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Curcuminoid–Piperine Combination Improves Radical Scavenging Activity in Women with Premenstrual Syndrome and Dysmenorrhea: A Post-hoc Analysis of a Randomized Clinical Study

Samira Karbasi[1], Saman Seyedabadi[1], Samira Mozaffari[1], Zahra Foroutan[2], Gordon A. Ferns[3], Asghar Zarban[1,4,*], and Afsane Bahrami[5,6,*]

[1]Cardiovascular Diseases Research Center, Birjand University of Medical Sciences, Birjand, [2]Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, [3]Division of Medical Education, Brighton & Sussex Medical School, Falmer, Brighton, United Kingdom, [4]Clinical Biochemistry Department, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, [5]Clinical Research Development Unit, Imam Reza Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, [6]Clinical Research Development Unit of Akbar Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Oxidative stress maybe involved in the patho-etiology of menstrual-associated complications. Curcuminoids, are polyphenolic natural compounds that have potentially important functional activities. This triple-blind, randomized, placebo-controlled trial was performed to investigate the effects of a curcuminoids on oxidative stress and antioxidant capacity in girls with premenstrual syndrome (PMS) and dysmenorrhea. Eighty young girls with both PMS and dysmenorrhea were randomly given either curcuminoids (500 mg+5 mg piperine) or a placebo daily, for a period from 7 days pre- until 3 days post- initiation of menstrual bleeding for 3 successive menstrual cycles. The total antioxidant capacity and free radical scavenging activity of serum and urine were quantified via ferric reducing/antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods, respectively. There were no significant differences between the placebo and curcumin groups, with respect to the age, dietary intake and biochemical/anthropometric indices ($p > 0.05$). The curcumin treatment significantly increased the free-radical scavenging activity of serum compared to the treatment with placebo ($p=0.031$). Although, no significant changes were found in serum and urinary levels of FRAP, DPPH and MDA between the groups ($p > 0.05$). Curcumin treatment did increase free-radical scavenging activity and antioxidant potential in girls with PMS and dysmenorrhea. Investigations with higher doses and duration of curcumin are required to verify our findings.

Key Words: *MDA; FRAP; DPPH; Menstruation; Dysmenorrhea*

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Corresponding Author:
Asghar Zarban
Cardiovascular Diseases Research Center, Birjand University of Medical Sciences, Birjand 9717853577, Iran
Tel: +98-5332381569
Fax: +98-56324380076
E-mail: azarban@bums.ac.ir

Afsane Bahrami
Clinical Research Development Unit, Imam Reza Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad 9137913316, Iran
Tel: +98-5137313292
Fax: +98-5138709201
E-mail: BahramiAF@mums.ac.ir

INTRODUCTION

Gynecological complaints such as premenstrual syndrome (PMS) and dysmenorrhea are frequently found among women of childbearing age and adversely affects well-being. PMS refers to wide spectra of recurrent and cyclic somatic, behavioral and mood manifestations that typically happen at the late luteal phase and relief with menstruation. Dysmenorrhea is abdominal bloating or cramping pain encountered pre-menstrually and throughout menstruation.[1] The pathogenesis of PMS and dysmenorrhea is not entirely clear, but inflammation and oxidative stress (OS) have been implicated in the etiology of menstrual associated psychological symptoms.[2]

Free radicals are highly reactive molecules including unpaired electrons which have the potential to damage essential cellular elements. Any physiological imbalance be-

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tween the formation of free radicals and pro-oxidants with the biological antioxidant species causes oxidative stress (OS). These conditions mainly are associated with increased generation of reactive oxygen species (ROS) from molecular oxygen, and eventually cell damage and death. Under normal conditions, the body's antioxidant defense system and enzymes can detoxify the ROS. Excessive ROS production or low neutralization capacity of the endogenous antioxidants, possibly results in OS and the development of many human disorders.[3] OS can influence female fertility by affecting ovulation, fertilization, embryogenesis, and implantation. We recently reported that prooxidant–antioxidant balance (PAB) level, as a reliable marker of OS, was significantly elevated in adolescent girls with PMS and dysmenorrhea versus normal pairs.[4]

First-line traditional treatment for PMS and dysmenorrhea including NSAIDs may be ineffective, not tolerated, or trigger severe adverse reactions, and so worldwide attention has been attracted using complementary and alternative medicine for alleviating menstrual pain. Curcumin (CUR), as natural polyphenolic pigment extracted from underground rhizome of Curcuma longa, is widely used as a medicinal herb. CUR has hydroxyl and methoxy moieties, that can interact with different molecular processes and various enzymes; having pleiotropic biological properties, including antioxidant, anti-inflammation, antibacterial, boosting immune function, and antimutagenic effects.[5]

The antioxidant properties of CUR can neutralize by-products of oxygen metabolism attributed to the some its actions such as mitigation of lipid peroxidation, scavenging of superoxide, hydroxyl and peroxyl radicals, as well as chelating heavy metals.[6] A recent systematic review and meta-analysis concluded that CUR administration significantly improved anti-oxidant capacity by increasing total antioxidant capacity (TAC), decreasing MDA levels, as well as SOD activity.[7] There are several evidences supporting the potential therapeutic potency of CUR on dysmenorrhea and PMS symptoms and severity.[5,8]

The use of redox indicators for the clarification of the mechanisms of beneficial effect of CUR has begun to attract increasing attention. Recently, Silva-Santana reported that 12 weeks of intervention, CUR with piperine was more effective compared to CUR alone to decreasing the plasma levels of MDA.[9] Piperine is a bioactive alkaloid which used a to increase the bioavailability and intestinal absorption of CUR. It has been speculated that CUR in PMS woman can regulate neurotransmitters and biomolecule, antioxidant and anti-nociceptive roles and lowering OS.[10] To the best of our knowledge, the effect of CUR on OS and antioxidant status in PMS and dysmenorrhea patients has not been explored previously. Regarding anti-oxidant characteristics of CUR, the aim of this study was to assess the effects of CUR on the antioxidant properties of serum and urine in healthy girls with PMS and dysmenorrhea. We hypothesized that consuming curcumin with piperine would reduce OS and favorably affect the antioxidant capacity of young women.

METHODS AND MATERIALS

1. Study design

This randomized, triple-blind, placebo-controlled clinical trial was undertaken in 80 healthy young female with PMS and dysmenorrhea recruited from December 1, 2020 through to March 1, 2021. Subjects with PMS and dysmenorrhea were selected from the dormitories of four universities in Birjand, Iran.

The study protocol was approved by our university (code: IR.BUMS.REC.1400.128), and registered at Iranian Registry of Clinical Trial (IRCT20191112045424N1). The current investigation was a sub-study from another research trial.[11] Based on the former study,[12] the sample size was estimated at a power of 80%, α=0.05 and the expectations value of 2.1 mmol/L as the change of serum TAC as the main variable. We determined that 30 participants per group were required as the sample size, and considering 15% drop-out rate, 40 patients per group were required initially.

Women with the following characteristics were included: aged 18-24 years, single, having regular menstrual cycle (length between 21-35 days) and normal bleeding pattern, and having moderate/severe PMS and primary dysmenorrhea. Subjects were omitted if they planned to get married in the near future, or were diagnosed with any history of acute or chronic diseases, were on any medication, or supplement during the trial period.

2. Diagnosis of PMS and dysmenorrhea

Menstrual patterns and cyclicity were assessed using a questionnaire consisting of questions about menarcheal age, menstruation cycle dates and duration of menstruation.[13] The presence and severity of PMS and dysmenorrhea was diagnosed by gynecologist according to PMS screening tool (PSST) and visual analogue scale (VAS). The PSST included 19 items concerning to different premenstrual bothering symptoms, each of which had to be rated with respect to the presence and intensity with scale (0-3) which provides a total point ranging from 0-57. Volunteers who had scores of $\geq$20 from PSST were counted as moderate to severe PMS cases. The severity of dysmenorrhea pain intensity based on VAS scored from 0 (no pain) to 10 (most intense pain).[14] Women who experienced severe dysmenorrhea pain (score $\geq$8) and PMS (PSST $\geq$20) were defined as women having both PMS and dysmenorrhea were eligible to participate in this trial.

3. Intervention

The CUR capsules comoprised 500 mg C3 Complex curcuminoids plus 5 mg piperine (Sami Labs Ltd., Bangalore, India). Placebo capsules contained 500 mg lactose powder +5 mg piperine and were similar with CUR capsules concerning size, shape, color and texture. Subjects received the capsules for ten days per month (from 7 days pre- to three days after menstrual bleeding) for three consecutive menstrual cycles. Compliance and possible adverse events

were probed in all subjects during trial by telephone fallow-up.

Due to the triple-masked design of study, the CUR and placebo capsules (designated as "code A" or "code B") were put into similar unlabeled bottles. A statistician prepared a randomization list by NCSS software through the simple block randomization. The eligible volunteers with an even number on the registration list were allocated to "code A" group; the remaining cases were put into the "code B" group. Coding keys were available to study principal investigator via mail after the final analysis. Researchers, patients, and statistical analysts were blinded to the grouping participants until the analysis of the results.

4. Blood sample collection

Ten mL blood and urine after overnight fasting was gathered at baseline and at the end of trial for each participant. Blood samples were centrifuged at 10,000 g for 15 minute to separate serum. Serum and urine specimens were stored at −70℃ in our laboratory until laboratory measurement. Serum and urinary DPPH, FRAP, MDA indices were measured twice: once three days before to the start of supplementation and once within three days after to the receiving last capsule.

5. Laboratory measurement

1) Total antioxidant capacity (TAC): Serum TAC was measured using the ferric reducing/antioxidant power (FRAP) method described by Benzie and Strain.[15] This procedure is based on the reducing ability of a ferric–tripyridyl triazine (Fe^{3+}-TPTZ) complex to its ferrous (Fe^{2+}) colored appearance in the presence of antioxidant compounds. TAC measure of samples was described in μmolTAC/L. For urine samples, the specimens diluted 1:10 and the findings are demonstrated in μmolTAC/mg creatinine.

2) Free radical scavenging activity: The free radical scavenging activity in samples was assessed via the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method that explained by Janaszewska and Bartosz.[16] The results are reported in mmol trolox equivalent/L. The urine tests were performed in reaction mixes including 250 μL of DPPH reagent and 10 μL urine (the samples diluted 1:10) and blank samples. The results are showed in mmol trolox equivalent/mg creatinine.

3) Malondialdehyde (MDA) assay: The thiobarbituric acid-reactive-substances (TBARS), a lipid peroxidation index, were estimated by the method described by Satoh.[17] The final product of fatty acid peroxidation, malonyldialdehyde (MDA), interacts with TBA to produce a colored complex. The TBARS measure of samples was indicated in μmolTBARs/L. For urine samples the results are presented in μmolTBARs/mg creatinine.

4) Assessment of other variables: The serum levels of fasting blood sugar, urea, creatinine (Cr), uric acid, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol, triglyceride, total cholesterol, alanine transaminase, aspartate transaminase, alkaline phosphatase, total bilirubin, direct bilirubin, albumin, total protein was measured using commercial kits (Pars Azmun, Iran) with auto-analyzer (Prestige 24i, Tokyo Boeki Ltd., Japan).

5) Dietary analysis: The dietary intake of volunteers participating was judged using a three-day recall food method at the first week and last week of the intervention. Diet plan4 software was employed for measuring of daily mean of intake within the trial.

6) Statistical analysis: Normality distribution of variables was verified using Kolmogorov–Smirnov test. The data for continuous variables were expressed as mean±SD. Parameters that did not verify the assumption of normal skewedness were analyzed with non-parametric tests and presented as median and interquartile range. The parameters were compared between the two intervention groups by recruiting independent sample t test or Mann–Whitney. The paired t-test or non-parametric Wilcoxon test for pair values were used to compare the pre- and post-intervention for all variables. The statistical significance of the any independent effects of supplementations on the main variables was detected by an ANCOVA taking the baseline value of each variable as a covariate. Correlation between variables was determined using Pearson or Spearman tests. The point for statistical significance was p-value < 0.05 and SPSS version 16.0 was recruited for statistical analysis.

RESULTS

1. General characteristics

Two hundred individuals were originally approached for this study. One-hundred and twenty persons were omitted from the trial as they failed to meet our criteria or were unwilling to participate. The eighty remaining subjects were randomly allocated to one of the 2 study groups. Of these a total of 77 subjects completed the study (Fig. 1). The baseline features of the participants of the two intervention

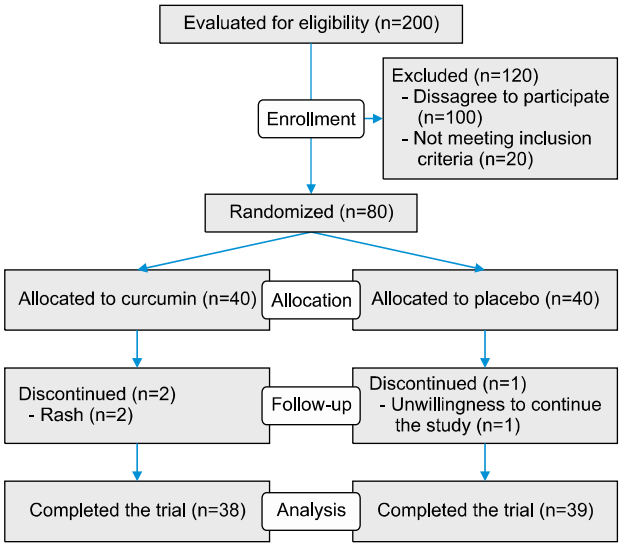

Fig. 1. Flow chart of trial.

arms are presented in Table 1. Two cases did not complete the follow-up in the CUR group due to the rash, and one in the placebo arm because of the personal reason.

2. Dietary intake

Dietary intakes of individuals were assessed before and at the end of the trial. The changes in the intakes of micro- and macro-nutrients at pre and post the supplementation had no significantly differed between the two arms ($p > 0.05$; Table 2).

3. Serum and urinary levels of FRAP, DPPH, and MDA

Baseline and post-trial values for redox biomarker in each study group are presented in Table 3. CUR significantly increased the free radical scavenging activity (DPPH %) of serum in subjects with PMS and dysmenorrhea compared to pairs received placebo (p=0.031). Whereas change in serum FRAP and MDA levels as well as FRAP/Cr, DPPH/Cr and MDA/Cr of urine was not different between subjects treated with CUR or placebo after intervention (Table 3).

4. Correlation between oxidative stress/antioxidant parameters and PSST and VAS scores

A significant decrement was observed in PSST score after supplementation in the CUR group (33.1±9.4 to 20.9±8.8, p<0.001; net changes: −10.6±12.9) and placebo groups (30.0±7.9 to 21.3±8.7, p<0.001; net changes: 9.3±9.5). Moreover, at the end of the trial, VAS scores were significantly lower in both intervention groups (8.3±2.2 to 4.7±2.6 in CUR group, p<0.001; 8.4±2.0 to 5.2±2.5, p<0.001 in placebo group). A significant correlation was found between changes in serum DPPH (△DPPH) level with changes in VAS (△VAS) score only in CUR group (r=−0.25; p=0.042; Table 4).

TABLE 1. Baseline characteristics of studied population

Parameters	Curcumin (n=38)	Placebo (n=39)	p-value*
Age, years	20.6±1.7	21.0±1.8	0.30
BMI, kg/m^2	21.0±2.7	20.7±3.0	0.56
Serum			
HDL-C, mg/dL	49.6±8.8	51.7±8.6	0.15
LDL-C, mg/dL	78.5±27.8	69.4±17.1	0.17
TC, mg/dL	153.5±24.7	149.9±24.2	0.38
TG, mg/dL	77.1±41.8	72.2±31.7	0.42
FBG, mg/dL	84.2±6.6	83.1±6.7	0.35
AST, IU/L	19.7±12.4	20.0±11.9	0.88
ALT, IU/L	16.7±12.2	16.9±11.2	0.82
ALP, U/L	187.2±35.8	190.7±47.4	0.62
T Bili, mg/dL	0.59±0.26	0.68±0.33	0.06
D Bili, mg/dL	0.30±0.14	0.33±0.16	0.05
Urea, mg/L	29.6±8.5	29.8±6.0	0.84
Creat, mg/dL	0.97±0.14	1.0±0.50	0.22
UA, mg/dL	3.4±3.1	3.2±0.76	0.48
Alb, g/dL	5.0±0.26	5.1±0.30	0.38
T protein, g/dL	7.9±0.42	8.0±0.46	0.53

Values are expressed as mean±SD. *Obtained from independent sample t-test. HDL: high-density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, TC: total cholesterol, TG: triglyceride, FBG: fasting blood glucose, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, T Bili: total bilirubin, D Bili: direct bilirubin, Creat: creatinine, UA: uric acid, Alb: albumin, T protein: total protein.

DISCUSSION

Our findings partially support our key hypotheses, that CUR supplementation increase serum DPPH levels in women with PMS and dysmenorrhea. This study is the first that has simultaneously evaluated the impacts of CUR on serum and urinary biomarkers of oxidative stress (MDA levels), and TAC (as measured by FRAP and % reduction of DPPH radical) in a group of young girls with PMS and dysmenorrhea.

Higher values of lipid hydroperoxide and lower TAC concentration have been reported in the patient with PMS compared to controls. In a recent investigation, women with menstrual cycle-associated complications such as PMS and dysmenorrhea compared to normal controls, a statistically significantly increased lipid peroxidation was reported.[18] In another study, women with PMS had a significantly higher urine 8-hydroxy-2-deoxyguanosine as an index of DNA damage marker versus healthy pairs.[19]

Antioxidants can have additive or synergistic affects, so the evaluation of antioxidant capacity may have a greater diagnostic value compared to the measurement of single antioxidants. The total antioxidant potency, known as "total antiradical activity", refers to the power of the biological system to scavenging of oxygen free radicals. Multiple tests have been proposed for quantification of total antioxidant activity. In order to correct measurement of total antioxidant activity, it is suggested to evaluate at least 2 different assays. In the present study, we recruited

TABLE 2. Changes in dietary intake analysis of subjects at baseline and after intervention

	Curcumin (n=38)	Placebo (n=39)	p-value*
Macronutrients (per day)			
Energy (kcal)	−33.1±75.9	10.3±69.8	0.07
Protein (g)	0.9±1.3	0.3±0.8	0.48
Carbohydrate (g)	3.0±9.1	5.9±13.7	0.64
Fat (g)	2.1±3.9	1.9±4.3	0.91
Dietary fiber (g)	−0.9±2.4	0.1±0.5	0.33
Micronutrients (per day)			
Zinc (mg)	−0.8±1.2	−0.6±1.1	0.59
Copper (mg)	−0.01±0.09	0.03±0.1	0.09
Selenium (mg)	2.5±4.6	2.3±3.8	0.83
Vitamin A (RE)	4.3±12.7	3.5±9.4	0.68
Vitamin E (mg)	−1.1±3.7	−2.2±4.9	0.09
Vitamin C (mg)	5.2±9.4	2.5±6.1	0.08

Values are expressed as mean±SD. *Obtained from independent sample t-test or or Mann–Whitney test.

TABLE 3. Comparison of main measures in treatment groups before and after intervention

Variables	Measurement period	Curcumin group (n=38)	Placebo group (n=39)	p[a]
Urinary FRAP (mmolTAC/mgCr)	Before intervention	6.6 (4.7-8.5)	6.9 (5.1-9.4)	0.39
	After intervention	3.7 (2.4-9.3)	4.1 (2.6-7.6)	0.69
	p[b]	0.58	0.25	
Urinary DPPH (mmol trolox equivalent/mg Cr)	Before intervention	1.9 (1.3-2.3)	1.9 (1.4-2.5)	0.63
	After intervention	0.85 (0.43-2.0)	1.0 (0.53-2.1)	0.81
	p[b]	0.34	0.24	
Urinary MDA (μmolTBARs/mg Cr)	Before intervention	0.58 (0.48-0.67)	0.54 (0.43-0.77)	0.96
	After intervention	0.61 (0.38-0.87)	0.61 (0.42-89)	0.49
	p[b]	0.34	0.91	
Serum FRAP (μmolTAC/L)	Before intervention	689.8±109.6	714.7±103.7	0.45
	After intervention	697.2±124.7	706.1±127.1	0.62
	p[b]	0.66	0.60	
Serum DPPH (mmol trolox equivalent/L)	Before intervention	91.5±59.1	98.9±52.3	0.55
	After intervention	121.7±84.7	101.1±69.1	0.031
	p[b]	0.038	0.69	
Serum MDA (mmolTBARs/L)	Before intervention	0.57 (0.44-0.76)	0.67 (0.59-0.90)	0.14
	After intervention	0.57 (0.44-0.70)	0.68 (0.49-0.88)	0.40
	p[b]	0.60	0.76	

Values expressed as mean±SD (parametric variables) or median and interquartile range (non-parametric variables). FRAP: Ferric reducing/antioxidant power, DPPH: 1,1-diphenyl-2-picrylhydrazyl, MDA: malondialdehyde, Cr: creatinine. [a]p-values indicate comparison between groups using independent sample t-test (parametric variables) or Mann–Whitney (non-parametric variables) at baseline and ANCOVA test after trial. [b]p-values indicate comparison within groups by using paired-sample t-test (parametric variables) or Wilcoxon test (non-parametric variables).

TABLE 4. Correlation coefficient between levels of oxidative stress/antioxidant parameters net change with PSST and VAS scores change in curcumin and placebo groups

Variables (net change)	Curcumin		Placebo	
	△PSST r	△VAS r	△PSST r	△VAS r
△ urinary FRAP	−0.15	−0.04	0.04	0.14
△ urinary DPPH	−0.18	0.02	0.02	0.12
△ urinary MDA	−0.19	−0.07	0.08	0.20
△ serum FRAP	−0.14	0.24	0.04	0.03
△ serum DPPH	0.01	−0.25*	0.19	0.26
△ serum MDA	−0.08	−0.03	−0.06	−0.08

*$p < 0.05$.

the most accepted: FRAP and DPPH. These are based on the identical approach; oxidants start a reaction which mainly detected spectrophotometrically. The existence of antioxidants in the specimens postpones the substrate's oxidation, which is in proportion to the antioxidant amount in the biological fluid. Nonetheless, the magnitude of single antioxidants to the total antioxidant value is diverse.[20] Quantification of scavenging function or hydrogen donors with DPPH stable radical is a fast, reliable, easy and most frequent technique to measure the free radical scavenger competency of different agents. DPPH is a free radical which has high stability at room temperature and solubilized in ethanol to yield a purple-colored compound. Decolouration was occurred in the attendance of an antioxidant.

We have for the first time reported that dietary CUR supplementation in patients with PMS and dysmenorrhea increase serum DPPH levels. Increased DPPH concentrations may indicate an elevated potency to eliminate free radicals, and so efficient protection against OS or ROS/RNS overproduction in women with PMS and dysmenorrhea. CUR demonstrates free radical quenching qualities due to the harbor of the phenolic, β-diketone, and the methoxy subsection in its chemical structure.[5] Two previous investigations suggested that the CUR+DPPH reaction mainly occurs via the successive proton loss electron transfer. The proportion of free radical scavenger ability of CUR was estimated to >69% at a concentration 0.1 mM.[21,22]

The reported effects of CUR supplements on oxidant or antioxidant systems have been inconsistent. Most of the evidence has suggested that CUR could enhance antioxidant capacities and mitigate oxidant molecules. This polyphenol has been found to prevent the production of ROS in different cell types such as macrophages and red blood cells.[23] In two randomized clinical trials, supplementation with CUR capsules contained piperine can improve serum TAC, superoxide dismutase activity and MDA concentrations versus the placebo.[24,25]

Contrary to these results, numerous reports support the oxidant effects of CUR. CUR promoted the release of superoxide radical in the extracellular space of BC-8 cells isolated from AK-5 cancer cells.[26] Some reports advocated the dual effects of CUR in the existence of different metals i.e., copper. Furthermore, CUR at low doses can protect against GSH decrement and at higher doses can decrease GSH con-

tents slowly. These findings suggested the both antioxidant and pro-oxidant role of CUR.[27]

In our trial, we did not detect any significant effect of CUR on serum and urine FRAP and MDA levels, indicating that CUR may not have ferric reducing antioxidant power as well as lipid peroxidation effects. In two other study, the PAB levels was not significantly different after 6-8 weeks of CUR+ piperine and phospholipid CUR supplementation among patients with NAFLD and metabolic syndrome, respectively.[28,29] In a meta-analysis including 308 participants supplemented with a mean of 645 miligram/day for average period of 67 days, CUR significantly elevated TAC (pooled standardized mean difference [SMD]=2.7, Z=2.00, p=0.045) and non-significantly decreased MDA level (SMD=−1.6, Z=−1.7; p=0.08).[12] CUR supplementation after 10 days in patients with sepsis significantly improved oxidative stress markers (MDA, catalase, SOD, and TAC) versus to the placebo group, while no considerable effect was found in the glutathione peroxidase concentrations.[30] These discrepancy between results may be due to the different target population and various preparations of CUR.

We also observed that amelioration in pain severity related to dysmenorrhea was significantly correlated with the increment of DPPH levels by the end of trial in group that received CUR. OS has been proposed in the reason of chronic pelvic pain and lower back pain. On the other hand, antioxidants can reduce pain in different situations. Antioxidants when administrated with known with or without analgesics, have been found to reduce the free radical–mediated nociception.[2] Our study indicated that administration of CUR to young women not only increased free radical scavenging potential but also led to decreasing dysmenorrhea pain in these women.

In this current study, piperine was added to promote the bioavailability of CUR. Piperine is an alkaloid which found in black pepper as a member of the piperaceae family. Piperine have anti-inflammatory, antitumor, anti-microbial, and most notably piperine is used as the bioavailability booster. Interestingly, piperine did not present any antioxidant action.[31] Therefore, this could have had not a remarkable effect on our final results.

This study suggests that CUR supplementation, due to its antioxidant properties is one of the well-studied and presents a various beneficial health outcome. However, our findings were not entirely clear following this intervention study (CUR 500 mg per day for three 10-days courses), as antioxidant profiles were not significantly improved in the group of patients with PMS and dysmenorrhea over this short time period. Another limitation was a relatively small sample size. This might have unfavorably affected the statistical power to find significant differences in the evaluated indices.

In conclusion, dietary CUR supplementation is a potential intervention to promote anti-oxidant potential and scavenging ROS in patients with PMS and dysmenorrhea. But our findings indicate that CUR dose and duration that was used in this study was insufficient to show improvements in several indicators of oxidative stress; so, further studies are required in these patients.

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CONFLICT OF INTEREST STATEMENT

None declared.

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