Sains Malaysiana 54(2)(2025): 557-567
http://doi.org/10.17576/jsm-2025-5402-20

Curcumin Improves the Expression of Phospholiphase C and Transient Receptor Potential Vanilloid Type 1 on Neuronal Myometrium in Dysmenorrhea Model

(Kurkumin Memperbaiki Ekspresi Fosfolipase C dan Potensi Reseptor Fana Vanilloid Jenis 1 pada Neuron Miometrium dalam Model Dismenorea)

MUKHOIROTIN[1,2], ABDUL KHAIRUL RIZKI PURBA[3,4,*], BAMBANG PURWANTO[5], ERNAWATI[6], HAMIMATUS ZAINIYAH[1,7] & LINA PATRICIA GUTJAHR[8]

[1]*Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Indonesia*
[2]*Department of Nursing, Faculty of Health Science, Universitas Pesantren Tinggi Darul Ulum (Unipdu), Indonesia*
[3]*Department of Pharmacology, Faculty of Medicine, Universitas Airlangga, Indonesia*
[4]*Department of Health Science, University Medical Center Groningen, University of Groningen, The Netherlands*
[5]*Department of Physiology and Medical Biochemistry, Faculty of Medicine, UniversitasAirlangga, Indonesia*
[6]*Department of Obstetric Gynecology, Faculty of Medicine, Universitas Airlangga, Indonesia*
[7]*Institute of Health Science, Ngudia Husada, Bangkalan, Madura, Indonesia*
[8]*Faculty of Medicine, Saarland University, Saarbrücken, Germany*

Received: 7 August 2024/Accepted: 20 November 2024

ABSTRACT

The pathogenesis of primary dysmenorrhea is closely related to increased expression of phospholipase C (PLC) and transient receptor potential vanilloid 1 (TRPV1) in myometrial nerve cells. Curcumin has traditionally been used to reduce the pain associated with dysmenorrhea. To date, the effect of curcumin on the expression of PLC and TRPV1 in myometrial pain sensitization has not been studied. This study aimed to determine the impact of curcumin on the expression of PLC and TRPV1 in the myometrial nerves of a primary dysmenorrhea model. The study involved a randomized post-test control group design using non-pregnant female Balb/c mice as a model of primary dysmenorrhea induced by estradiol benzoate and oxytocin. Thirty-five mice that met the inclusion criteria were divided into five groups: sham group (SG), receiving placebo; ibuprofen group (IG) receiving ibuprofen 100 mg/kg; Cur100 receiving curcumin 100 mg/kg; Cur200 receiving curcumin 200 mg/kg; and Cur400 receiving curcumin 400 mg/kg. Each drug was administered orally twice daily for 7 days. Finally, oxytocin was administered to induce a writhing response in the mice. PLC and TRPV1 expression were examined using double immunofluorescence staining. Data were analyzed using a one-way analysis of variance and Tukey's test. Curcumin significantly lowered PLC and TRPV1 expressions in the primary dysmenorrhea model compared to those in the SG ($p<0.05$). The best effect was observed in the Cur400 group with the PLC expression at 27.31 ± 7.42 ($p<0.001$), and TRPV1 expression at 33.21 ± 9.99 ($p<0.001$). In a primary dysmenorrhea model, Curcumin effectively improves PLC and TRPV1 expression in myometrial nerves.

Keywords: Curcumin; dysmenorrhea; PLC; reproductive health; TRPV1

ABSTRAK

Patogenesis dismenorea primer berkait rapat dengan peningkatan ekspresi phospholipase C (PLC) dan potensi reseptor fana vanilloid Jenis 1 (TRPV1) dalam sel saraf miometrium. Kurkumin secara tradisinya telah digunakan untuk mengurangkan kesakitan yang berkaitan dengan dismenorea. Sehingga kini, kesan kurkumin pada ekspresi PLC dan TRPV1 dalam pemekaan sakit miometrium belum dikaji. Penyelidikan ini bertujuan untuk menentukan kesan kurkumin terhadap ekspresi PLC dan TRPV1 dalam saraf miometrium model dismenorea primer. Penyelidikan ini melibatkan reka bentuk kumpulan kawalan ujian pasca rawak menggunakan tikus Balb/c betina tidak hamil sebagai model dismenorea primer yang disebabkan oleh estradiol benzoat dan oksitosin. Tiga puluh lima tikus yang memenuhi kriteria kemasukan dibahagikan kepada lima kumpulan: kumpulan sham (SG), menerima plasebo; kumpulan ibuprofen (IG) menerima ibuprofen 100 mg/kg, Cur100 menerima curcumin 100 mg/kg, Cur200 menerima curcumin 200 mg/kg dan Cur400 menerima curcumin 400 mg/kg. Setiap ubat diberikan secara oral dua kali sehari selama 7 hari. Akhirnya, oksitosin diberikan untuk mendorong tindak balas menggeliat pada tikus. Ekspresi PLC dan TRPV1 diperiksa menggunakan pewarnaan imunofluoresensi berganda. Data dianalisis menggunakan analisis varian sehala and ujian Tukey. Kurkumin menurunkan ekspresi PLC dan TRPV1 dengan ketara dalam model dismenorea primer berbanding dengan model SG ($p<0.05$). Kesan terbaik diperhatikan dalam

kumpulan Cur400 dengan ekspresi PLC pada 27.31 + 7.42 ($p<0.001$) dan ungkapan TRPV1 pada 33.21 ± 9.99 ($p<0.001$). Dalam model dismenorea primer, kurkumin berkesan meningkatkan ekspresi PLC dan TRPV1 dalam saraf miometrium.

Kata kunci: Dismenorea; kesihatan reproduktif; kurkumin; PLC; TRPV1

INTRODUCTION

The physiological cycle of menstruation continues throughout a woman's reproductive period. Up to 75% of women experience menstrual abnormalities. These abnormalities include dysmenorrhea which has become the most commonly reported symptom (Çinar et al. 2021). Menstrual cramps causing pain in the lower abdomen or pelvis, sometimes radiating to the inner thighs, legs, and lower back are known as dysmenorrhea. The symptoms usually manifest as diarrhea, vomiting, breast sensitivity, lethargy, fatigue, and sweating (Karout et al. 2021). Based on its pathophysiology, dysmenorrhea is classified as either primary and secondary. Primary dysmenorrhea is painful menstrual cramping that appears within 6-12 months after menarche without pelvic abnormalities. The condition persists for 48-72 h and the symptoms manifest in the suprapubic area before and/or during menstruation. Secondary dysmenorrhea arises from pelvic pathologies and/or underlying anatomical abnormalities, such as endometriosis, leiomyoma, pelvic inflammatory disease, and adenomyosis, typically developing years after menarche (Çinar et al. 2021; Wang et al. 2022).

The prevalence of dysmenorrhea in some countries remains high, with 69.8% in Nigeria, 75.9% in Zimbabwe, and 90.1% in Croatia (Esan et al. 2024; Horvat et al. 2023; Nyirenda et al. 2023). In terms of ethnicity, the prevalence of dysmenorrhea ranges from 16% to 91%, with 10% to 20% of patients experiencing severe menstrual disorders. These conditions are the primary reasons for frequent school absences, reduced concentration, lack of active class participation, hindered study and completion of homework, poor exam performance, and limited activity engagement (Mesele et al. 2022). Factors influencing dysmenorrhea include early onset of menarche, age less than 20 years, nulliparity, heavy menstrual flow, family history of dysmenorrhea, high body mass index (BMI), short or long menstrual cycle interval, premenstrual syndrome, stress, anxiety, depression, low level of social support, smoking, lifestyle and personal habits, physical activity, and irregular menstrual cycles (heavy, frequent, and prolonged periods) (Hu et al. 2020; Mammo, Alemayehu & Ambaw 2022; Mesele et al. 2022; Wang et al. 2022).

The pathogenesis of primary dysmenorrhea is closely related to the increase of prostaglandin F2α and leukotrienes (Harel 2012). Increased prostaglandin levels can cause uterine contractions, reduce blood flow to the myometrium, lead to ischemia, and increase peripheral nerve sensitivity (Barcikowska et al. 2020). Moreover, leukotrienes may enhance uterine pain and nerve sensitivity (Hillard 2006). Pain perception can also be determined by stretching receptor activation (Bakhsh et al. 2022). Activation of receptors and channels on nociceptor afferent Aδ and C fiber terminals generates action potentials that travel to the spinal cord and then to the brain for conscious perception of pain (HelloBio 2022).

Inflammatory mediators produced by damaged immune cells activate prostaglandin EP, bradykinin B1/2, and cytokine receptors. Serotonin and adenosine triphosphate (ATP) activate the 5-$HT_{2A/3}$ and P2X3 receptors, respectively. These receptors activate phospholipase C (PLC) and protein kinase C (PKC), causing intracellular calcium (Ca^{2+}) release, Ca^{2+}, and sodium (Na^{+}) influx, and inhibition of potassium (K^{+}) influx. Inflammatory mediators result in peripheral sensitization, increased gene translation, and the opening of ion channels and receptors, with a decreased transient receptor potential vanilloid type 1 (TRPV1) channel activation threshold due to PKC phosphorylation. A decrease in the TRPV1 channel activation threshold opens the TRPV1 channel, causing Ca^{2+} and Na^{+} influx. The influx of Ca^{2+} and Na^{+} along with the release of intracellular Ca^{2+} leads to an increase in intracellular Ca^{2+}, resulting in pain excitation and heightened pain sensitivity (HelloBio 2022). TRVP1 is located on nociceptive nerves at the terminals of Aδ and C fibers of free afferent nerves that play a role in the transmission of pain impulses (Elokely et al. 2016).

Drug therapy and complementary medicine are often used to manage dysmenorrhea (Sharghi et al. 2019). Non-steroidal anti-inflammatory drugs (NSAIDs) and oral contraceptive pills (OCPs) are the most commonly used drugs. These drugs reduce pain by inhibiting prostaglandin production and release. However, long-term use of NSAIDs results in the side effects of headache, dizziness, drowsiness, loss of appetite, nausea, vomiting, gastrointestinal bleeding, acute asthma, dysuria, and acne (Navvabi Rigi et al. 2012). OCPs suppress ovulation, decrease endometrial proliferation, and provide an endocrine environment similar to the early stages of the proliferative phase of the menstrual cycle, when prostaglandin levels are at their lowest. Low prostaglandin levels lead to few uterine cramps. Complementary medicine includes herbs; yoga; relaxation; psychotherapy; massage; hypnosis; vitamins E, B, and C, supplements (calcium and magnesium) as well as acupressure; and acupuncture (Sharghi et al. 2019).

The primary naturally occurring polyphenol in the rhizome of *Curcuma longa* or turmeric is curcumin, which has long been utilized to ease dysmenorrhea-related pain. Several studies have demonstrated that curcumin administration decreases the duration and intensity of dysmenorrhea (Bahrami et al. 2021; Pichardo

et al. 2020; Tabari et al. 2020). Curcumin significantly reduced the average PLC in a diabetic rat model induced by streptozotocin (STZ) (Fattah et al. 2020), along with inhibiting visceral nociception by antagonizing TRPV1 in rats with colorectal distension (Zhi et al. 2013). Additionally, curcumin blocked TRPV1 activation in capsaicin-treated rats, inhibiting TRPV1-mediated pain hypersensitivity (Yeon et al. 2009). The oxytocin-administered mouse model is widely recognized as a frequently used pharmacodynamic experimental mouse model in primary dysmenorrhea research (Yang et al. 2015). Several studies have demonstrated that curcumin effectively decreases the intensity of menstrual pain (dysmenorrhea). Unfortunately, the effects of curcumin on the expression of the PLC and TRPV1 proteins, which are involved in the mechanism of dysmenorrhea, remain unclear. This study investigated the effects of curcumin on the expression of PLC and TRPV1 in the myometrial nerves of a mouse model of primary dysmenorrhea.

MATERIALS AND METHODS

ANIMALS

Female Balb/c mice (*Mus musculus*), weighting 20 ± 2 g and aged 6-8 weeks from the Faculty of Veterinary Medicine's laboratory, Universitas Airlangga is used in this study. Animals were housed in a 12-h light/dark cycle chamber at 23 ± 1 °C with food and water provided *ad libitum* (Hong et al. 2022; Yang et al. 2015). All experimental and animal welfare procedures were performed in accordance with the care and use guidelines of the Faculty of Veterinary Medicine's Laboratory, Universitas Airlangga. The study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya (approval no. 2). KEH.109.07.2023.

The sample size was determined based on the number of replicates or replications using Lemeshow's formula as follows:

$$N = \frac{2\sigma^2(Z1-\alpha + Z1-\beta)^2}{(\mu 1 - \mu 2)^2}$$

where N is the minimum sample size; Z1-α is the standard deviation value, 1.65 at α = 0.05; Z1-β is the standard deviation value, 0.85 at β = 0.20; σ^2 is the standard deviation of means of treatment and control groups (4.07); μ1 is the mean of treatment group (11.38); μ2 is the mean of control group (5.00); and $N = \frac{2(4,07)^2(1,65+0,85)^2}{(6,38)^2}$; N ≥ 5,09 ~ 6

The minimum sample size required based on the calculation results was six mice (*Mus musculus*). Number of replicates with correction factors.

$$n' = n \times 1/(1-f),\ n' = 6 \times 1/(1-0.1),\ n' = 6.67 \sim 7$$

where n' is the number of replications following the correction; and f is the correction factor based on treatment risk (0.1). The minimum sample size required for each group after accounting for the correction factor was seven Balb/c mice (*Mus musculus*).

CHEMICALS AND REAGENTS

The materials used in this research include 1) Estradiol benzoate (E0329, Tokyo Chemical Industry Co. LTD, Japan) and oxytocin (Interchemie Werken, Holland); 2) Curcumin (C1386, Sigma-Aldirch); 3) Ibuprofen (Novapharin, Gresik Indonesia); 4) Corn Oil; 5) Mouse monoclonal anti-calcitonin gene-related peptide (CGRP) (4901): sc-57053, Santa Cruz Biotechnology, Inc.; 6) Rabbit CGRP polyclonal antibody (E-AB-93381; Elabscience); 7) Rabbit anti-PLC gamma 1 (Tyr783) polyclonal antibody (bs-3343R, Bioss Antibodies); 8) Mouse VR1 (E-8) sc-398417 Santa Cruz-Biotex.

ANIMAL TREATMENT

The mice (35 animals) were acclimatized to laboratory conditions for 1 week with food and water *ad libitum* before treatment. The mice included in this study were in the estrus phase as determined by vaginal smear examination. Mice were randomly divided into five groups (n = seven per group): sham group (SG), ibuprofen group (IG), first treatment group (Cur100), second treatment group (Cur200), and third treatment group (Cur400). Primary dysmenorrhea was modeled in 35 mice by subcutaneously (sc) injecting estradiol benzoate for 10 days (10 mg/kg on days 1 and 10, and 5 mg/kg on days 2-9). On day 11, the mice were injected intraperitoneally (i.p.) with oxytocin (100 U/kg) to induce dysmenorrhea (Peng et al. 2020). On days 4 to 10, SG was administered corn oil (0.5 mL), IG was administered ibuprofen (100 mg/kg) and corn oil (0.5 mL), Cur100 was administered curcumin 100 mg/kg and corn oil (0.5 mL), Cur200 was administered curcumin 200 mg/kg and corn oil (0.5 mL), and Cur400 was administered curcumin 400 mg/kg ad 0.5 mL corn oil. Each drug was administered twice daily by oral gavage. On day 11, a writhing response was observed within 30 min of oxytocin injection (Liu et al. 2022; Peng et al. 2020; Yu et al. 2019). Writhing response is the key indicator of pain in the primary dysmenorrhea model (Yang et al. 2015).

EXAMINATION OF VAGINAL SMEAR

Vaginal smears were prepared using the dab method with a cotton bud. The cotton bud was dipped in 0.9% sodium chloride (NaCl) and the tip was inserted into the vaginal opening of the mice and rotated slowly. The tip of the cotton bud was then applied to an object glass that had been dabbed with 0.9% NaCl solution, and a thin and evenly distributed smear was prepared. The preparation was fixed using 70% alcohol for 5 min and stained with 1% Giemsa dye for 2 min to color the vaginal smear. The preparations

were then washed under running distilled water and dried. After drying, the preparations were observed under a stereomicroscope at 400 x magnification (Sulastri, Wiratmini & Suriani 2014).

EXAMINATION OF PLC EXPRESSION AND TRPV1 EXPRESSION

PLC and TRPV1 expressions were examined using immunofluorescence. The immunofluorescence examination procedure included: 1) Tissue slides (5-7 µm thick) were heated at 60 °C for 60 min. They were then sequentially immersed in xylol solution (2 × 10 min), absolute alcohol (2 × 10 min), 90% alcohol (1 × 5 min), 80% alcohol (1 × 5 min), 70% alcohol (1 × 5 min), and sterile aquades (3 × 5 min); 2) Antigen retrieval was performed using citrate buffer. Slides were submerged in a pH 6.0 buffer citrate chamber and heated for 20 min at 95 °C in a water bath. The slide was removed from the water bath and allowed to cool to room temperature for approximately 20 min; 3) Phosphate-buffered saline (PBS) was used to wash the slides three times for 5 min; 4) Following that the slides were washed with 0.1% Triton-X 100 PBS for 1 × 5 min; 5) Slides were incubated with 1% bovine serum albumin (BSA) for 30 min at room temperature; 6) The BSA solution was removed; 7) The prepared sample was incubated with primary antibody overnight, at 4 °C. Primary antibodies for PLC expression were mouse monoclonal anti-CGRP (4901) (sc-57053, Santa Cruz Biotechnology, Inc.) and rabbit anti-PLC gamma 1 (Tyr783) polyclonal antibody (bs-3343R, Bioss Antibodies). For TRPV1 expression examination, the following antibodies were used: Rat CGRP polyclonal antibody monoclonal anti-CGRP (4901): sc-57053, Santa Cruz Biotechnology, Inc, TRPV1 polyclonal antibody (PA1-29770, Thermo Fisher Scientific); 8) The prepared sample was washed with PBS for 3 × 5 min; 9) Incubated with secondary antibody. For PLC examination goat anti-rabbit immunoglobulin (Ig) G H & LTIRTC (AB6718, ABCAM, Red) and anti-mouse FITC were used (Abcam, Green) for CGRP; TRPV1 examination was performed using anti-mouse FITC (Abcam, Green) and goat anti-rabbit IgG H & LTIRTC (AB6718, ABCAM, Red) for CGRP, 1:1000, for 30 min at room temperature; 10) The slides were washed with PBS for 3 × 5 min; 11) Incubated with 4′,6-diamidino-2-phenylindole 1:1000 for 5 min; 12) Washed with PBS for 3 × 5 min; 13) Covered with mounting medium and cover glass; 14) Observed with fluorescence microscope (Olympus IX71, Japan).

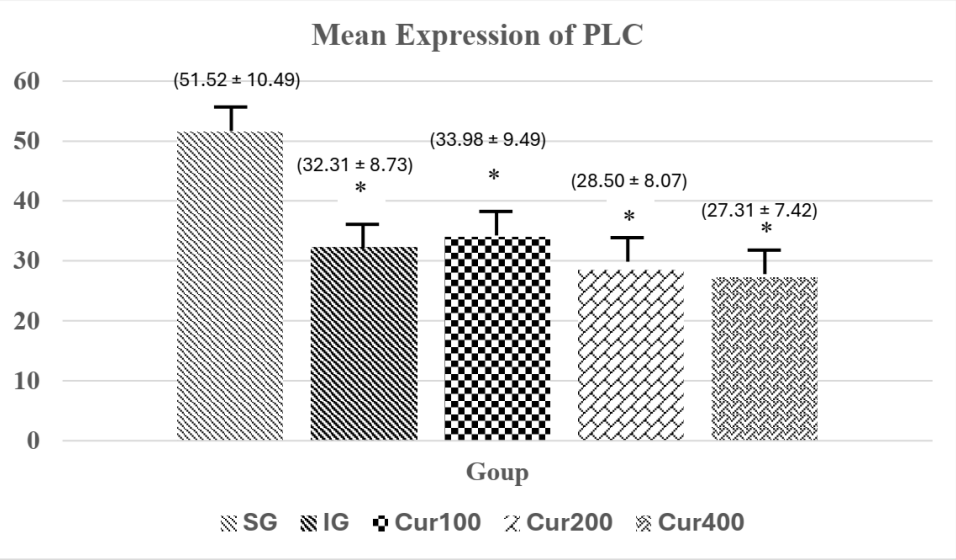
DATA ANALYSIS

Data were analyzed using the One-way analysis of variance statistical test and the Tukey test to determine differences between groups with a significance level of $\alpha \leq 0.05$ by using SPSS version 26.0.

RESULTS AND DISCUSSION

CURCUMIN IMPROVED PLC EXPRESSION OF NEURONAL MYOMETRIUM

Average PLC expression and the effect of curcumin on the myometrial nerve in a dysmenorrhea mouse model are presented in Figure 1. The expression of PLC in the



Data are presented as mean±standard deviation (n=7). *p<0.05, vs sham group

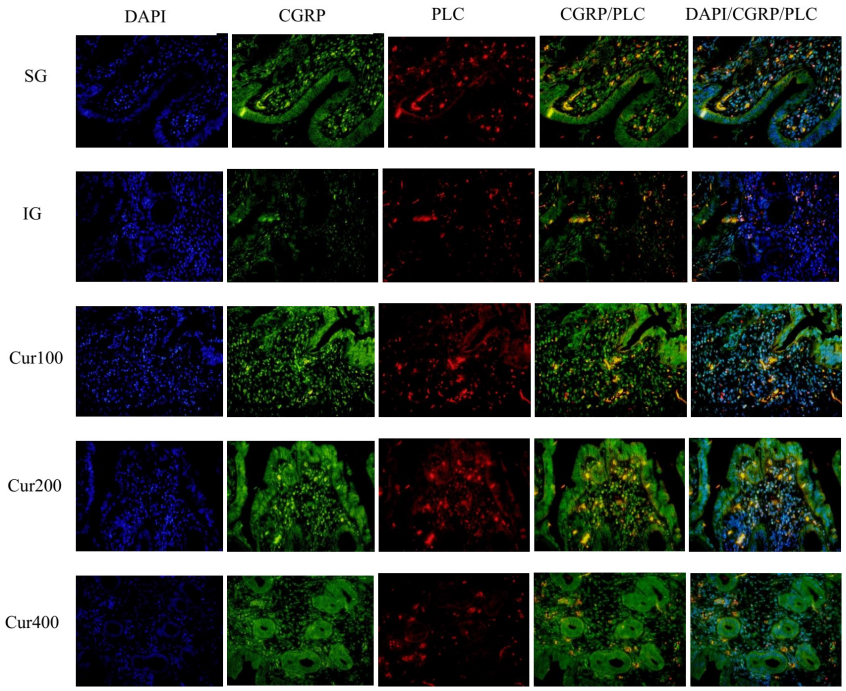
FIGURE 1. Effect of curcumin on phospholipase C expression in myometrial nerves in primary dysmenorrhea model

myometrial nerves of primary dysmenorrhea model mice was assessed using double immunofluorescence staining (Figure 2).

The highest average PLC expression was observed in the SG group (51.52), and the lowest was observed in the Cur400 group (27.31). The Cur100 group had an expression of 33.98, the IG group had 32.31, and the Cur200 group demonstrated 28.50. Significant differences were observed in PLC expression across all groups compared to that in the SG group ($p<0.05$). The effect of curcumin was not significantly different from that of ibuprofen ($p>0.05$). This indicated that curcumin administration improved PLC expression in myometrial nerves in the primary dysmenorrhea model.

The main polyphenol naturally occurring in the rhizomes of *Curcuma longa* (turmeric) is called curcumin (Kaur et al. 2024). Curcumin is non-toxic and has various therapeutic properties, including antioxidant, analgesic, anti-inflammatory, and antiseptic activities (Dewi et al. 2024). Clinical evidence has demonstrated that curcumin has analgesic and anti-inflammatory effects (Pichardo et al. 2020). The anti-inflammatory and neurotransmitter-modulating properties of curcumin reduce the severity of premenstrual syndrome symptoms (Khayat et al. 2015). Moreover, curcumin is also effective in reducing the duration and intensity of menstrual pain (Khayat et al. 2015; Tabari et al. 2020). The results showed that curcumin effectively improved PLC expression in the myometrial nerves of mice with primary dysmenorrhea.

Increased levels of prostaglandins and leukotrienes cause uterine contractions and result in ischemia by reducing the blood supply to the myometrium, causing primary dysmenorrhea. Additionally, they increase the sensitivity of peripheral nerves, lowering the pain threshold, resulting in pain (Harel 2012). Prostaglandin receptors activate protein kinase A (PKA), PLC, and PKC which leads to an increased influx of Ca^{2+} and Na^{+}, the release of intracellular Ca^{2+}, and the inhibition of K^{+} influx (HelloBio 2022). PLC is a cytoplasmic protein that regulates the levels of phosphatidylinositol-4,5-bisphosphate (PIP2) in cells by localizing inside or outside lipid rafts in the plasma membrane and catalyzing the hydrolysis of the phosphorylated form of phosphatidyl inositol in response to cellular stimuli (Kadamur & Ross 2013). Additionally, PLC cleaves PIP2 to produce diacylglycerol (DAG), activating PKC, and inositol-1,4,5-trisphosphate (IP3). IP3 in the endoplasmic reticulum induces the release of Ca^{2+} from intracellular stores within organelles (Bill & Vines 2020). PKC phosphorylation leads to a decrease in the activation threshold of TRPV1 channels; consequently, opening the channels, leading to Ca^{2+} and Na^{+} influx. The influx of Ca^{2+} and Na^{+} as well as the release of intracellular Ca^{2+} causes intracellular Ca^{2+} to rise, resulting in pain excitation (HelloBio 2022). PKC plays a major role in TRPV1 sensitization, with PKC-induced TRPV1 phosphorylation



SG, sham group; IG, ibuprofen group; Cur100, first treatment; Cur200, second treatment; Cur400, third treatment. Representative immunofluorescence staining of the myometrial nerves with 4′,6-diamidino-2-phenylindole (blue), calcitonin gene-related peptide (green), and PLC (red)

FIGURE 2. Immunofluorescence double staining for examination of phospholipase C (PLC) expression in myometrial nerves

enhancing responses to capsaicin, acid, and heat. The three major PKC phosphorylation residues in the rat TRPV1 were S502, T704, and S800. TRPV1 S800 is a polymodal-sensitizing residue. Phosphorylation of TRPV1 S800 may alter the modulatory capacity of the C-terminal domain, resulting in hypersensitivity to polymodal stimuli (Wang et al. 2015).

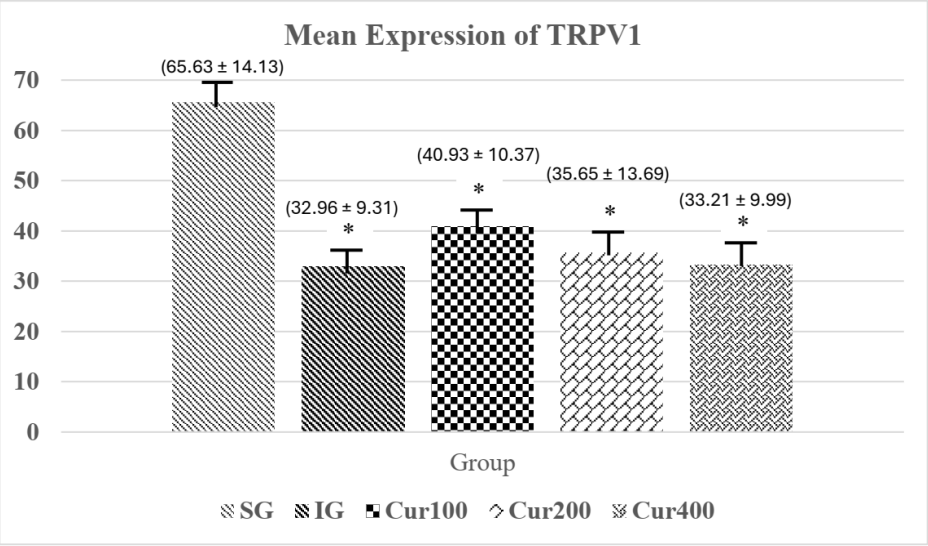
The results showed that curcumin effectively improved PLC expression in myometrial nerves in the primary dysmenorrhea model. The least improvement in PLC expression was observed in the Cur400 group, followed by the Cur100, PG, and Cur200 groups. The mechanism of action of curcumin in reducing PLC may be mediated through anti-inflammatory pathways and direct suppression of PLC. The anti-inflammatory effects of curcumin are mediated through the inhibition of cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS) (Menon & Sudheer 2007; Park 2010). Curcumin inhibits prostaglandin E2 production by suppressing the upregulation of COX-2 (Moriyuki et al. 2010). This pathway involves the mechanisms underlying the anti-inflammatory and analgesic effects of ibuprofen. However, we were unable to confirm the COX-2 expression in this study.

COX-2 is a key factor that regulates prostaglandin synthesis (Marjoribanks et al. 2015). Curcumin inhibited COX-2, thereby decreasing prostaglandin production. A decrease in prostaglandins also reduces PLC activation by prostaglandin receptors, resulting in a decrease in the entry of Ca^{2+} and Na^{+}, as well as a reduction in intracellular Ca^{2+} release. The decrease in intracellular Ca^{2+} reduces pain excitation, thereby lowering pain perception. In this study, the dysmenorrhea model group with the lowest PLC expression was the Cur400 group compared to the expression in the groups administered Cur100 and Cur200. The findings of this study are supported by outcomes of the previous research, which states that the administration of curcumin can weaken the inflammatory response in acrolein-induced human endothelial cells by inhibiting COX-2 expression and prostaglandin synthesis (Lee et al. 2020) and significantly decrease the average PLC expression in a diabetic rat model induced by STZ injection (Fattah et al. 2020). Curcumin attenuates pain-related neurotransmitters (substance P [SP]) and suppresses immune responses and prostaglandin E2 synthesis by suppressing COX-2, modulating purinergic and chemokine receptors, and activating the opioid system (Uddin et al. 2021).

CURCUMIN IMPROVED TRPV1 EXPRESSION OF NEURONAL MYOMETRIUM

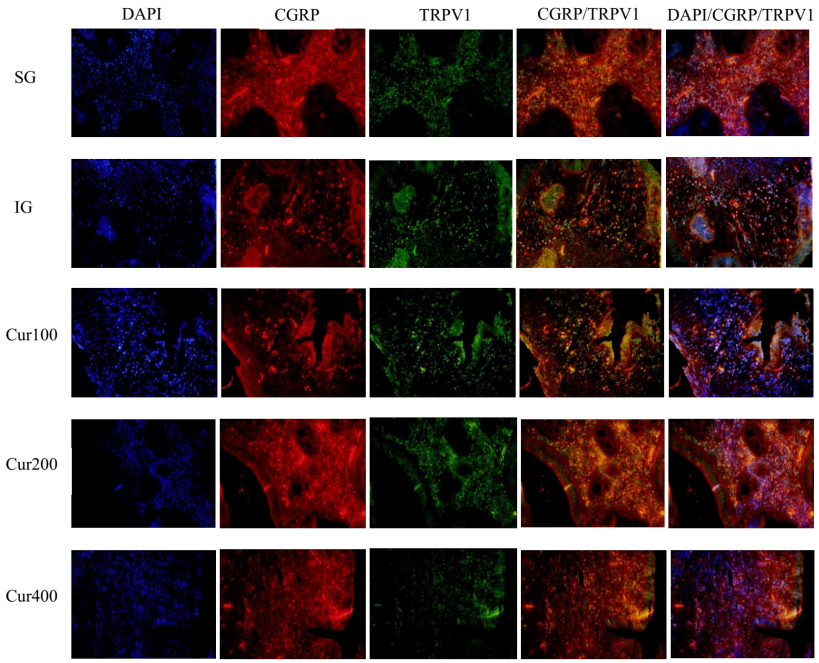
Average TRPV1 expression and the effect of curcumin on the myometrial nerve in the dysmenorrhea mouse model are presented in Figure 3. The expression of TRPV1 in the myometrial nerves of mice with primary dysmenorrhea was examined using double immunofluorescence staining (Figure 4).

The highest mean TRPV1 expression was observed in SG (65.63), followed by Cur 100 (40.93), Cur200 (35.65), Cur400 (33.21), and IG (32.96). A significant difference was noted in TRPV1 expression in all groups compared with that in the SG group ($p<0.05$). The effect of curcumin was not significantly different from that of ibuprofen



Data are presented as mean±standard deviation (n=7). *p<0.05, vs SG

FIGURE 3. Effect of curcumin on transient receptor potential vanilloid 1 expression in myometrial nerves in primary dysmenorrhea model



SG: Sham group, IG: Ibuprofen group, Cur100: First treatment group, Cur200: Second treatment group, Cur400: Third treatment group. Representative immunofluorescence staining of myometrial nerves with 4′,6-diamidino-2-phenylindole (blue), calcitonin gene-related peptide (red), and TRPV1 (green)

FIGURE 4. Immunofluorescence double staining for examination of transient receptor potential vanilloid 1 (TRPV1) expression in myometrial nerves

($p>0.05$). This suggests that curcumin administration improves TRPV1 expression in myometrial nerves in a primary dysmenorrhea model.

The results confirmed that curcumin effectively improved TRPV1 expression in myometrial nerves in the primary dysmenorrhea model. TRPV1 is expressed in all sensory ganglia (dorsal root ganglia [DRG], trigeminal ganglia [TG], vagal ganglia, and nodose ganglia) and in the terminal portion of free afferent nerve Aδ and C fibers, which may contain various neuropeptides including SP and/or CGRP (Jara-Oseguera, Simon & Rosenbaum 2010). Furthermore, TRPV1 plays a role in various pathologies, making this channel protein a formidable therapeutic target for pain management drugs (Jara-Oseguera, Simon & Rosenbaum 2010). Curcumin may also inhibit visceral nociception via TRPV1 antagonism (Zhi et al. 2013). The vanilloid moiety of curcumin is believed to be important for the activation of TRPV1, which plays a significant role in nociception (Yeon et al. 2009). The lowest TRPV1 decrease was noted in the primary dysmenorrhea mouse model treated with Cur400.

The results of this study are consistent with those of previous studies, which showed that curcumin blocks capsaicin-induced TRPV1 activation. This suggests that curcumin inhibits TRPV1-mediated pain hypersensitivity (Yeon et al. 2009). In addition, a study conducted by Yang et al. (2017) on visceral hyperalgesia and inflammation in a rat model of ulcerative colitis demonstrated that the oral administration of curcumin relieved visceral hyperalgesia in rats with dextran sulfate sodium-induced colitis. The anti-hyperalgesic effect was partly mediated by the downregulation of colonic expression and phosphorylation of TRPV1 on afferent fibers projecting from peptidergic and non-peptidergic nociceptive neurons of the dorsal root ganglion.

Curcumin is a natural product that is well-tolerated with minimal or no toxicity during short- and long-term use (Verma et al. 2017). The therapeutic efficacy of curcumin against nociceptive inflammation and neuropathic pain has been reported in several animal models (Dasuni Wasana et al. 2022). In humans, curcumin exhibits an antinociceptive effect and is safe even at very high doses (Lee et al. 2013). Curcumin has been reported to decrease mean PLC in a diabetic rat model (Fattah et al. 2020), meanwhile, also activating, sensitizing, and inhibiting TRPV1 expression. These effects may contribute to the antinociceptive and anti-inflammatory properties of curcumin (Nalli et al. 2017). TRPV1 responds to capsaicin at high temperatures, affecting the thermal sensation and pain signal transmission (Kwon et al. 2021).

The results demonstrated that curcumin effectively improved the expression of PLC and TRPV1 in myometrial neurons. PLC mediates the transduction of neurotransmitter signals across the membrane via hydrolysis of PIP2, which produces the secondary messengers IP3 and DAG. Low PLC levels result in low levels of IP3 leading to impaired Ca^{2+} release, as well as low intracellular Ca^{2+} levels. Thus, failing to perform normal neuronal function in the cerebral cortex and cerebellum (Kumar et al. 2010). DAG activates PKC, whereas, phosphorylation of PKC reduces the activation threshold of TRPV1 channels, causing Ca^{2+} and Na^{+} influx as TRPV1 channels open. The influx of Ca^{2+} and Na^{+} and the release of intracellular Ca^{2+} lead to an increase in intracellular Ca^{2+}. Consequently, pain excitation occurs, leading to an increase in pain intensity (HelloBio 2022).

Low expression of PLC and TRPV1 in this study reduced Ca^{2+} release and decreased intracellular Ca^{2+} levels, thereby decreasing pain sensitization. This suggests that curcumin can reduce pain sensitization in myometrial neurons in a primary dysmenorrhea model. The results of this study are supported by those of previous research, which demonstrated that curcumin produces an antinociceptive effect by decreasing PLC expression in the cerebral cortex and cerebellum of diabetic rats (Kumar et al. 2010) and by directly blocking TRPV1 in the TG and DRG of mice (Yang et al. 2018; Yeon et al. 2009).

The strength of this study is that it is the first to elucidate the mechanism by which curcumin reduces pain sensitization in primary dysmenorrhea. Decreased expression of PLC and TRPV1 following curcumin treatment explains the decrease in pain sensitization. In dysmenorrhea, PLC and TRPV1 play key roles in pain desensitization. A limitation of this study is that the pain sensitization process likely occurred through mechanisms beyond just PLC and TRPV1. No other pathways for pain sensitization were observed in this study. In the future, research to confirm pain sensitization through the PKA and PKC pathways in a dysmenorrhea model will be necessary. Given the effects of large doses of curcumin on the desensitization of myometrial neurons, considering modifications to the delivery system for orally administered curcumin is necessary. Curcumin has a potential synergistic effect in improving PLC and TRPV1 in a dysmenorrhea model; therefore, curcumin should be considered in further investigations when co-administered with conventional medicine.

CONCLUSION

The interactions of curcumin with the TRPV1 and PLC pathways play crucial roles in its antinociceptive and modulatory effects on pain perception. This interaction influences the activity of TRPV1 channels, which are sensitive to PLC-mediated signaling. Notably, curcumin modulates TRPV1-mediated synaptic responses by activating the PLC pathway, which can serve as a potential antinociceptive agent.

ACKNOWLEDGMENTS

The researchers would like to thank the Rector of Universitas Airlangga and the Dean of the Faculty of Medicine of Universitas Airlangga for providing support for this research.

REFERENCES

Bahrami, A., Zarban, A., Rezapour, H., Agha Amini Fashami, A. & Ferns, G.A. 2021. Effects of curcumin on menstrual pattern, premenstrual syndrome, and dysmenorrhea: A triple-blind, placebo-controlled clinical trial. *Phytotherapy Research* 35(12): 6954-6962. https://doi.org/10.1002/ptr.7314

Bakhsh, H., Algenaimi, E., Aldhuwayhi, R. & AboWadaan, M. 2022. Prevalence of dysmenorrhea among reproductive age group in Saudi Women. *BMC Women's Health* 22(1): 1-14. https://doi.org/10.1186/s12905-022-01654-9

Barcikowska, Z., Rajkowska-Labon, E., Grzybowska, M.E., Hansdorfer-Korzon, R. & Zorena, K. 2020. Inflammatory markers in dysmenorrhea and therapeutic options. *International Journal of Environmental Research and Public Health* 17(4): 1191. https://doi.org/10.3390/ijerph17041191

Bill, C.A. & Vines, C.M. 2020. Phospholipase C. *Advances in Experimental Medicine and Biology* 1131: 215-242. https://doi.org/10.1007/978-3-030-12457-1_9.

Çinar, G.N., Akbayrak, T., Gürşen, C., Baran, E., Üzelpasacı, E., Nakip, G., Bozdağ, G., Beksaç, M.S. & Özgül, S. 2021. Factors related to primary dysmenorrhea in Turkish women: A multiple multinomial logistic regression analysis. *Reproductive Sciences* 28(2): 381-392. https://doi.org/10.1007/s43032-020-00289-1

Dasuni Wasana, P.W., Hasriadi, Muangnoi, C., Vajragupta, O., Rojsitthisak, P., Rojsitthisak, P. & Towiwat, P. 2022. Curcumin and metformin synergistically modulate peripheral and central immune mechanisms of pain. *Scientific Reports* 12: 9713. https://doi.org/10.1038/s41598-022-13647-7

Dewi, F.R.P., Tambunan, U.V.D., Bari, P.A., Farid, M.A., Anjani, N.A., Wahyuningsih, S.P.A., Saik, A.Y.H., Keong, Y.Y., Lim, V., Tan, W.N. & Alshawsh, M.A.M. 2024. Formation of inclusion complex of curcumin and tetrahydrocurcumin prevents angiogenesis by inhibiting VEGF activity: An *in-silico* study. *Sains Malaysiana* 53(3): 653-665. https://doi.org/10.17576/jsm-2024-5303-13

Elokely, K., Velisetty, P., Delemotte, L., Palovcak, E., Klein, M.L., Rohacs, T. & Carnevale, V. 2016. Understanding TRPV1 activation by ligands: Insights from the binding modes of capsaicin and resiniferatoxin. *Proceedings of the National Academy of Sciences of the United States of America* 113(2): E137-E145. https://doi.org/10.1073/pnas.1517288113

Esan, D.T., Ariyo, S.A., Akinlolu, E.F., Akingbade, O., Olabisi, O.I., Olawade, D.B., Bamigboye, T.O. & Ogunfowokan, A.A. 2024. Prevalence of dysmenorrhea and its effect on the quality of life of female undergraduate students in Nigeria. *Journal of Endometriosis and Uterine Disorders* 5: 100059. https://doi.org/10.1016/j.jeud.2024.100059

Fattah, S.A., Waly, H., El-enein, A.A., Kamel, A. & Labib, H. 2020. Mesenchymal stem cells versus curcumin in enhancing the alterations in the cerebellar cortex of streptozocin-induced diabetic albino rats. The role of GFAP, PLC and α-synuclein. *Journal of Chemical Neuroanatomy* 109: 101842. https://doi.org/10.1016/j.jchemneu.2020.101842

Harel, Z. 2012. Dysmenorrhea in adolescents and young adults: An update on pharmacological treatments and management strategies. *Expert Opinion on Pharmacotherapy* 13(15): 2157-2170. https://doi.org/10.1517/14656566.2012.725045

HelloBio. 2022. *Signaling Pathways in Pain*. https://hellobio.com/media/pdf/Technical-resource/Signaling pathways in pain.pdf

Hillard, P.J.A. 2006. Consultation with the specialist: Dysmenorrhea. *Pediatrics in Review* 27(2): 64-71. https://doi.org/10.1542/pir.27-2-64

Hong, F., He, G., Zhang, M., Yu, B. & Chai, C. 2022. The establishment of a mouse model of recurrent primary dysmenorrhea. *International Journal of Molecular Sciences* 23(11): 6128. https://doi.org/10.3390/ijms23116128

Horvat, M., Pavan Jukić, D., Marinović, L., Bursać, D., Ribić, R., Neuberg, M. & Bursać, D. 2023. Prevalence of primary dysmenorrhoea and its impact on academic performance among Croatian students during the COVID-19 pandemic. *Obstetrics and Gynecology International* 2023: 2953762. https://doi.org/10.1155/2023/2953762

Hu, Z., Tang, L., Chen, L., Kaminga, A.C. & Xu, H. 2020. Prevalence and risk factors associated with primary dysmenorrhea among Chinese female university students: A cross-sectional study. *Journal of Pediatric and Adolescent Gynecology* 33(1): 15-22. https://doi.org/10.1016/j.jpag.2019.09.004

Jara-Oseguera, A., Simon, S. & Rosenbaum, T. 2010. TRPV1: On the road to pain relief. *Current Molecular Pharmacology* 1(3): 255-269. https://doi.org/10.2174/1874467210801030255

Kadamur, G. & Ross, E.M. 2013. Mammalian phospholipase C. *Annual Review of Physiology* 75: 127-154. https://doi.org/10.1146/annurev-physiol-030212-183750

Karout, S., Soubra, L., Rahme, D., Karout, L., Khojah, H.M.J. & Itani, R. 2021. Prevalence, risk factors, and management practices of primary dysmenorrhea among young females. *BMC Women's Health* 21: 392. https://doi.org/10.1186/s12905-021-01532-w

Kaur, K., Al-Khazaleh, A.K., Bhuyan, D.J., Li, F. & Li, C.G. 2024. A review of recent curcumin analogues and their antioxidant, anti-inflammatory, and anticancer activities. *Antioxidants* 13(9): 1092. https://doi.org/10.3390/antiox13091092

Khayat, S., Fanaei, H., Kheirkhah, M., Moghadam, Z.B., Kasaeian, A. & Javadimehr, M. 2015. Curcumin attenuates severity of premenstrual syndrome symptoms: A randomized, double-blind, placebo-controlled trial. *Complementary Therapies in Medicine* 25(3): 318-324. https://doi.org/10.1016/j.ctim.2015.04.001

Kumar, T.P., Antony, S., Gireesh, G., George, N. & Paulose, C.S. 2010. Curcumin modulates dopaminergic receptor, CREB and phospholipase c gene expression in the cerebral cortex and cerebellum of streptozotocin induced diabetic rats. *Journal of Biomedical Science* 17(1): 43. https://doi.org/10.1186/1423-0127-17-43

Kwon, D.H., Zhang, F., Suo, Y., Bouvette, J., Borgnia, M.J. & Lee, S.Y. 2021. Heat-dependent opening of TRPV1 in the presence of capsaicin. *Nature Structural and Molecular Biology* 28(7): 554-563. https://doi.org/10.1038/s41594-021-00616-3

Lee, J.Y., Shin, T.J., Choi, J.M., Seo, K.S., Kim, H.J., Yoon, T.G., Lee, Y.S., Han, H., Chung, H.J., Oh, Y., Jung, S.J. & Shin, K.J. 2013. Antinociceptive curcuminoid, KMS4034, effects on inflammatory and neuropathic pain likely via modulating TRPV1 in mice. *British Journal of Anaesthesia* 111(4): 667-672. https://doi.org/10.1093/bja/aet176

Lee, S.E., Park, H.R., Jeon, S., Han, D. & Park, Y.S. 2020. Curcumin attenuates acrolein-induced cox-2 expression and prostaglandin production in human umbilical vein endothelial cells. *Journal of Lipid and Atherosclerosis* 9(1): 184-194. https://doi.org/10.12997/jla.2020.9.1.184

Liu, C., Li, X., Zhou, C., Liang, Y., Zhang, X., Liu, Y., Zhao, Z. & Ma, X. 2022. Effects of ginger-partitioned moxibustion on the expression levels of PGF2α, E2, P, and mRNAs of PGF2αR and E2R in rats with primary dysmenorrhea due to cold-dampness stagnation. *Journal of Acupuncture and Tuina Science* 20(2): 104-110. https://doi.org/10.1007/s11726-022-1301-0

Mammo, M., Alemayehu, M. & Ambaw, G. 2022. Prevalence of primary dysmenorrhea, its intensity and associated factors among female students at high schools of Wolaita Zone, Southern Ethiopia: Cross-sectional study design. *International Journal of Women's Health* 14: 1569-1577. https://doi.org/10.2147/IJWH.S384275

Marjoribanks, J., Ro, A., Farquhar, C. & Proctor, M. 2015. Nonsteroidal anti-inflammatory drugs for dysmenorrhoea (Review). *Cochrane Database of Systematic Reviews* 2015(7): CD001751. . https://doi.org/10.1002/14651858.CD001751.pub3.www.cochranelibrary.com

Menon, V.P. & Sudheer, A.R. 2007. Antioxidant and anti-inflammatory properties of curcumin. *Advances in Experimental Medicine and Biology* 595: 105-125. https://doi.org/10.1007/978-0-387-46401-5_3

Mesele, T.T., Dheresa, M., Oljira, L., Wakwoya, E.B. & Gemeda, G.M. 2022. Prevalence of dysmenorrhea and associated factors among Haramaya University students, Eastern Ethiopia. *International Journal of Women's Health* 14: 517-527. https://doi.org/10.2147/IJWH.S333447

Moriyuki, K., Sekiguchi, F., Matsubara, K., Nishikawa, H. & Kawabata, A. 2010. Curcumin inhibits the proteinase-activated receptor-2-triggered prostaglandin E2 production by suppressing cyclooxygenase-2 upregulation and Akt-dependent activation of nuclear factor-κB in human lung epithelial cells. *Journal of Pharmacological Sciences* 114(2): 225-229. https://doi.org/10.1254/jphs.10126SC

Nalli, M., Ortar, G., Schiano Moriello, A., Di Marzo, V. & De Petrocellis, L. 2017. Effects of curcumin and curcumin analogues on TRP channels. *Fitoterapia* 122: 126-131. https://doi.org/10.1016/j.fitote.2017.09.007

Navvabi Rigi, S., Kermansaravi, F., Navidian, A., Safabakhsh, L., Safarzadeh, A., Khazaeian, S., Shafie, S. & Salehian, T. 2012. Comparing the analgesic effect of heat patch containing iron chip and ibuprofen for primary dysmenorrhea: A randomized controlled trial. *BMC Women's Health* 12: 25. https://doi.org/10.1186/1472-6874-12-25

Nyirenda, T., Nyagumbo, E., Murewanhema, G., Mukonowenzou, N., Kagodora, S.B., Mapfumo, C., Bhebhe, M. & Mufunda, J. 2023. Prevalence of dysmenorrhea and associated risk factors among university students in Zimbabwe. *Women's Health* 19: 17455057231189549. https://doi.org/10.1177/17455057231189549

Park, J. 2010. Anti-carcinogenic properties of curcumin on colorectal cancer. *World Journal of Gastrointestinal Oncology* 2(4): 169. https://doi.org/10.4251/wjgo.v2.i4.169

Peng, Y., Zheng, X., Fan, Z., Zhou, H., Zhu, X., Wang, G. & Liu, Z. 2020. Paeonol alleviates primary dysmenorrhea in mice via activating CB2R in the uterus. *Phytomedicine* 68: 153151. https://doi.org/10.1016/j.phymed.2019.153151

Pichardo, E., Liborio-Kimura, T., Caballero, M.E., Kandany, V.N., Mena, L., Ferreira-Filho, E., Almeida, E.D., Navia, S.B., Zelniker, M., Matsuura, D., Ying, C.T.Q., Calabria, B.S.R., De Almeida, G., Mushtaq, K., Abdallah, A., Doomi, A., Antonio, E.P. & Tamayo, A. 2020. ESCAPE pain trial - The effects of Curcumin in pain relief in women diagnosed with primary dysmenorrhea: A triple-blinded, placebo-controlled, phase II, randomized clinical trial protocol. *Principles and Practice of Clinical Research* 6(2): 25-32. https://doi.org/10.21801/ppcrj.2020.62.5

Sharghi, M., Mansurkhani, S.M., Ashtary-Larky, D., Kooti, W., Niksefat, M., Firoozbakht, M., Behzadifar, M., Azami, M., Servatyari, K. & Jouybari, L. 2019. An update and systematic review on the treatment of primary dysmenorrhea. *Jornal Brasileiro de Reproducao Assistida* 23(1): 51-57. https://doi.org/10.5935/1518-0557.20180083

Sulastri, S., Wiratmini, N.I. & Suriani, N.L. 2014. Panjang siklus estrus mencit (*Mus musculus* L.) yang diberi pemanis buatan aspartam secara oral. *Jurnal Biologi* 18(2): 69-72. https://doi.org/jurnal.harianregional.com/bio/id-16839

Tabari, N.S., Kheirkhah, M., Mojab, F. & Salehi, M. 2020. An investigation of the effect of curcumin (turmeric) capsule on the severity and duration of dysmenorrhea in students of Iran University of Medical Sciences. *Journal of Evolution of Medical and Dental Sciences* 9(46): 3444-3451. https://doi.org/10.14260/jemds/2020/755

Uddin, S.J., Hasan, M.F., Afroz, M., Sarker, D.K., Rouf, R., Islam, M.T., Shilpi, J.A. & Mubarak, M.S. 2021. Curcumin and its multi-target function against pain and inflammation: An update of pre-clinical data. *Curr. Drug Targets* 22(6): 656-671. https://doi.org/10.2174/1389450121666200925150022

Verma, S., Mundkinajeddu, D., Agarwal, A., Chatterjee, S.S. & Kumar, V. 2017. Effects of turmeric curcuminoids and metformin against central sensitivity to pain in mice. *Journal of Traditional and Complementary Medicine* 7(2): 145-151. https://doi.org/10.1016/j.jtcme.2016.04.001

Wang, L., Yan, Y., Qiu, H., Xu, D., Zhu, J., Liu, J. & Li, H. 2022. Prevalence and risk factors of primary dysmenorrhea in students: A meta-analysis. *Value in Health* 25(10): 1678-1684. https://doi.org/10.1016/j.jval.2022.03.023

Wang, S., Joseph, J., Ro, J.Y. & Chung, M-K. 2015. Modality-specific mechanisms of protein kinase C–induced hypersensitivity of TRPV1. *Pain* 156(5): 931-941. https://doi.org/10.1097/j.pain.0000000000000134

Yang, L., Cao, Z., Yu, B. & Chai, C. 2015. An *in vivo* mouse model of primary dysmenorrheal. *Experimental Animals* 64(3): 295-303. https://doi.org/10.1538/expanim.14-0111

Yang, M., Wang, J., Yang, C., Han, H., Rong, W. & Zhang, G. 2017. Oral administration of curcumin attenuates visceral hyperalgesia through inhibiting phosphorylation of TRPV1 in rat model of ulcerative colitis. *Molecular Pain* 13: 1744806917726416. https://doi.org/10.1177/1744806917726416

Yang, Z., He, C., He, J., Chu, J., Liu, H. & Deng, X. 2018. Curcumin-mediated bone marrow mesenchymal stem cell sheets create a favorable immune microenvironment for adult full-thickness cutaneous wound healing. *Stem Cell Research and Therapy* 9: 21. https://doi.org/10.1186/s13287-018-0768-6

Yeon, K.Y., Kim, S.A., Kim, Y.H., Lee, M.K., Ahn, D.K., Kim, H.J., Kim, J.S., Jung, S.J. & Oh, S.B. 2009. Curcumin produces an antihyperalgesic effect via antagonism of TRPV1. *Journal of Dental Research* 89(2): 170-174. https://doi.org/10.1177/0022034509356169

Yu, L., Yi-qin, W., Ling-yu, C., Bin-qian, M., Xiao-xian, W., Yao, X. & Biao, T. 2019. Effect of electroacupuncture on NF-κB and NLRP3 rats with primary dysmenorrhea inflammasome in uterine tissues of rats with primary dysmenorrhea. *Journal of Acupuncture and Tuina Science* 17(4): 215-222. https://doi.org/10.1007/s11726-019-1117-8

Zhi, L., Dong, L., Kong, D., Sun, B., Sun, Q., Grundy, D., Zhang, G. & Rong, W. 2013. Curcumin acts via transient receptor potential vanilloid-1 receptors to inhibit gut nociception and reverses visceral hyperalgesia. *Neurogastroenterology & Motility* 25(6): e429-e440. https://doi.org/10.1111/nmo.12145

*Corresponding author; email: khairul_purba@fk.unair.ac.id