ORIGINAL ARTICLE

Curcumin Improved Superoxide Dismutase Concentration but Failed to Decrease Malondialdehyde Concentration After a Series of Estradiol Benzoate Injection in Mice

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ABSTRACT

Introduction: Estradiol benzoate is widely used to mimic dysmenorrhea in mice, inducing oxidative stress and inflammation during menstruation. Since curcumin is a potent natural antioxidant, it presumably decreases dysmenorrhea and oxidative stress during menstruation. This study aims to investigate the effect of curcumin on superoxide dismutase (SOD) and malondialdehyde (MDA) concentration in estradiol benzoate-induced dysmenorrhea in mice. **Methods:** Forty mice were randomly grouped into five groups. The first three groups were treated with curcumin twice a day with 100 mg/kg (Dys+Cur100), 200 mg/kg (Dys+Cur200), and 400 mg/kg (Dys+Cur400) in corn oil. The next two groups were treated with either 100 mg/kg ibuprofen (Dys+Ibu) or corn oil as placebo (Dys). Oxytocin was used to induce a writhing response to pain in mice. On day 11, blood serum was taken for examination of SOD and MDA concentration in mice. **Results:** Significant differences were observed between the SOD concentration in the Dys+Cur100 vs. Dys group ($p < 0.05$). The MDA concentration did not differ between groups ($p > 0.05$). Although the antioxidant effect of curcumin failed to lower the MDA concentration, it succeeded in elevating the SOD concentration in mice treated with 100 mg curcumin. **Conclusion:** The antioxidant activity of curcumin improved SOD levels but not MDA levels in dysmenorrhea-induced mice, highlighting the indirect effect of curcumin on dysmenorrhea treatment.
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Keywords: Curcumin, Dysmenorrhea, Estradiol, Antioxidant, Oxidative stress

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INTRODUCTION

The prevalence of primary dysmenorrhea varies significantly across countries, with reports ranging from approximately 41.7% to 92.3% of the female population. In China, 41.7% of the population has experienced primary dysmenorrhea, compared with 55–88% in Turkey. In students in Northern Ghana, the prevalence of dysmenorrhea was found to be 83.6%, with more than half describing pain lasting less than three days as moderate. In Ethiopia, 64.7% of female students reported experiencing primary dysmenorrhea, 61% of which also reported menstrual pain of moderate intensity, while 50.7% reported lower abdominal pain. In Bafoussam, Cameroon, the prevalence of primary dysmenorrhea was 71.9%, among which 11.6%, 52.5%, and 35.9% reported mild, moderate, and severe pain, respectively. In Saudi Arabia, 92.3% of women have reported primary dysmenorrhea (1–8). In Indonesia, the prevalence of primary dysmenorrhea is 54.89% (9). Dewi et al. (2021) found 83.9% prevalence of dysmenorrhea among students at the University of Riau, Indonesia(10). Risk factors for primary dysmenorrhea include family history of dysmenorrhea, obesity, younger age, shorter or longer menstrual cycle intervals, irregular menstrual cycles, early menarche (before the age of 12), heavy menstrual flow, smoking, alcohol consumption, stress, anxiety and depression, and low social support (5,6,11). The use of mice as a model of primary dysmenorrhea via oxytocin induction is widely accepted as a valid experimental protocol (12,13). Hong et al. (2022)

described a pioneering mice model of recurrent primary dysmenorrhea induced via estrogen combined with oxytocin. The mice model fully characterized the clinical features associated with primary dysmenorrhea over two consecutive cycles (13). Severe acute abdominal pain is the primary clinical symptom of primary dysmenorrhea, with writhing response being a key indicator, and writhing times were used to evaluate model and drug trial effects (14).

The pathogenesis of primary dysmenorrhea is closely related to increasing levels of prostaglandin F2α (PGF2α) and leukotrienes and the release of large amounts of oxygen free radicals (15). Prostaglandins increase free radicals and decrease endogenous antioxidant responses that trigger oxidative stress. One marker of oxidative stress is an increase in malondialdehyde (MDA) concentration and a decrease in endogenous antioxidant concentration as determined by measuring superoxide dismutase (SOD) (16). MDA is formed during membrane lipid peroxidation and used as a sign of tissue injury (17). Oxidative stress markers are higher among individuals with dysmenorrhea than individuals with painless menstruation (18). Primary dysmenorrhea has been associated with an increase in lipid peroxidation and a decrease in the antioxidant concentration (16,19).

Turmeric has long been used as a remedy to reduce uterine contractions that cause menstrual pain (20). Phenolic compounds found in turmeric show potential as antioxidants. The active compound in turmeric is curcumin, which reduces oxidative stress by scavenging free radicals and preventing the production of reactive oxygen species (ROS) (21). Several studies have found that the administration of curcumin can reduce the severity and duration of dysmenorrhea (22,23). Curcumin has been shown to significantly increase SOD and decrease MDA in mice with oxidative stress-induced Parkinson's disease (24). However, until now, the effect of curcumin on SOD and MDA concentrations in mice model dysmenorrhea has not been known. This study investigated the effect of curcumin on SOD and MDA concentration in mice with primary dysmenorrhea models.

MATERIALS AND METHODS

Animals

Mice (*Mus musculus*) Balb strain/c female (weighing 20 ± 2 g) aged 6-8 weeks were obtained from Faculty of Veterinary Medicine laboratory, Universitas Airlangga. The animals were placed in a 12-hour light/dark cycle with a temperature at 23 ± 1°C with ad libitum access to food and water (13,14). All mice were injected by estradiol benzoate to get dysmenorrhea mice model. All experimental procedures were performed in line with the care and use guidelines of the Faculty of Veterinary Medicine laboratory, Airlangga University. The protocol for the use of animals was approved by the Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga Surabaya (approval no. 2.KEH.109.07.2023).

Chemical and Reagents

Induction of primary dysmenorrhea was performed using estradiol benzoate (E0329; Tokyo Chemical Industry Co. LTD, Japan) and oxytocin (Interchemie Werken, Holland). Curcumin was purchased from Sigma-Aldrich (C1386) and ibuprofen from Novapharin (Gresik Indonesia). Mouse superoxide dismutase, Cu-Zn, SOD (cat. no. E2608Mo-48T), and mouse malondialdehyde MDA ELISA Kit 4 (cat. no. E0625Mo-48T) were obtained from Bioassay Technology Laboratory (Zhejiang, China).

Animal Treatment

Mice were initially acclimatized in laboratory conditions for one week with ad libitum access to food and water. All mice were determined to be in the estrus phase by vaginal smear examination, as a pre-treatment prerequisite. Next, the mice were randomly assigned to five groups: negative control group (Dys), positive control group (Dys+Ibu), first treatment group (Dys+Cur100), second treatment group (Dys+Cur200), and third treatment group (Dys+Cur400). Forty mice (all groups) were injected with estradiol benzoate subcutaneously (sc) for ten days (10 mg/kg on day 1 and 10 and 5 mg/kg on day 2 to 9). On day 11, the mice were injected intraperitoneally (ip) with oxytocin (100 U/kg) to induce dysmenorrhea (26). Estradiol benzoate is often used to induce primary dysmenorrhea, and the writhing response triggered by oxytocin is used to simulate the primary symptoms (13,14,25,26). Estradiol benzoate is a natural estradiol derivative, used to increase uterine sensitivity to oxytocin and stimulate uterine smooth muscle contractions. Oxytocin is a small peptide hormone, directly binding to oxytocin receptors on the cytoplasmic membrane of uterine smooth muscle which promotes massive production of prostaglandins that stimulate excessive uterine smooth muscle contractions that are characteristic of primary dysmenorrhea (27).

On days four to ten, 0.5 ml of corn oil was administered orally via sonde to the negative control group, while ibuprofen (100 mg/kg) and corn oil (0.5 ml) was administered orally via sonde to the positive control group. The first, second, and third treatment groups received 100 mg/kg of curcumin and 0.5 ml of corn oil, 200 mg/kg of curcumin and 0.5 ml of corn oil, and 400 mg/kg of curcumin and 0.5 ml corn oil, respectively, administered orally via sonde. Treatment was administered twice a day in each group. On day 11, all groups were observed for a writhing response within 30 minutes of oxytocin injection (28–30). Writhing is a specific pain response observed in models of primary dysmenorrhea (14). The writhing response mainly consists of abdominal wall contraction and pelvic rotation, followed by the stretching of the hind limbs (13,14). The parameter for the writhing response

is writhing number. The writhing number was defined as the number of writhes observed in each group within 30 min after oxytocin injection (31). The writhing responses were observed by at least two research teams and documented using video.

SOD and MDA Test

After observation of the writhing response, blood samples were taken intracardiac using a 3-cc syringe. The samples were transferred into a test tube and centrifuged at 3000 rpm for ten minutes. The resulting serum was placed into an Eppendorf and stored at −40°C. Examination of SOD and MDA levels was performed with the reagents mouse superoxide dismutase, Cu-Zn, SOD, and mouse malondialdehyde MDA ELISA Kit 4.

Statistical Analysis

Data were analyzed using one-way analysis of variance and Tukey's post-hoc tests to determine differences in each group with a significance level of $p \leq 0.05$ (32).

RESULTS

Estradiol Benzoate-induced Dysmenorrhea in Mice

The observation of the writhing response indicated that the administration of estradiol benzoate successfully induced dysmenorrhea in the mice. The value of the writhing response in the control group (Dys) was higher than that in the treatment group. The standard deviation of the writhing response in the Dys group was found to be higher, possibly due to differences in the variability of hormone responses and differences in mice sensitivity to pain (33,34). The results of the analysis test showed a significant difference in the writhing response of all groups compared with the Dys group (Fig. 1).

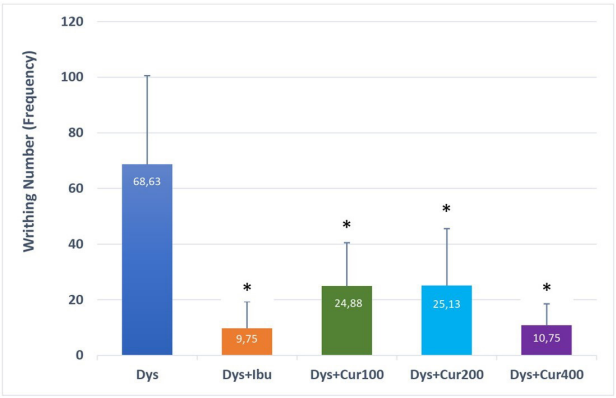

Fig. 1: Estradiol benzoate-induce dysmenorrhea in mice. Data represent the mean ± standard deviation (n = 8). *p < 0.05 vs. Dys. Dys, Dysmenorrhea; Dys+Ibu, Dysmenorrhea + ibuprofen (100 mg/kg); Dys+Cur100, Dysmenorrhea + curcumin (100 mg/kg); Dys+Cur200, Dysmenorrhea + curcumin (200 mg/kg); Dys+Cur400, Dysmenorrhea + curcumin (400 mg/kg).

Effect of Curcumin on SOD Concentration

The highest average SOD concentration was obtained in the Dys+Cur100 group (1.394 ng/mL) and the lowest SOD concentration was obtained in the Dys group (0.954 ng/mL). Significant differences were observed between the SOD concentrations in the Dys+Cur100 vs. Dys groups ($p = 0.010$) (Fig. 2). These results indicate that the administration of 100 mg/kg curcumin twice a day was effective in increasing the SOD concentration in dysmenorrhea model mice.

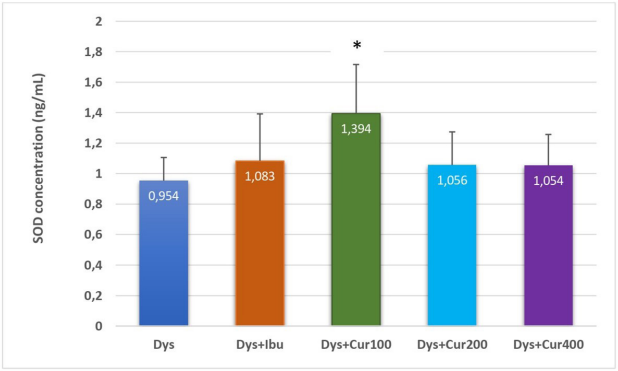

Fig. 2: Effect of curcumin on superoxide dismutase (SOD) concentration in mice with dysmenorrhea. Data represent the mean ± standard deviation (n = 8). *p < 0.05 vs. Dys. Dys, Dysmenorrhea; Dys+Ibu, Dysmenorrhea + ibuprofen (100 mg/kg); Dys+Cur100, Dysmenorrhea + curcumin (100 mg/kg); Dys+Cur200, Dysmenorrhea + curcumin (200 mg/kg); Dys+Cur400, Dysmenorrhea + curcumin (400 mg/kg).

Effect of Curcumin on MDA Concentration

The MDA concentration in the Dys+Ibu group (0.610 nmol/mL) was lower than that in the Dys group (0.777 nmol/mL). The concentration of MDA did not vary between groups ($p > 0.05$). The lowest MDA value was observed in the Dys+Ibu group compared with the other treatment groups (Fig. 3). This indicate that the administration of ibuprofen (100 mg/kg) and curcumin (100, 200, and 400 mg/kg) twice a day did not reduce the MDA concentration in dysmenorrhea model mice. However, the average MDA concentration in the positive control group (Dys+Ibu) given ibuprofen (100 mg/kg) showed the lowest average MDA concentration compared to the negative control group (Dys) and all treatment groups. This shows that the administration of ibuprofen (100 mg/kg) reduced the MDA concentration compared to all other treatment group.

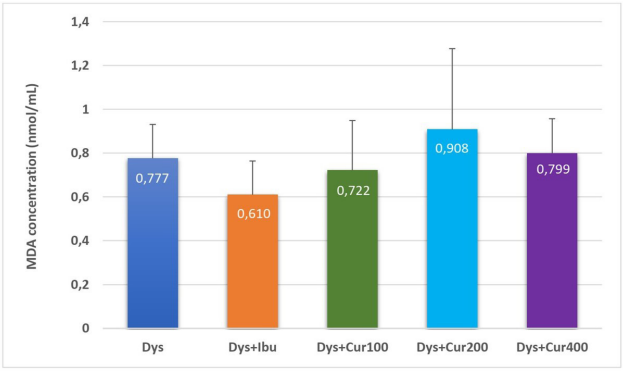

Fig. 3: Effect of curcumin on malondialdehyde (MDA) concentration in mice with dysmenorrhea. Data represent the mean ± standard deviation (n = 8). Dys, Dysmenorrhea; Dys+Ibu, Dysmenorrhea + ibuprofen (100 mg/kg); Dys+Cur100, Dysmenorrhea + curcumin (100 mg/kg); Dys+Cur200, Dysmenorrhea + curcumin (200 mg/kg); Dys+Cur400, Dysmenorrhea + curcumin (400 mg/kg).

DISCUSSION

Primary dysmenorrhea is a condition with a higher prevalence among young women that causes loss of productivity and absenteeism from school, disrupting daily life, especially in the first days of the menstrual cycle (25). The experimental animal model of dysmenorrhea used in this study consisted of prior estrogenization of female mice and the administration of oxytocin to induce a writhing response. This nociceptive behavior was observed up to 30 minutes after oxytocin injection (14). The appearance of a writhing response indicates that the dysmenorrhea model was successfully established (26). In primary dysmenorrhea, there was an increased endometrial secretion, namely PGF2α and leukotriene concentration, during menstruation (35). Cyclooxygenase (COX) is involved in the synthesis of prostaglandin, while COX-2 activation leads to PGF2α production (36). Increased levels of prostaglandins and leukotrienes induce uterine contractions, decrease blood flow to the myometrium resulting in ischemia, and increase peripheral nerve sensitivity, lowering the pain threshold (35). The activation of the COX-2 pathway influences the development of primary dysmenorrhea, increases inflammatory responses, and triggers oxidative stress (36).

The levels of SOD in the mice of the Dys group were found to be the lowest compared to the other treatment groups. The SOD concentration in the Dys group acted as a baseline and reference for other groups. SOD levels are known to decrease in primary dysmenorrhea (16,19). Low SOD levels can increase the risk of oxidative stress due to oxidative radicals in an individual. Superoxide dismutase breaks the chain of oxidative radical reactions to form hydrogen peroxide (H2O2), which triggers the lipid peroxidation of cell membranes (37).

Lipid peroxidation of the cell membrane in turn triggers phospholipase enzyme activity, producing arachidonic acid from damaged membrane phospholipids (15). The activity of the enzyme phospholipase triggers inflammation by producing prostaglandins and leukotrienes (35). Higher activity of lipid peroxidation elevate the oxidative stress marker. The injection estradiol benzoate protocol induce HIF-1α, hypoxia sygnaling pathway. Under hypoxia, HIF-1α inhibit endogenous antioxidant expression (16). Our findings confirmed that the SOD level in the Dys group was the lowest. Low levels of SOD in the Dys group mice failed to prevent the peroxidation of membrane lipids that trigger inflammation. This suggests that the inflammatory activity was highest in mice in the Dys group. Unfortunately, the degree of inflammatory activity could not be experimentally confirmed in this study.

Our findings indicate that the SOD concentration in mice in the Dys+Ibu group with anti-inflammatory (ibuprofen) intervention was higher than those in the Dys control group. In mice with atherosclerosis, ibuprofen inhibits the COX enzyme and inhibits the formation of free radicals during prostaglandin synthesis. It may also be responsible for reduced lipid peroxidation, increased SOD, and catalase concentration (38). This provides a basis for treating inflammation in dysmenorrhea via therapeutic modalities that increase SOD concentration. SOD is an enzyme synthesized via the nuclear factor E2-related factor 2 (Nrf2)-antioxidant reaction elements (ARE) signaling activity. The stimulation of Nrf2-ARE signaling activity enhances genomic responses to the synthesis of endogenous antioxidant molecules, such as SOD, catalase, and glutathione peroxidase (39). Compounds known to stimulate Nrf2-ARE signaling activity include resveratrol in berries, epigallocatechin gallate in green tea, and curcumin in turmeric (40). In the present study, the effect of curcumin on SOD concentration in mice with dysmenorrhea was evaluated.

The results showed that higher SOD concentrations were found in mice treated with curcumin compared with the control group. The highest SOD concentration was observed in mice treated with 100 mg/kg curcumin. However, our findings revealed that any increased in the curcumin concentration that is more than 100 mg/kg may not give any effects in further increasing the SOD concentration. Curcumin is known to increase SOD synthesis via Nrf2-ARE activity, requiring at least 24 hours of intervention before oxidative stress occurs (41). Repeatability of curcumin intervention is necessary to increase the effectiveness of genomic responses on the synthesis of endogenous antioxidant molecules. This protocol is a reference for curcumin interventions in mice models with dysmenorrhea. These results are consistent with those of previous studies, which found that curcumin increases serum SOD and reduces serum lipid peroxide (42). Other studies have also found that the oral administration of 300 mg/kg curcumin per day increased the SOD concentration and decreased the MDA concentration in rats suffering from chronic

aflatoxicosis induced by Aflatoxin B1 (43).

The concentration of MDA in curcumin administered dysmenorrhea model mice did not differ when compared with that of the Dys group. In primary dysmenorrhea, inflammation and endothelial dysfunction is characterized by lipid peroxidation and is an indication of oxidative stress (44). Oxidative stress is an imbalance between the production of free radicals/ROS and antioxidant defenses, thus supporting prooxidants. The main target of ROS is polyunsaturated fatty acids in membrane lipids, resulting in lipid peroxidation, subsequently damaging cell structure and function (37). Lipid peroxidation is identified by elevated plasma MDA levels (45). Excessive ROS defeats the endogenous antioxidant defense system (46), induces lipid peroxidation, and is responsible for damage to DNA and cell membranes (47). Previous studies have reported increased MDA levels in dysmenorrhea (15,18,19).

In this study, curcumin treatment failed to decrease the MDA concentration in mice with primary dysmenorrhea. Curcumin reduced ROS production via nicotinamide oxidase adenine dinucleotide phosphate (NADPH) and increased the levels of antioxidant enzyme activity in association with the Nrf2-Keap1 pathway. The results of a meta-analysis study showed that curcumin intervention was effective in decreasing the MDA concentration and increasing the antioxidant concentration in individuals under oxidative stress conditions. The reduction of oxidative stress with curcumin intervention depends on the dosage and duration of treatment. Piperine with curcumin intervention can increase the potency of curcumin in the antioxidant defense system (48). The results of this study were in line with those of previous studies in which curcuminoid intervention was found to prevent protein oxidation, but did not prevent lipid peroxidation in mice with exercise-induced muscle damage. The concentration of MDA in the muscles of treated mice was no different to that of the control group ($p = 0.092$). This suggests that protein oxidation is more closely related to muscle breakdown mechanisms than lipid peroxidation. The inhibition of protein oxidation is known to be better for preventing muscle breakdown than lipid peroxidation (49).

The mechanism of non-steroidal anti-inflammatory drugs (NSAIDs) action was associated with oxidative stress and inflammation-mediated COX inhibition. The COX pathway is known to be activated by ROS (peroxide and superoxide anion) (37). The inhibition of COX-2 activity indicates a prevention of membrane lipid peroxidation process (50). NSAIDs scavenge free radicals and prevent ROS activity by reducing the levels of oxidative stress. These findings did not align with those of previous studies, including a study in which ibuprofen significantly reduced lipid peroxidation among mice with atherosclerosis who were given ibuprofen (200 mg/kg) per oral with a high-cholesterol diet for six days (38).

Lastly, curcumin treatment was found to improve the endogenous antioxidant defense via SOD concentration, even if it did not alter the oxidative stress marker MDA. Curcumin failed to lower the MDA concentration of the dysmenorrhea mice model. It revealed that curcumin was not as scavenger to induce a direct effect in oxidative stress resilation. Curcumin needs more time to show the antioxidant effect in dysmenorrhea mice model. This represents a limitation of this study, which will need to be verified by further research. Additionally, curcumin treatment was beneficial for elevated antioxidant defense and reduced pain in dysmenorrhea, even though the mechanism behind it remains poorly understood.

CONCLUSION

The antioxidant activity of curcumin was demonstrated to improve the SOD but not MDA levels of dysmenorrhea-induced mice. These findings suggest that curcumin shows potential as a therapeutic approach for the treatment of dysmenorrhea via indirect effects. It is suggested that consumption of curcumin at the early menstrual period may help in reducing the risk of having/developing dysmenorrhea among those who have been experiencing dysmenorrhea during menstruation.

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