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Therapeutic Efficacy of Wet Cupping on Uterine Inflammation in Primary Dysmenorrhea: An *In Vivo* Investigation Targeting Bilateral L4/L5 Vertebrae

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Abstract

Primary dysmenorrhea (PD) is a prevalent problem in gynecology affecting the quality of life for young women. This medical condition is often responsible for the absence of young women from school or work, increasing the risk of developing hyperemesis gravidarum in the future. Furthermore, PD causes pain due to the activation of the local PGF2α pathway in the endometrium, which triggers oxidative stress processes, uterine ischemia and spasmodic uterine contractions. The first-line therapies for PD, Nonsteroid anti-inflammatory drugs (NSAIDs) and oral contraceptives, can cause side effects such as headache, nausea, intestinal ulcers and platelet abnormalities. To overcome the impact of PD, cupping has been proven effective as an alternative pain therapy with minimal side effects. Therefore, this study aimed to evaluate the effects of cupping on writing latency threshold time, PGF2α levels and FP receptor expression in PD model rats. The experiment was conducted using female Sprague Dawley rats to analyze the effects of wet cupping therapy (WCT) on inflammatory repair in the uterus. The method used was a pre- and post-test control group design, with 35 female rats randomly divided into 5 groups. These consisted of normal control (NC), negative control (C−), positive control (C+) + ibuprofen 7.2 mg, as well as treatment groups, comprising dry cupping (DC) and WCT. The results showed that writhing time threshold of WCT was the same as C+ ($p = 0.368$), while PGF2α decreased in DC (43.43 pg/mL, $p = 0.008$) and WCT (189.25 pg/mL, $p = 0.0154$). Similar to C+, WCT significantly decreased serum PGF2α levels compared to C−. WCT also influenced the expression of PTGFR subunit similarly to NC, showing the capacity to control the inflammatory pathway. The study suggested that WCT was a promising therapeutic strategy for PD by decreasing PGF2α and PTGFR expression.

Keywords: Primary dysmenorrhea, Wet cupping therapy, Dry cupping, PGF2α, Prostaglandin F receptor expression

Introduction

Primary dysmenorrhea (PD) is a prevalent complaint among young and adult women frequently disregarded, misdiagnosed and inadequately addressed. This medical condition is characterized by excruciating lower abdominal cramps lasting for approximately 3 days, which start before or at the onset of menstruation. Due to the widespread occurrence, PD remains a pertinent issue in Gynecology, focusing on the female reproductive system. It also contributes to decreasing the quality of life for young women, serving as the main cause of the absence from work or school, thereby increasing the risk of developing future hyperemesis gravidarum [1-3]. The abnormal contractions of the uterus caused by the local endometrial release of prostaglandins are the source of the pain and discomfort. Chemicals released at the uterus induce uterine ischemia and oxidative stress mechanisms [4], as supported by previous investigations on the release of pro-inflammatory chemicals during PD [5]. Globally, the prevalence of dysmenorrhea varies from 50 to 95 %, indicating a considerable incidence [6]. According to World Health Organization (WHO) data, 90 % of women, totaling 1,769,425, experience dysmenorrhea, with over 50 % in every country enduring the effects. The prevalence in women of childbearing age varies with an estimated average of 44, 45 - 95, 51, 52 - 64, 60 - 80, 73, 80, 84.2 and 94 % in China, UK, Singapore, Mexico, USA, Sweden, Western Australia, Thailand and Egypt, respectively.

Dysmenorrhea is responsible for significant financial losses due to therapy costs, medical care, disruption of daily activities and decreased productivity [5]. Additionally, 15 - 20 % of women affected are unable to perform normal daily activities during each menstrual period [6]. For example, in USA, approximately 140 million working hours are lost annually due to dysmenorrhea, with a significant decrease in the work output among women who intend to maintain commitments during menstrual cramps. In Japan, there is an economic loss of $ 4.2 billion, which is a major cause of short-term school, and work absenteeism, and poor quality of life [7]. This medical condition affects daily activities and socioeconomic status associated with the risk of future hyperemesis gravidarum (HG). According to a study from the State University of New York, women with a history of dysmenorrhea in adolescence and adulthood are 5 times more susceptible to experience HG [8]. This is because prostaglandins and cytokines induced by excessive nausea and vomiting are related to severe dysmenorrhea. Therefore, early diagnosis and therapy in patients with a history of severe dysmenorrhea reduce morbidity associated with HG [4].

Circular hormonal changes on the axis of the hypothalamus-pituitary-gonad are the main determinant of menstrual cycles, regulated by a sophisticated feedback system [9]. Initially, the pituitary gland regularly and progressively releases follicle-stimulating and luteinizing hormones in response to gonadotropin-release hormones from the hypothalamus. This phenomenon triggers the ovaries' follicles to grow and mature, oocyte maturation, as well as the release of progesterone and estrogen [10]. Generally, the menstrual cycle consists of several phases, which include menstruation and follicular, characterized by increasing estrogen levels, comprising the first half. Meanwhile, the second half is the luteal phase, with a peak in progesterone secretion. When fertilization does not occur, progesterone levels decrease approximately 3 days before the onset of menstrual bleeding [11]. This decrease in progesterone and estradiol, initiates menstruation by triggering the release of the corpus luteum, leading to the production of progesterone, which vanishes when egg cells are not fertilized. Subsequently, lytic enzymes in lysosomes and acid phosphatase are released into the cytoplasm when progesterone levels are lowered, leading to the production of prostaglandins. The inflammatory response induced by reduced progesterone increases menstrual bleeding and endometrial bleaching, triggering a complex response including the immune, circulatory, and hormonal systems. Moreover, a previous study showed that prostaglandins played a significant role in increased pain in PD [12].

Nonsteroid anti-inflammatory drugs (NSAIDs) and combined hormonal contraceptives (COCs) are first-line therapies that reduce prostaglandin synthesis and secretion [13]. Specifically, ibuprofen operates by blocking enzymes in the central nervous system that produce prostaglandins, such as cyclooxygenase. This creates an analgesic effect, which reduces sensitivity to pain receptors induced by pain mediators such as bradykinin, histamine, serotonin, prostacyclin, prostaglandins, hydrogen ions and potassium. By inhibiting COX-1 and COX-2, ibuprofen reduces prostaglandin synthesis, which is correlated with tissue damage, inflammation and analgesic effects [14,15]. Although drugs are used to treat various health conditions, the usage is often associated with side effects. For example, non-selective inhibition of COX enzymes can lead to gastrointestinal symptoms, making long-term use of NSAIDs contraindicated for patients with peptic ulcers and bleeding. Similarly, COCs also increase the risk of deep vein thrombosis, hindering recommendations for patients with cardiovascular disease and breast cancer [16]. NSAIDs or oral contraceptive pills can lead to a series of adverse effects on the digestive tract, liver and kidneys causing water retention, nausea and endometriosis [17]. Temporary interventions, including [18] stretching therapy, acupuncture and TENS (Transcutaneous Electrical Nerve Stimulation) therapy have been developed to reduce dysmenorrhea, but the application failed to provide sustained relief [19]. This has led to the recommendation of herbal therapy, such as gingerol, to reduce dysmenorrhea. However, when used along with other herbs of similar effect, there is a tendency for complicated dosage control, quality and drug interactions [20-27].

Due to the limitations of oral contraceptives and NSAIDs, traditional medicine therapies, such as cupping therapy have been identified as an effective alternative and are becoming increasingly popular since 3,000 SM to treat a variety of diseases, including pain and dysmenorrhea [28,29]. Although the mechanism of action remains unknown, cupping is generally accepted as a pain therapy method [30], with pain reduction mainly assessed through the Visual Analog Scale (VAS) and the numerical rating scale (NRS). The investigation of the mechanism of action in uterus stimulated by oxytocin and estradiol benzoate requires experimentation on animal model, as human trials are unfeasible. Therefore, this study aimed to explore the effects of cupping on the treatment of pain, particularly PD, using.

Materials and methods

Experimental animals

In this study, female Sprague Dawley (SD) rats aged 8 - 10 weeks weighing 180 ± 20 g were maintained at the Laboratory of Integrated Research and Assessment-Service for Research and Development of Preclinical Animal Experiments (LPPT-LP3HP) Unit 4, Gadjah Mada University, Yogyakarta (Accredited with Laboratory Accreditation Certificate No. LP-1502-IDN - SNI ISO/IEC 17025:2017). Rats were given 7 days to adapt and housed in animal cages with free access to Bio Rat (Citra Feed) 10 %/kg rat weight and reverse osmosis (RO) water ad libitum. The air-conditioned room was set at an ambient temperature of 19 - 25 °C and relative humidity of 70 - 80 %, light of 300 lux, and illumination set to a cycle of 12 h light and 12 h dark (light cycle from 6:00 am - 6:00 pm). Subsequently, cages were placed in a quiet room and noise from outside was minimized. These cages are made of Polycarbonate with a size of 18.98×10.51×8.27 in^3 (482×267×210 mm^3) and a floor area of 940 cm^2/145.7 in^2, accommodating 3 rats each. Ethical approval for animal use protocol was obtained from the Research Ethics Commission of the Faculty of Medicine, Sebelas Maret University, with Ref. No.: 162/UN27.06.11/KEP/EC/2022 dated November 22, 2022. Additionally, efforts were made to minimize the number of animal used and the experiment adhered to the guidelines provided by the Integrated Research and Assessment Laboratory-Laboratory for Research and Development of Preclinical Animal Experiments (LPPT-LP3HP) Unit 4, Gadjah Mada University, Yogyakarta.

Chemicals and reagents

Female Sprague Dawley rats were provided with cages and reversed osmosis water. In this study, standard rats feed (Bio Rat, Citra Feed) was purchased from PT. Citra Ina Feed mill Jakarta, Indonesia. Subsequently, the quantitation of PG-F2α (catalog: ER1257) was conducted using the Fine test kit sourced from Wuhan Fine Biotech Co., Ltd., China, supplemented by enzyme-linked immunosorbent assay (ELISA) kits. Estradiol Benzoate, oxytocin and ibuprofen were purchased from Sigma Aldrich, USA: E8515, O3251 and 14883, respectively. Prostaglandin F2 alpha Receptor Polyclonal Antibody was purchased from Invitrogen (cat: PA5-111890, Carlsbad, California).

Study design and construction of PD rats

This study used a randomized control trial, consisting of female rats with pre and post-test control group design. The sample size was calculated using G*Power online application available at https://www.gpower.hhu.de/ [31], resulting in a total of 30 rats in 5 groups. To anticipate potential dropout, a formula was adopted from [32]).

$$n^1 = \frac{n}{(1-f)}$$

where n = Sample size, f = estimated proportion of drop out (10 %, f = 0, 10) and finally obtained 7 female rats per group.

The selection of female rats in this study was based on inclusion criteria, namely aged 8 - 10 weeks old, body weight of 180 ± 20 g, and overall good health. Meanwhile, the exclusion criteria were sickness, low activity, weight loss or death. A sample of 35 female rats was obtained using a random sampling method from the existing rat population at the Animal Laboratory Center of the Center for Integrated Studies and Assessment of Gadjah Mada University. These samples were randomly divided into 5 groups, namely normal control (NC, n = 7), PD (n = 7), ibuprofen therapy (IT, n = 7), cupping therapy (CT, n = 7) and wet cupping therapy (WCT, n = 7). To induce PD model, rats in PD, IT, CT and WCT groups were injected subcutaneously and intraperitoneally with estradiol benzoate and oxytocin. Specifically, rats were injected with estradiol benzoate for 10 days (5 mg/kg body weight on days 1 and 10, 3 mg/day on days 2-9) and 3 U/kg body weight per rat was induced with oxytocin on day 11.

Wet Cupping Therapy (WCT)

Group C+ received NSAIDs therapy (7.2 mg/200 g body weight, single dose) orally on days 2, 5 and 11. Meanwhile, dry cupping (DC) group received application of a 2 cm diameter cup with 0.04 MPa negative pressure on intact skin in the right and left back areas (bilateral L4/L5 vertebrae), immobilized for 5 min. In WCT group, initial cupping included the application of a 2 cm diameter cup with a negative pressure of 0.04 MPa on intact skin in the right and left back areas (bilateral L4/L5 vertebrae), stationary for 5 min. This was followed by 10 punctures using a 21 G needle, with a depth ranging from ≤ 0.1 to 2 mm. The second method was performed using the same procedure for 5 min with a negative pressure of 0.04 MPa and therapy was administered on days 5 and 11 [33,34].

Assessment of PD model

Measurement of writhing threshold

Writhing threshold time is the duration at which rats show a response to an intraperitoneal oxytocin injection, observed from the first writhing reaction. This measurement entails observing each rat and

recording the time using a stopwatch from the moment of the intraperitoneal oxytocin injection to the first writhing reaction. Moreover, several indicators showing a successful PD modeling in first writhing reaction include abdominal contraction, concavity, extension of the body and hind limbs, rotation of 1 limb and uterine contraction [35,36].

Detection of biochemical indexes in serum

During anesthesia with Ketamine (40 - 100 mg/kg) administered intraperitoneally, approximately 0.5 - 0.8 mL of blood was collected through medial canthus puncture. The sample was incubated for 30 min at room temperature followed by blood centrifugation at 3,000 rpm, 4 °C for 15 min, and the resulting serum was stored at −80 °C. PGF2α levels were determined using ELISA kit according to the manufacturer's instructions, while optical density (OD) readings were taken using a Microplate Reader at a wavelength of 450 nm [37,38].

Detection of biochemical indexes in the uterus with immunohistochemical detection

After achieving anesthesia and entering an unconscious state induced by drug administration, rats were sacrificed on day 11 by decapitation [37]. Furthermore, the expression of the prostaglandin F receptor in the uterus was examined according to the specific requirements and instructions of the Prostaglandin F2 alpha Receptor Polyclonal Antibody kit. Tissue sections of 4 μm were stained with Immunohistochemical (IHC) for histopathological examination of the uterus [39]. Digital images of uterine morphology at 400×.

Statistical analysis

In this study, statistical analysis was performed using GraphPad Prism 9.3 software. Before analyzing significant differences among groups, Shapiro-Wilk test was applied to verify the normality and homogeneity of numerical data. Mean ± standard deviation (SEM) was used to express normal data, while median (lower quartile, upper quartile) was applied for non-normally distributed data. The results of the normality test showed that the data were not normally distributed. Therefore, the corresponding Wilcoxon test was used to determine serum PGF2alfa levels, and Kruskal-Wallis test was used for comparison between groups, followed by Mann-Whitney test. A *p*-value of less than 0.05 was considered statistically significant.

Results and discussion

Results

The results showed that WCT significantly increased writhing latency threshold time.

Table 1 Rat writhing threshold time.

Groups	Median	Kruskall-Wallis	Median Dif ± Post Hoc (Mann-Whitney)			
			C−	C+	DC	WCT
NC	0		−4 ± 0.0003*	−7 ± 0.0003*	−4 ± 0.0003*	−5 ± 0.002*
C−	4			−3 ± 0.038*	0 ± 0.418	−3 ± 0.018*
C+	7	0.0008*			3 ± 0.440	2 ± 0.368
DC	4					−1 ± 0.465
WCT	7					

Notes: *) = significant at an α value of 95 %

The median writhing threshold ratio showed a significant increase in C+ (7) and WCT (7) groups, respectively compared to the C− (4) group. However, C+ and WCT were unable to match NC group, which did not receive estradiol benzoate and oxytocin induction, indicating the absence of writhing latency in NC (0) group, as shown in **Table 1** and **Figure 1**.

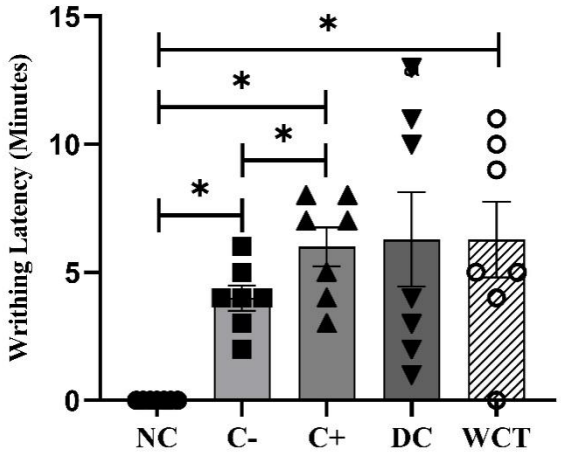

Figure 1 The threshold writhing time (latency) for different groups. These consist of NC (normal control), C− (primary dysmenorrhea), C+ (primary dysmenorrhea receiving standard ibuprofen therapy), DC (primary dysmenorrhea receiving dry cupping) and WCT (primary dysmenorrhea group receiving wet cupping). *) $p < 0.05$, shows a significant difference.

Wet cupping therapy reduces PGF2α in estradiol benzoate and oxytocin-induced PD

This study was conducted to evaluate the effect of WCT on the inflammatory process and the levels of Prostaglandin F2-alpha (PGF2α). The results showed that the median PGF2α levels significantly decreased in NC (115.7), C+ (166.4), DC (82.4) and WCT (264.1) groups, but increased in C− group (−200.2), as presented in **Figure 2(A)**. Based on the comparison, DC (−180, $p = 0.0131$) and WCT (−145, $p = 0.0055$) groups did not perform as well as C+ group due to variation in PGF2α, with WCT group showing the highest level before therapy (**Figure 2(B)**). **Table 3** shows the section on pre- and post-therapy, where the average decrease in PGF2α levels from the largest group was found in WCT (264.1), C+ (166.4), NC (115.7) and DC (82.4).

Table 2 Effect of cupping on PGF2α levels (pg/mL).

Groups	$PGF_{2\alpha}$ (pg/mL)		*p*-value	Median Dif ± Post Hoc (Mann-Whitney)[d)]			
	Pre	Post		C−	C+	DC	WCT
NC	297.6	181.9	0.0247*[b)]	−371 ± 0.0003*	−227 ± 0.0006*	−407 ± 0.0003*	−371 ± 0.0003*
C−	352.6	552.8	0.0156*[c)]		144 ± 0.0003*	−35.9 ± 0.486	−0.48 ± 0.4371
C+	575	408.6	0.017*[b)]			−180 ± 0.0131*	−145 ± 0.0055*
DC	671.1	588.7	0.327				35.42 ± 0.5
WCT	817.3	553.2	0.042*[b)]				
p-value		< 0.001*[a)]					

Notes: *) = significant at an α value of 95 % using [a)] Kruskal-Wallis test, [b)] Paired t-test, [c)] Wilcoxon test, [d)] Mann-Whitney

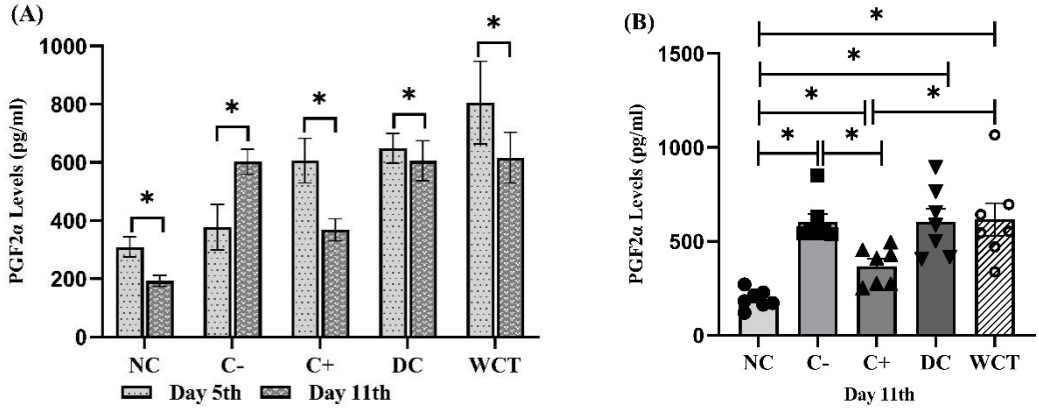

Figure 2 The results of a pathway analysis of PGF2α using ELISA. (A) shows the levels of PGF2α (in pg/mL) before and after the test for each group. The groups include NC (normal control), C− (primary dysmenorrhea), C+ (primary dysmenorrhea with ibuprofen), DC (primary dysmenorrhea with dry cupping), WCT (primary dysmenorrhea with wet cupping). *) shows a significant difference at an alpha value of 95 % using a Dependent sample t-test. (B) shows PGF2α levels (in pg/mL) for each group. *) shows a significant difference at an alpha value of 95 % using Mann-Whitney test.

Wet cupping therapy has reduced PTGFR expression in estradiol benzoate and oxytocin-induced PD

This study showed that FP receptors were present in the cell nucleus and cytoplasm of rats' uterine myometrial stroma. According to **Figure 3**, the expression of PTGFR was very low in NC group, but strong in the cell nucleus and cytoplasm of rat uterine myometrial stroma in C− group compared to NC, C+, DC and WCT. Additionally, PTGFR expression in the myometrial stroma of rats in C+ and WCT groups was lower compared to DC. The results showed a significant decrease in prostaglandin F expression in cell nuclei and cytoplasm of inflamed uterine myometrial stroma in WCT group (3.25 ± 0.0087) compared to C− group, as presented in **Table 3** and **Figure 4**. DC (0.38 ± 0.2013) and WCT (0.5 ± 0.4024) groups showed similar efficacy to C+ group.

Table 3 Effect of cupping on PTGFR expression in endometrium.

Groups	Median	Kruskal-Wallis	Median Dif ± Post Hoc (Mann-Whitney)			
			C-	C+	DC	WCT
NC	0.38		−5.125 ± 0.0003*	−2.375 ± 0.0664	−2 ± 0.0283*	−1.875 ± 0.0385*
C−	5.5			2.75 ± 0.0682	3.13 ± 0.2279	3.25 ± 0.0087*
C+	2.75	0.0185*			0.38 ± 0.2013	0.5 ± 0.4024
DC	2.38					0.13 ± 0.2383
WCT	2.25					

Notes: *significant at a value of α = 95 %

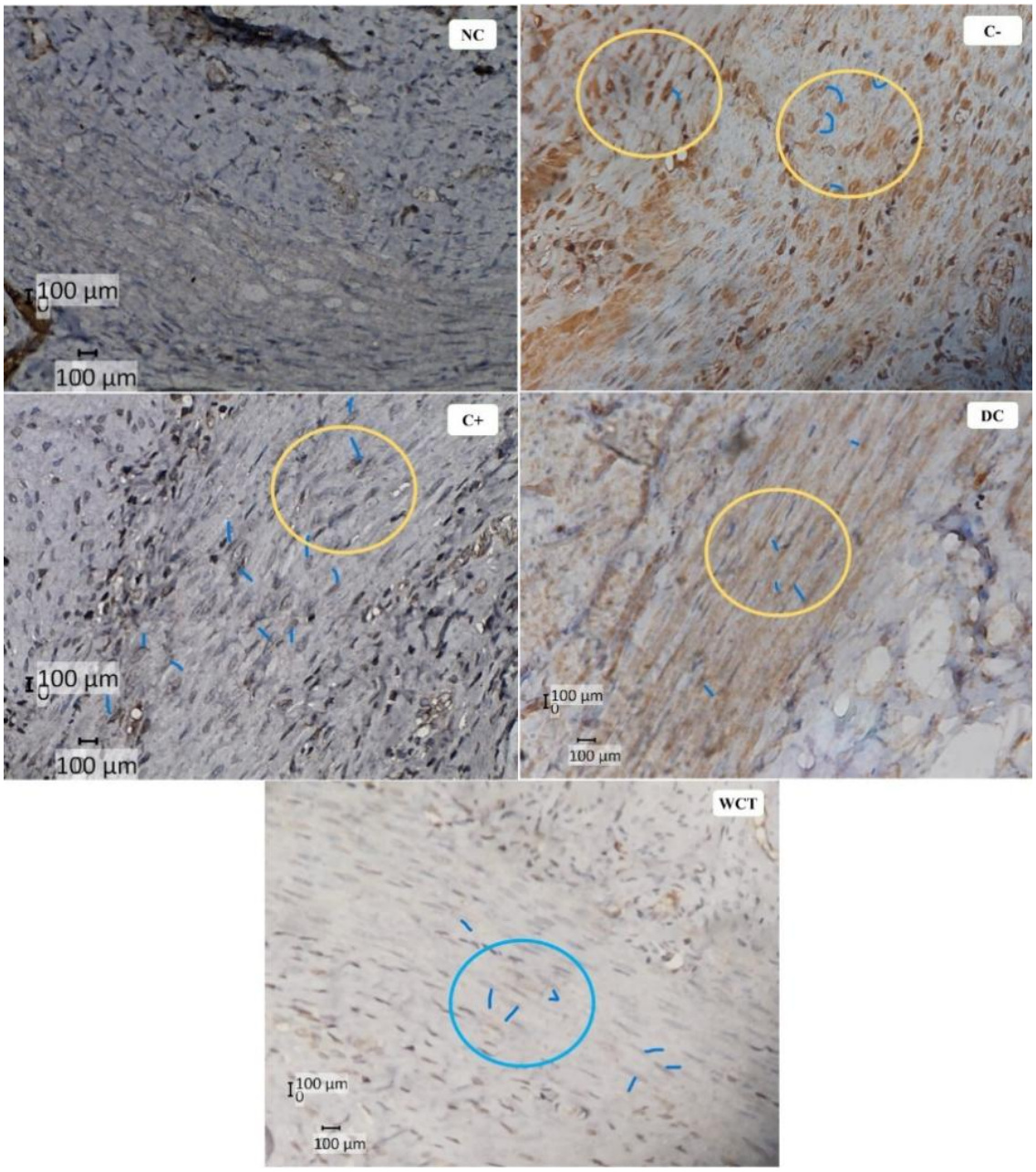

Figure 3 PTGFR Expression in Endometrium. The experiment included 5 groups, namely NC: Normal control, C−: Primary dysmenorrhea, C+: Primary dysmenorrhea received ibuprofen standard therapy, DC: Primary dysmenorrhea received dry cupping; WCT: Primary dysmenorrhea group received wet cupping. Immunohistochemistry (IHC) staining was used to paint the preparations and the observations were made using an Olympus CX33 microscope equipped with a sigma digital camera. The images were processed using Optil Lab Viewer Image Raster software at 400× magnification. The yellow and blue circles show the expression of prostaglandin F protein in the cell nucleus and cytoplasm of rat uterine myometrial stroma.

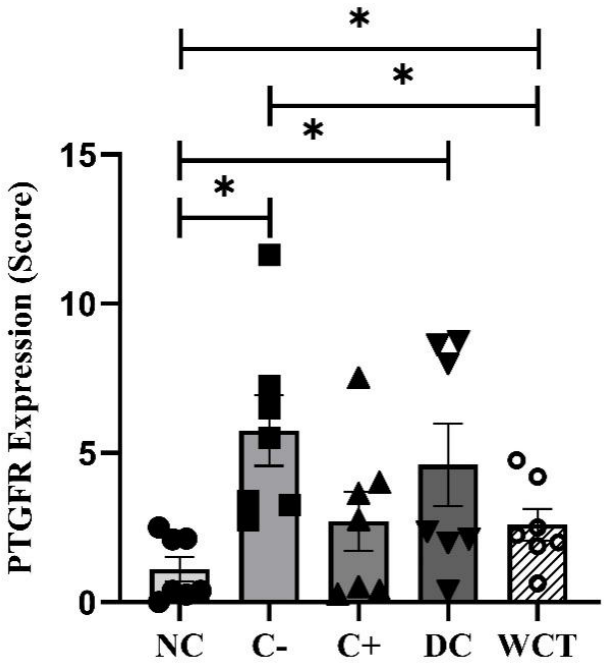

Figure 4 PGF2α expression between different study groups in Estradiol Benzoate-induced PD model. PTGFR expression intensity in each group. Notes: NC: Normal control; C−: Primary dysmenorrhea; C+: Primary dysmenorrhea received ibuprofen standard therapy; DC: Primary dysmenorrhea received dry cupping; WCT: Primary dysmenorrhea group received wet cupping; *) = Significant at α value of 95 % using Mann-Whitney test.

Discussion

The results of writhing threshold time in this study showed significant differences between the treatment groups ($p < 0.001$), with the median time of each group ranging from C− (4.00), to DC (4.00), C+ (7.00) and WCT (7. 00). The same median was observed in WCT and C+ groups, showing the potential to prolong the occurrence of writhing latency threshold time in EB-injected and oxytocin-induced PD model rats. However, C+ and WCT groups were unable to match NC group, which was not given estradiol benzoate and oxytocin induction, showing the absence of writhing occurrence in rats in NC group (0).

This study showed that the median PGF2α levels decreased significantly in NC (115.7), C+ (166.4), DC (82.4) and WCT (264.1) groups. However, in C− group, PGF2α levels increased (−200.2), and the greatest decrease was found in WCT group (264.1), followed by C+ (166.4), NC (115.7) and DC (82.4). In comparison, DC (−180, $p = 0.0131$) and WCT (−145, $p = 0.0055$) groups showed less efficiency compared to C+ group. This was attributed to the difference in the administration of PGF2α levels before therapy, with the highest observed in WCT group.

FP receptors were found in the cell nucleus and cytoplasm of rat uterine myometrial stroma induced by estradiol benzoate and oxytocin. Although PTGFR expression was very low in NC group, a high level was observed in the cell nucleus and stromal cytoplasm in C− group compared to NC, C+, DC and WCT groups. In myometrial stroma of rats in C+ and WCT groups, PTGFR expression was also lower than DC. The results showed a significant decrease in prostaglandin F expression in cell nuclei and cytoplasm of inflamed uterine myometrial stroma in WCT group (3.25 ± 0.0087) compared to C− group.

Dysmenorrhea is a health condition characterized by severe pain in the uterus during menstruation, which often presents as recurrent lower abdominal pain. This condition is often categorized as PD when not accompanied by other pathological problems [40]. In women experiencing PD, there is an increase in the levels of substances that signal inflammation, such as vasopressin, prostaglandins PGE2, PGF2α and leukotrienes in the endometrial fluid. PGF2α is a substance that stimulates uterine muscle contraction and functions as a powerful vasoconstrictor. Meanwhile, leukotrienes promote stimulation and vasoconstriction of the uterine muscles, increasing the sensitivity of nerve fibers that cause pain. Vasopressin stimulates uterine activity, reduces blood flow to the uterus and constricts the uterine blood vessels in laboratory settings. The underlying cause of these elevated levels is still unknown, but uterine muscle activity is influenced and amplified by prostaglandin synthesis. PGF2 and PGE2 can also cause smooth muscle contractions in the bronchi, intestines and blood vessels, leading to bronchial constriction, nausea, vomiting, diarrhea and increased blood pressure [40].

Therapy of PD pain remains challenging due to its chronic nature and side effects. Moreover, primary therapy such as NSAIDs or oral contraceptive pills can cause various side effects on the gastrointestinal tract, liver and kidneys, leading to water retention, nausea and endometriosis [17]. Long-term NSAIDs often cause gastrointestinal symptoms and are contraindicated in patients with peptic ulcers and bleeding. Similarly, oral contraceptive increases the risk of deep vein thrombosis and is contraindicated in patients with cardiovascular disease and breast cancer [16]. Despite several mitigating efforts, temporary interventions have failed to provide a permanent cure, with symptoms recurring after drug withdrawal [18]. Several other methods developed to reduce dysmenorrhea, such as stretching therapy, acupuncture and TENS (Transcutaneous Electrical Nerve Stimulation) therapy, have not been proven effective [19]. To overcome this limitation, the administration of herbal therapy such as Gingerol is highly recommended due to the associated benefits and easy availability. However, there is a high tendency for dose control, quality and drug interactions, particularly when taken with other herbs that have the same effect [22,24,27].

Due to the limitations of existing dysmenorrhea therapies, cupping therapy has been proven as a viable alternative potentially becoming popular for pain therapy, including PD. Cupping therapy is a therapeutic method that uses a vacuum created under a small container applied to the skin's surface to alleviate pain. This activity provides healthcare professionals with an in-depth exploration of cupping therapy, an ancient healing method that has been applied in contemporary medicine. Generally, there are DC and WCT, both including skin suction with differences in blood drawing. In DC, no blood draw is performed, but blood is sucked into the cup in WCT due to skin injury, thereby serving as a superior therapy [41,42]. WCT can also restore normal physiology and remove pathological causative substances (CPS), while DC dilutes and redistributes CPS to new sites [43].

The modeling of dysmenorrhea in this study is similar to the previous analysis, using a well-established animal model to enhance the understanding of PD mechanisms. The application of PGF2α hypersensitivity model observed for 11 days showed a strong pain hypersensitivity after interventions. As a pointer of PD, twisting responses (abdominal contraction, concavity, trunk and hind limb extension, 1 limb rotation, uterine contraction) were observed [35,36,44]. Moreover, EB injection has been proven effective in inducing a significant increase in PGF2α levels, serving as a marker for PD induction. PGF2α is also a potent vasoconstrictor produced by the endometrium that acts directly on smooth muscle fibers, reducing vessel diameter, and causing constriction of arcuate vessels, leading to local hypoxia of endometrial tissue. Additionally, it acts as an agonist in the myometrium, inducing contraction by activating FP receptors and mobilizing intracellular Ca^{2+}. Writhing threshold time is considered a key indicator because intense acute abdominal pain is the main clinical symptom of PD. A comprehensive evaluation of writhing latency showed that cupping therapy had the strongest pain-reducing effect, as indicated by longer

writhing threshold time compared to rats in the model group. This indicator was also used to evaluate the model, serving as an index of behavioral observation of the analgesic effect in dysmenorrhea model rats [45,46].

Previous studies reported that WCT could increase the pain threshold in Complete Freund's Adjuvant (CFA)-induced experimental animals. However, the exact mechanism of cupping therapy to reduce pain is still not fully understood. A frequently discussed mechanism is the negative pressure effect, which can improve blood circulation, the immune system, pain threshold and anaerobic metabolism, alter skin biomechanics and reduce inflammation. According to the theory of Al-Bedah *et al.* [47], mechanical and physiological signals generated by stress on the skin caused by negative pressure lead to gene expression. Immunomodulation theory states that changes in the microenvironment by skin stimulation can change biological signals and activate the neuroendocrine-immune system. Another possibility is the function of WCT as an analgesic through the central nervous system by stimulating the release of enkephalins and dynorphins. Cupping causes local damage to the skin, thereby activating nociceptors that stimulate noxious inhibitory control and the secretion of β-endorphin hormone to provide anti-pain effects, induce comfort and relaxation at the systemic level to reduce pain through the limbic system. Mechanical stimulation also induces strain on collagen fibers and autonomic nerves, stimulating the production of neuropeptides, neurohormones and activation of the neuroendocrine system [48]. The results are supported by further investigations, where WCT increases withdrawal latency and decreases the expression of GABA-A receptors in the spinal cord in chronic constriction injury rat model [42]. An increase in GABA-A receptor expression positively correlates with increased withdrawal latency time (TWL) in the pain threshold test. The major inhibitory neurotransmitter γ-aminobutyric acid (GABA) has regulatory control over neuronal excitability levels. Therefore, reduced GABAergic activity may increase neuronal activity in the anterior cingulate cortex (ACC), contributing to neuropathic pain [49,50]. WCT produces transient analgesic effects blocked by naloxone, suggesting that endogenous opioid release can be the underlying mechanism. Due to the small sample size and low initial analgesic impact, statistical insignificance might have occurred. An endorphin up-regulator known as Complete Freud's adjuvant has been found to improve analgesia. Further studies using a larger sample size, animal pain models and multiple cupping sessions are recommended to explore the mechanism of cupping therapy [51].

Al-Tawarah [52] stated that WCT induced a significant reduction in several inflammatory markers, including pre- and post-inflammatory factors. This intervention also induced the efficiency of antioxidant parameters known as defense systems against various reactive oxygen species (ROS) [52]. Prostaglandins have been shown to directly affect uterine contractions, which are responsible for dysmenorrhea. According to clinical analysis, the level of PGF2α in dysmenorrhea patients is significantly higher compared to asymptomatic controls, therefore an increased level of PGF2α/PG-E2 has been considered an indicator of clinical diagnosis for PD. Another study found that the uterine PGF2α/serum PG-E2 ratio was significantly increased by 53 and 46 % in model group, suggesting the association of PD with an imbalance in uterine PGF2α and E2 (PG-E2) concentrations [12].

Although several theories have been, the precise therapeutic mechanism of cupping has not been identified. According to Taibah's theory, WCT emulates an artificial kidney, where the high-pressure filtration of hydrophilic and hydrophobic materials enhances blood volume, capillary filtration rate, as well as clearance of filtered and interstitial fluid. This process generates pressure gradients and traction forces on the capillary blood and lymphatic walls in cupping area to drain solutes and fluids in the interstitial space through the skin openings. Consequently, the augmented blood circulation and lymph flow directs the collected filtered fluid containing disease-related substances, including prostaglandins and inflammatory mediators to the cupping area under negative pressure. The process can help restore homeostasis in the

body, leading to increased blood flow, removal of toxins, restoration of neuroendocrine balance, improved oxygen supply and tissue perfusion. The accompanying scratches can enhance innate and acquired immunity by stimulating inflammatory cell migration and the release of endogenous opioids [43,53].

Conclusions

In conclusion, this study showed that the administration of therapeutic WCT could increase writhing threshold time, lower PGF2α and PTGFR expression. This showed the effectiveness of cupping therapy in lowering inflammation in PD disease by inhibiting pro-inflammatory cytokines such as PGF2α and healing the uterus through the cyclooxygenase pathway. Moreover, further studies were recommended to explore the mechanism of WCT' on PD caused by oxytocin and estradiol benzoate.

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