1.42	0.816
BMI, kg/m^2	20.05 ± 0.28	20.49 ± 0.46	0.415
Age at menarche, yr	12.93 ± 0.14	13.06 ± 0.16	0.539
Menstrual cycle, days	29.47 ± 0.42	29.65 ± 0.34	0.742
Proportion of patients			0.985
Mild patients	20 (27.4)	18 (24.6)	
Moderate patients	27 (37.0)	26 (40.0)	
Severe patients	26 (35.6)	25 (35.4)	
VAS scores			
All patients	5.19 ± 0.28	5.35 ± 0.32	0.813
Patients with mild pain	2.65 ± 0.11	2.5 ± 0.13	0.425
Patients with moderate pain	4.33 ± 0.13	4.38 ± 0.17	0.775
Patients with severe pain	8.04 ± 0.22	8.43 ± 0.26	0.359
Beta-endorphin, pg/mL	28.45 ± 0.87	28.81 ± 0.76	0.756
PGF2α, pg/mL	44.71 ± 1.27	45.28 ± 1.50	0.770
Oxytocin, pg/mL	219.13 ± 5.46	219.22 ± 6.30	0.991
Vasopressin, pg/mL	1627.37 ± 50.29	1602.42 ± 53.31	0.734

Data are presented as mean ± SEM or number (percentage).

Abbreviations: DKP, Dingkun pill; BMI, body mass index; VAS, visual analog scale; PGF2α, prostaglandin F2 alpha.

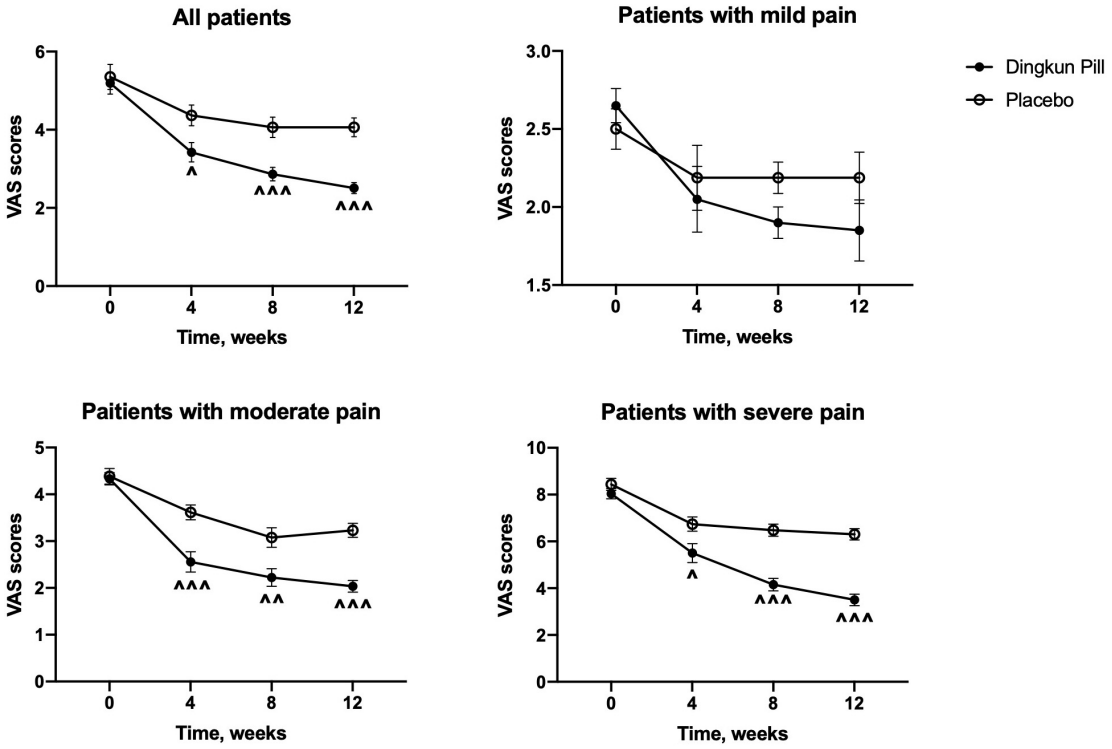

Fig. 2. Changes in mean scores of visual analogue scale over time in patients and patients with different degrees of dysmenorrhea treated with Dingkun Pill and placebo. Data are expressed as mean ± SEM; intergroup significance during the treatment, ^ $p < 0.05$, ^^ $p < 0.01$, ^^^ $p < 0.001$.

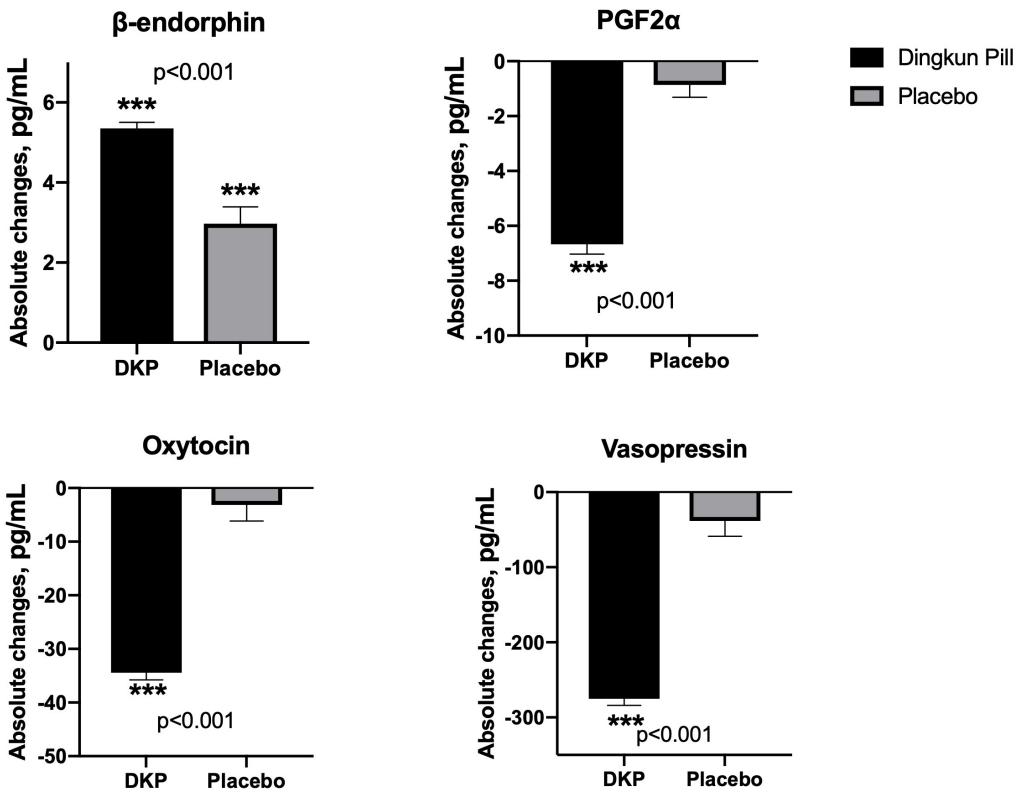

Fig. 3. Mean absolute changes in pain-associated biochemical factors in Dingkun Pill group and placebo group. Data are presented as mean ± SEM; intragroup significance before and after the treatment, *** $p < 0.001$.

pain score with objective parameters. No abnormal results were found in safety indexes after the intervention. Therefore, our study suggests that DKP has an analgesic effect on dysmenorrheal pain with little systemic toxicity.

DKP has exhibited diverse pharmacological activities by targeting multiple pathways through its multiple ingredients [21,22]. Previously, 234 chemical compounds were isolated and identified from the 30 crude ingredients of DKP; most of the ingredients were triterpenoid saponins, flavonoids, and alkaloids [21]. Notably, *in vitro* experiments suggested the triterpenoid saponins extracted from Ilex oubescens roots were able to inhibit nitric oxide (NO) and prostaglandin E_2 (PGE_2) production [23]. Flavonoids were also found with anti-inflammatory effects by inhibiting the release of PGs [24]. Serological examination in rats further confirmed the 18 prototype constituents in 6 primary ingredients namely Ginseng Radix et Rhizoma Rubra, Rhizoma Ligustici Chuanxiong, Rhizoma Corydalis, Radix Glycyrrhizae, Angelicae Sinensis, and Radix Scutellariae. Those original ingredients, identified in other TCMs, have anti-inflammatory, and immunomodulatory properties and promote blood circulation and estrogen-receptor activation for pain management [18].

Although DKP has a wide clinical application in polycystic ovarian syndrome, female subfertility, and menstrual disorders, studies investigating the efficacy of DKP in PD are extremely scarce [25,26]. There is only one randomized controlled study that investigated the role of DKP in dysmenorrhea [20]. Ma *et al*. [20] found that DKP remarkably relieved the pain severity in women experiencing dysmenorrhea; a significantly higher proportion of participants in the DKP group achieved over 50% reduction in VAS scores compared with the control group. In addition, the DKP group also showed a more significant decrease in the serum levels of PGF2α and endothelin which are the contributing factors of myometrial hyperactivity than the control group. However, their study used another TCM (Fuke Zaizao capsule) as positive drug control, rather than an inert one, and no allocation concealment was mentioned. Given that pain sensation can be influenced by psychological factors [27], such limitations compromised the scientific merit of their findings. Our study corroborates the benefit of DKP in treating PD by direct comparison with a placebo group. The pain-relieving effect of DKP mainly existed in women with moderate and severe pain while it was not effective in patients with mild symptoms. However, even in mild cases, the gaps in pain reduction between groups also increased with time, suggesting there might be a potential benefit of DKP if the treatment duration is extended.

Based on previous findings, many components in DKP have been found involved in the symptomatic regression for pain management. For example, the biologically active constituents such as baicalein, ethyl acetate fraction, and glabridin in Radix Scutellariae, Angelicae Sinensis, and

Table 2. Changes in ovarian hormonal parameters between DKP group and placebo group during menstruation.

Hormone variables	DKP group (n = 73)	Placebo group (n = 69)	*p* value
FSH, IU/L			
Baseline	6.57 ± 0.20	6.71 ± 0.23	0.641
12th week	6.33 ± 0.23	7.11 ± 0.29	0.037
LH, IU/L			
Baseline	4.50 ± 0.31	4.59 ± 0.25	0.813
12th week	4.53 ± 0.28	5.05 ± 0.31*	0.228
Estradiol, pg/mL			
Baseline	37.86 ± 1.98	37.14 ± 2.39	0.815
12th week	47.04 ± 2.92 **	48.50 ± 5.60 *	0.815
Testosterone, ng/mL			
Baseline	0.40 ± 0.02	0.41 ± 0.03	0.765
12th week	0.41 ± 0.02	0.40 ± 0.03	0.905

Data are expressed as mean ± SEM.

Abbreviations: DKP, Dingkun pill; FSH, follicular stimulation hormone; LH, luteinizing hormone. *$p < 0.05$, **$p < 0.01$, when compared with basal values in the same group.

Table 3. Changes in uterine artery indices between DKP group and placebo group.

Uterine blood flow indices		DKP group (n = 73)	Placebo group (n = 69)	*p*-value
Left PI	Baseline	2.57 ± 0.12	2.54 ± 0.09	0.860
	12th week	2.02 ± 0.09 ***	2.48 ± 0.09	0.001
Right PI	Baseline	2.61 ± 0.13	2.67 ± 0.13	0.635
	12th week	2.17 ± 0.08 **	2.50 ± 0.09	0.011
Left RI	Baseline	0.85 ± 0.01	0.85 ± 0.01	0.758
	12th week	0.78 ± 0.01 ***	0.85 ± 0.02	<0.001
Right RI	Baseline	0.83 ± 0.01	0.86 ± 0.05	0.546
	12th week	0.80 ± 0.02	0.81 ± 0.01	0.784
Left S/D	Baseline	7.00 ± 0.34	6.96 ± 0.27	0.930
	12th week	5.16 ± 0.21 ***	6.68 ± 0.33	<0.001
Right S/D	Baseline	7.00 ± 0.42	6.87 ± 0.30	0.882
	12th week	5.13 ± 0.29 ***	6.68 ± 0.34	0.001

Data are presented as mean ± SEM.

Abbreviations: DKP, Dingkun pill; PI, pulsatility index; RI, resistance index; S/D, the ratio between peak systolic and end-diastolic flow velocity.

Intragroup significance **$p < 0.01$, ***$p < 0.001$, when compared with basal values in the same.

Radix glycyrrhizae have been found to reduce the COX-2 mRNA or protein expression, and to inhibit the release of PG [28–30]. *In vitro* experiments indicated both Radix Scutellariae and Radix Glycyrrhizae had a spasmolytic effect on the smooth muscle of rat uterine tissue [31,32]. Furthermore, as the primary extract of Rhizoma Corydalis, tetrahydropalmatine displays antinociceptive action on different types of pain syndromes by inhibiting the protein expression of inducible nitric oxide synthase (iNOS) which is responsible for the inflammatory cascade [33,34]. Future research is needed to unravel the intricate molecular mechanism behind the antinociceptive effect of DKP in PD.

Though the pathogenesis of PD has not been fully elucidated to date, previous studies have shown that this complicated process is modulated by many biological factors [35]. In general, PGF2α, vasopressin, and oxytocin are associated with increased contractility of smooth muscle in the uterus and vessels [2]. Numerous studies have shown that PD is correlated with an elevated concentration of PGF2α in both serum and endometrium, and the pain severity seemed to be positively related to its level [36]. Oxytocin and vasopressin are mainly secreted from the posterior lobe of the pituitary, supraoptic, and paraventricular nuclei of the hypothalamus. Both hormones are potent stimulants of uterine contraction, leading to a restriction on uterine blood supply [37]. Similar to previous findings, the present study showed the serum concentrations of PGF2α, vasopressin, and oxytocin were significantly lower in the DKP group compared to the control group, suggesting that DKP exerts the analgesic function through an integral regulation of vasoconstrictive substances [20].

Conversely, beta-endorphin, a neurotransmitter primarily produced in the anterior lobe of the pituitary gland, has morphine-like effects on opioid receptors throughout the body by inhibiting the irritation of peripheral somatosensory fibers [38]. It's been believed that anxiety disorders induced by the painful event are related to decreased peripheral beta-endorphin [39]. Fedele *et al.* [27] discovered women suffering from PD had positive feedback to placebo and postulated that a surge in endogenous beta-endorphin might result in short-term attenuation. Intriguingly, we indeed detected a significant elevation of 3.05 ± 0.43 pg/mL in serum beta-endorphin from baseline in the sham group. It's speculated that the placebo treatment may induce positive expectations which attenuate the mental stress during painful episodes and thereby restore the beta-endorphin levels. Moreover, the growth in beta-endorphin concentrations was significantly higher in the DKP group as compared with the placebo control. This finding indicates that beta-endorphin could probably involve the regulation of the pain matrix in PD as a pain reliever during DKP treatment. Briefly, the underlying mechanism of DKP in pain suppression is mediated through a variety of biochemical factors, and generally, those substances are predominantly regulated at the molecular level. Therefore, in-depth exploration is needed to investigate which signaling and metabolic pathways are involved in the pain-relieving process during DKP treatment.

Doppler sonographic studies suggest dysmenorrheal women have higher uterine impedance than eumenorrheic women throughout the whole menstrual cycle [40]. In the

Table 4. Safety evaluation of DKP and placebo.

Parameters	DKP Group (n = 73)			Placebo Group (n = 69)		
	Baseline	Week 12	*p*-value	Baseline	Week 12	*p*-value
SP, mmHg	107.9 ± 1.1	106.7 ± 1.0	0.338	108.4 ± 1.2	108.1 ± 1.3	0.771
DP, mmHg	67.6 ± 0.8	69.3 ± 0.9	0.108	69.2 ± 1.0	69.6 ± 1.4	0.775
HR, bpm	77.2 ± 1.5	76.8 ± 1.2	0.369	76.3 ± 1.3	76.8 ± 1.2	0.346
WBC, 10^9/L	5.51 ± 0.15	5.57 ± 0.17	0.741	5.48 ± 0.17	5.46 ± 0.18	0.884
N, %	58.84 ± 1.08	60.21 ± 0.94	0.162	57.84 ± 0.97	58.66 ± 1.10	0.243
HGB, g/L	128.91 ± 1.17	130.79 ± 1.02	0.062	129.75 ± 1.41	128.70 ± 1.26	0.160
PLT, 10^9/L	242.18 ± 6.50	243.32 ± 7.27	0.780	245.45 ± 6.95	257.56 ± 6.67	0.015
ALT, IU/L	11.45 ± 0.59	11.65 ± 0.52	0.693	11.20 ± 0.75	11.75 ± 0.66	0.430
AST, IU/L	16.11 ± 0.73	15.96 ± 0.39	0.839	16.02 ± 0.42	16.03 ± 0.36	0.976
BUN, mmol/L	3.90 ± 0.12	3.91 ± 0.10	0.970	4.02 ± 0.13	3.98 ± 0.11	0.767
Cr, umol/L	59.10 ± 1.01	59.90 ± 1.24	0.320	59.59 ± 1.12	57.42 ± 1.13	0.012

Variables are described as mean ± SEM.

Abbreviations: DKP, Dingkun pill; SP, Systolic pressure; DP, Diastolic pressure; HR, heart rate; WBC, White blood cell; N, neutrophil; HGB, Hemoglobin; PLT, Platelet; ALT, Serum alanine aminotransferase; AST, Serum aspartate aminotransferase; BUN, Urea nitrogen; Cr, Creatinine.

present study, patients in the DKP group showed favorable changes in hemodynamic parameters, inhibition of vasoconstrictors, and improvement in uterine blood flow (spasmolytic effect of DKP). Similar to our findings, a previous study observed that DKP treatment decreased PI and RI of the uterine artery [20,41]. However, it remains unclear whether this vasodilative function is a direct effect of DKP or secondary sequalae indirectly mediated by the diminishing levels of vasoconstrictors.

Up to date, our study is the first and largest, randomized, double-blinded, placebo-controlled trial investigating the analgesic effect of DKP on PD based on a clinical setting. Notably, in this research, not only the subjective pain rating score but also the objective indicators, biochemical substances involved in pain pathogenesis and uterine artery flow indices reflecting vascular contractility, were evaluated thoroughly, which provided a more comprehensive understanding of the bioactive effect of DKP. Additionally, we included patients with different severity ranging from mild to severe pain and performed subgroup analysis. This approach ensured less selection bias and offered more pragmatic information of reference in daily practice.

Despite those advantages mentioned above, the results of our study should be interpreted with caution considering the following limitations. First, we took the blood sample for the measurement of biochemical parameters associated with pain perception, which might to some extent cause potential bias since the serum concentration of these biofactors could be regulated by systemic metabolic status. Further clinical trials investigating samples from menstruation blood or endometrial biopsy should be encouraged because they might provide more specific details on the local effect of DKP on the uterus. Second, the subjects who participated in our study are all Chinese Han women, which make it hard to extrapolate these therapeutic effects to all women of different ethnicity. Third, although the PD diagnosis in our study has been clinically verified via personal history, pelvic examination, and ultrasonographic screening, some pathological conditions that may cause secondary dysmenorrhea, like peritoneal endometriosis, can barely be identified at the very early stage. Therefore, the possibility of recruiting a dysmenorrheic woman with organic pathological conditions cannot be completely ruled out. Fourth, no pilot study has been done to conducted in this study. Last but not least, there are still major gaps in knowledge about the mechanism of the antinociceptive effect that DKP had on PD. In the current study, we did not explore the intervention of active components in DKP on the targets of different biological functions involved in this painful disorder. Additional bench research on the DKP targets of pain genesis is expected to elucidate its potential pain-killing mechanism. Hopefully, based on that, further trials might be proposed to explore whether PD patients who are refractory to first-line treatment could benefit from DKP treatment.

5. Conclusions

In conclusion, a pronounced reduction of pain intensity was observed in patients with PD administrating DKP, compared with the placebo, especially for patients with moderate to severe symptoms. With the elevation in beta-endorphin and inhibition of PGF2α, oxytocin, and vasopressin, its therapeutic effect seems to be associated with a synergistic combination of vasoconstrictors and neurological stress hormone. Investigation into the molecular mechanism of DKP behind pain attenuation may provide further evidence for alternative therapy in PD management.

Abbreviations

PD, Primary dysmenorrhea; DKP, Dingkun pill; VAS, visual analog scale; PG, prostaglandins; PGF2α, prostaglandin F2 alpha; PGE_2, prostaglandin E_2; FSH, follicular stimulating hormone; LH, luteinizing hormone; TCM, traditional Chinese medicine; ELISA, enzyme-linked immunosorbent assay; RI, resistance index; PI, pulsatility index; S/D, peak-systolic and end-diastolic; SEM, standard error of the mean; NO, nitric oxide; iNOS, inducible nitric oxide synthase; Lif, leukemia inhibitory factor.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

AS contributed to the conceptualization and design of the study, the data collection and interpretation. SZ and XD wrote and editing the manuscript. SZ and XM contributed to the protocol planning, data collection and analysis. SZ, XD, JG, YD and YW contributed to patient recruitment and data collection. All authors contributed to editorial changes in the manuscript. All authors read and approved the final version of the paper.

Ethics Approval and Consent to Participate

All patients recruited were fully informed, and the informed consent was signed by each participant. he protocol of this trial was evaluated and approved by the Ethics Committee of Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science (No.ZS-1913).

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Conflict of Interest

The authors declare no conflict of interest.

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