Double-blind, randomized, placebo-controlled pilot study of the phosphodiesterase-3 inhibitor cilostazol as an adjunctive to antidepressants in patients with major depressive disorder

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Aims: Cilostazol (CLS) has shown antidepressant effect in cardiovascular patients, post-stroke depression, and animal models through its neurotrophic and anti-inflammatory activities. Consequently, we aimed to investigate its safety and efficacy in patients with MDD by conducting double-blind, randomized, placebo-controlled pilot study.

Methods: 80 participants with MDD (DSM-IV criteria) and Hamilton Depression Rating Scale (HDRS) score >20 were treated with CLS 50 mg or placebo twice daily plus escitalopram (ESC) 20 mg once daily for six weeks. Patients were evaluated by HDRS scores (weeks 0, 2, 4, and 6). Serum levels of CREB1, BDNF, 5-HT, TNF-α, NF-κB, and FAM19A5 were assessed pre- and post-treatment.

Results: Co-administration of CLS had markedly decreased HDRS score at all-time points compared to the placebo group (p < 0.001). Early improvement, response, and remission rates after 6 weeks were significantly higher in the CLS group (90%, 80%) than in the placebo group (25%, 65%, 50% respectively) (p < 0.001). Moreover, the CLS group was superior to the placebo group in modulation of the measured neurotrophic and inflammatory biomarkers.

Conclusion: CLS is safe and effective short-term adjunctive therapy in patients with MDD with no other comorbid conditions.

Trial registration ID:NCT04069819.

Keywords
adjunctive therapy, cilostazol, CREB/BDNF, inflammatory markers, major depressive disorder

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1 | INTRODUCTION

Major depressive disorder (MDD) is a common mental disorder with serious socioeconomic consequences on daily life and health care costs. Although the advent of newer monoamine pathways targeted antidepressants, nearly fifty percent of patients have no response to the first-line antidepressant therapy. Therefore, augmentation strategy using agents with novel mechanism of action and therapeutic targets at the start of the therapy could provide additional therapeutic benefits for patients with MDD.

Several studies have shown the importance of cyclic adenosine monophosphate (cAMP) cascade in MDD. It has been noted that the cAMP is downregulated in MDD and upregulated by antidepressant. Medications that increase the expression level of cAMP could activate the transcription of cAMP response element-binding protein (CREB). As a result, activation of CREB increases the expression of brain-derived neurotrophic factor (BDNF), which has an important function in neuronal development and synaptic plasticity. Several findings have demonstrated that BDNF may be involved in the antidepressants therapeutic action.

On the other hand, cumulative evidence has shown that an increased inflammatory response of the central nervous system (CNS) plays a critical role in MDD pathophysiology. Pro-inflammatory cytokines such as interleukin-1beta (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) are elevated in patients with MDD and decreased after therapy. Furthermore, these cytokines can disrupt the synthesis of 5-hydroxytryptamin (5-HT) and glutamatergic transmission, which are profoundly implicated in the pathophysiology of MDD. FAM19A5 is a novel peptide-like chemokine that is highly expressed in the brain and developed during neurogenesis. Increased serum level of FAM19A5 has been linked to neuroinflammation and neurodegeneration in patients with MDD.

Cilostazol (CLS), a selective phosphodiesterase-3 (PDE-3) inhibitor, acts as an antiplatelet agent with neurotrophic and anti-inflammatory properties. It has strong pleiotropic effects by restoring cAMP/CREB signaling and stimulating BDNF gene expression. It showed antidepressant action in post-stroke depression and in animal models through inhibition of neurodegeneration and promotion of neurogenesis. Besides CLS can overcome the inflammation-based hypothesis for the development of MDD by its ability to suppress TNF-mediated nuclear factor kappa B (NF-κB) and the release of cytokines. These findings suggest it to be beneficial adjunctive therapy for patients with MDD.

In this trial, we supposed that CLS could exert antidepressant effect in patients with MDD. Therefore, we aimed to investigate its safety and efficacy in the treatment of patients with MDD with no other comorbid conditions by conducting double-blind, randomized, placebo-controlled pilot study of CLS as adjunctive agent. Furthermore, we aimed to evaluate its neurotrophic and anti-inflammatory activities in those patients.
enclosed opaque packets. Placebo tablets were supplied by Sigma Pharmaceutical Company, Menoufia, Egypt, and they were indistinguishable from CLS in their size, color, and shape. The patients, the physician who referred the patient, care provider, the statistician, and the assessor were all blinded to treatment allocation. Patients were excluded from the trial if they missed their trial medication for a week.

2.5 | Intervention

Patients were randomly assigned to receive either CLS 50 mg tablet twice daily or placebo tablets in the same way adjunctive to ESC 20 mg once daily for six consecutive weeks. The medications were distributed by the pharmacy, and the returned pills were counted.

2.6 | Outcomes

The primary outcome was the 17-items HDRS score was recorded at the baseline and after 2, 4, and 6 weeks from the starting the medications. Early improvement was defined as 20% reduction in HDRS total score in the first 2 weeks, response rate (≥50% decrease in the HDRS total score), and remission rate (HDRS total score ≤7). Moreover, a checklist was used to monitor the adverse drug reactions and medication adherence. The patients were followed up weekly by phone to assess their compliance with the medications in addition to counting the remaining pills. The secondary outcome measurements were the serum levels of CREB1, BDNF, 5-HT (5-hydroxytryptamine), TNF-κB, and FAM19A5 that measured pre-and post-treatment to assess the biological effect of the used drugs.

2.7 | Measurements

Morning blood samples (5 ml) were drawn from all patients by venepuncture in plain vacutainers at the same time (8:00 a.m.) following an eight-hour fast. The vacutainers were then centrifuged at 4500×g for 15 min to obtain separated serum samples, which were transferred to Eppendorf tubes and maintained in a deep freezer at −80°C until analysis. Serum levels of CREB1, BDNF, 5-HT, TNF-κB, and FAM19A5 were measured using specific commercial ELISA kits purchased from MyBioSource, Inc. (USA). The measurements were carried out according to the manufacturers’ instructions using Biotek ELx800 UV-Vis Microplate Reader (USA).

2.8 | Statistical analysis

IBM® SPSS® Statistics version 22 software (IBM Corp., Armonk, NY, USA) was used to analyze the data. Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were expressed as number (percentage). For continuous variables, the Student’s t test was used for independent samples, and if the t-tests’ normality assumptions are not met, the Wilcoxon rank sum test was used. The Shapiro–Wilk and Kolmogorov–Smirnov tests were used to assess the assumption of normality. For categorical data, the chi-square test or Fisher’s exact test was used as appropriate. Treatment efficacy tests were done at two-sided significance level of 0.05. Mixed-effects model repeated measures (MMRM) analysis of covariance (ANCOVA) was used for comparing the end-point score in HDRS total score. The change between the two groups in HDRS score was compared using two-way analysis of variance (ANOVA) with-repeated measures (group as inter-subject factor) with four measurements as within-subject factor. Bonferroni correction was done for multiple comparisons. Moreover, ANCOVA was done to compare the change in the biomarkers after six weeks in the two groups. Graphs were performed using GraphPad Prism 6.01 software (GraphPad Software, La Jolla CA, USA).

3 | RESULTS

Figure 1 shows that after screening 140 patients for selection criteria, 60 patients were excluded from the study because they had another active medical problem or refused to participate in the study. Eighty eligible patients were assigned to either CLS plus ESC (n = 40) or placebo plus ESC (n = 40). Table 1 shows the demographic and baseline data of the patients. Two weeks after the beginning of the study, eight patients dropped out due to non-compliance with the procedures; four were in the placebo group and four were in the CLS group. These dropped subjects were included in the HDRS analysis but were omitted from the biomarker analysis. The HDRS baseline scores between the two groups were not statistically different (mean ± SD for placebo was 22.6 ± 2.6, for CLS was 22.9 ± 2.3, $t = 0.289$, df = 78, $p = 0.387$).

3.1 | Effect on HDRS Score (primary outcome)

The MMRM analysis showed a statistically significant decrease in the HDRS total score in CLS group compared to the placebo group after 2, 4, and 6 weeks of the treatment (least squares mean difference [LSMD] = 2.83, $p = 0.001$), [LSMD] = 3.74, $p = 0.001$), ([LSMD] = 3.99, $p = 0.001$), respectively as shown in Figure 2. The two-factor ANOVA analysis showed that the difference between the two treatments was statistically significant, as indicated by the effect of group; the inter-subject factor (F(1, 70) = 60.67, $p = 0.02$, $\eta^2 = 0.64$). The difference between the two treatments was significant as indicated by the effect of groups-by-time interaction (F(3, 210) = 56.89, $p = 0.000$, $\eta^2 = 0.448$). The early improvement was a statistically significant higher in the CLS group than the placebo group (90% in the CLS group vs 25% in the placebo group, $p < 0.001$). The CLS group also showed statistically significant improved response to the treatment by the fourth and sixth week. The response rate for CLS group was 90% vs 65% for placebo group after six weeks.
3.2 | Effect on neurotrophic and inflammatory biomarkers (secondary outcome)

The difference between the CLS and the placebo groups, in the baseline serum levels of CREB1, BDNF, 5-HT, TNF-α, NF-κB, and FAM19A5, was statistically nonsignificant (Table 3). Using ANCOVA after six weeks of treatment, the CLS group showed a statistically substantial increase in the serum levels of CREB1, BDNF, and 5-HT compared with the placebo group ((F (1, 70) = 79.43, p = 0.001, η² = 0.531), (F (1, 70) = 69.3, p = 0.004, η² = 0.497), and (F (1, 70) = 67.3, p = 0.001, η² = 0.49), respectively). On the other hand, the serum levels of TNF-α, NF-κB, and FAM19A5 were statistically significant lower in the CLS group compared to the placebo group ((F (1, 70) = 118.19, p = 0.002, η² = 0.629), (F (1, 70) = 108.86, p = 0.003, η² = 0.608), and (F (1, 70) = 99.34, p = 0.001, η² = 0.586), respectively).

After six weeks of treatment, CREB1, BDNF, and 5-HT serum levels were statistically significant higher in comparison with their baseline data as reflected by the effect of groups-time interaction ((F (1, 70) = 55.67, p = 0.001, η² = 0.442), (F (1, 70) = 65.67, p = 0.001, η² = 0.484), and (F (1, 70) = 70.45, p = 0.001, η² = 0.501), respectively).

3.3 | Clinical side effects

The difference between the CLS and the placebo groups in terms of the frequency of the side effects was statistically nonsignificant (Table 4). Over the period of the trial, 15 side effects were recorded; the most common of which was headache (20% placebo, 22.5% CLS). The other reported side effects were transient and spontaneously resolved.

4 | DISCUSSION

The previously published human studies regarding the antidepressant effect of CLS have been involved patients with cardiovascular diseases associated with MDD or post-stroke depression. Therefore, and to our knowledge, this study is the first double-blind, randomized, and placebo-controlled pilot trial that evaluates the adjunctive role of CLS in the management of patients with MDD with no other comorbid conditions.

It has been reported that using combined medications at the beginning of the treatment of MDD patients may provide additional...
therapeutic benefits as ~50% of the patients do not respond to the first-line antidepressants.\textsuperscript{2,3} Although study designs make direct comparison a difficult issue, the response rate of 90% in the CLS combination group is consistent to that of previous studies (89%–92%) including simvastatin,\textsuperscript{31} metformin,\textsuperscript{32} or pentoxifylline\textsuperscript{12} as adjuvant therapies in MDD patients over 6 and 12 weeks. Furthermore, the remission rate of 80% in our study is also consistent with the 59%–85% remission rates in the above-mentioned trials.\textsuperscript{12,31,32} Moreover, the rapid reduction in the HDRS score in the first two weeks in the CLS group is consistent to that of previous studies (89%–92%).\textsuperscript{12,31,32} Regarding the response and remission rates in the placebo group, which were 65% and 50%, they were also comparable to the previously reported response and remission rates for monotherapy in two published studies, which were 58%–76%, and 27%–64%, respectively.\textsuperscript{12,34,35}

Several evidences indicated a correlation between MDD and both PDEs and inflammatory pathways.\textsuperscript{11,36} Thus, higher response and remission rates in the CLS combination group could be related to its neurotrophic and antiinflammatory actions, which resulted in a significant increase in the CREB1, BDNF, and 5-HT serum levels along with a significant decrease in the TNF-α, NF-κB, and FAM19A5 serum levels.\textsuperscript{20,22,29,37,38} CLS could improve brain plasticity by modulating the levels of neurotrophic factor, like BDNF, via CREB activation as reported in preclinical and clinical studies.\textsuperscript{22,29,39} Different clinical studies have showed that BDNF could mediate the antidepressants’ therapeutic activities by enhancing the neuronal plasticity as MDD patients have a decreased level of BDNF, which was restored to the normal levels by the antidepressant therapy.\textsuperscript{9,10}

### TABLE 1 Demographic and baseline characteristics of the participants

| Characteristic                | Placebo group (n = 40) | Cilostazol group (n = 40) | Statistical value |
|------------------------------|------------------------|---------------------------|-------------------|
| Age (years)                  | 38.1 ± 10.02           | 37.05 ± 9.4               | t = 0.456, df = 78, p = 0.324 |
| Gender                       |                        |                           |                   |
| Male                         | 9 (22.5%)              | 10 (25%)                  | X^2 = 0.209, df = 1, p = 0.647 |
| Female                       | 31 (77.5%)             | 30 (75%)                  | X^2 = 0.209, df = 1, p = 0.647 |
| Smoking                      | 12 (30%)               | 10 (25%)                  | X^2 = 0.044, df = 1, p = 0.833 |
| Weight (kg)                  | 76.93 ± 8.67           | 77.88 ± 8.56              | t = 0.567, df = 78, p = 0.286 |
| Height (cm)                  | 173.38 ± 10.96         | 172.68 ± 10.84            | t = 0.433, df = 78, p = 0.333 |
| BMI (kg/m\(^2\))             | 23.74 ± 1.67           | 23.18 ± 1.57              | t = 1.12, df = 78, p = 0.133 |
| Marital status               |                        |                           |                   |
| Single                       | 15 (37.5%)             | 13 (32.5%)                | X^2 = 0.334, df = 1, p = 0.563 |
| Married                      | 15 (37.5%)             | 18 (45%)                  | X^2 = 0.334, df = 1, p = 0.563 |
| Divorced                     | 10 (25%)               | 11 (27.5%)                | X^2 = 0.334, df = 1, p = 0.563 |
| HDRS score                   | 22.6 ± 2.6             | 22.9 ± 2.3                | t = 0.289, df = 78, p = 0.387 |
| Prothrombin time (second)    | 12 ± 0.7               | 11 ± 0.87                 | t = 0.239, df = 78, p = 0.406 |
| Episodes of depression       |                        |                           |                   |
| First                        | 30 (75%)               | 31 (77.5%)                | X^2 = 0.209, df = 1, p = 0.647 |
| Second                       | 10 (25%)               | 9 (22.5%)                 | X^2 = 0.209, df = 1, p = 0.647 |
| Drugs used in last episode   |                        |                           |                   |
| Paroxetine                   | 3 (7.5%)               | 1 (2.5%)                  | X^2 = 0.201, df = 1, p = 0.653 |
| Fluoxetine                   | 3 (7.5%)               | 1 (2.5%)                  | X^2 = 0.201, df = 1, p = 0.643 |
| Escitalopram                 | 4 (10%)                | 4 (10%)                   | X^2 = 0.201, df = 1, p = 0.653 |

Notes: Data presented as mean ± SD. Chi-square test was used for categorical data and student t-test was used for continuous data.

Abbreviations: BMI, body mass index; HDRS score, Hamilton Depression Rating Scale score.

**FIGURE 2** Baseline-to-Endpoint Changes in Hamilton Depression Rating Scale (HDRS) Total Score. Data presented as mean ± SD. Mixed-effects model repeated measures (MMRM) analysis of covariance (ANCOVA) was used for comparing the end-point score in HDRS total score. HDRS, Hamilton Depression Rating Scale (HDRS)
The high serum level of FAM19A5 in MDD has been reported, which reflects the activation of neuroinflammatory processes and increased production of pro-inflammatory cytokines such as TNF-α, IL-6, or IL-1β. Moreover, preclinical studies have shown that these cytokines could activate the N-methyl-D-aspartate receptor (NMDA) receptors, thus increasing excitotoxicity and reducing neurogenesis as well as the BDNF. Our findings were in agreement with other studies, which have shown that CLS directly inhibits...
cytokines’ expression triggered by NF-κB activation.\textsuperscript{24,38,42} As a consequence, the reduced level of the pro-inflammatory cytokines resulted in an increased bioavailability of 5-HT through regulation of its metabolic pathways.\textsuperscript{44,45}

Furthermore, ESC monotherapy could increase the levels of CERB/BDNF as reported in preclinical and clinical studies.\textsuperscript{36–48} ESC also has antiinflammatory effect, which mediated by reducing the pro-inflammatory cytokines.\textsuperscript{49} These changes were reflected in a high response rate to ESC, which is consistent with previous studies indicated that ESC was effective in reducing depressive symptoms when compared to placebo.\textsuperscript{50,51}

The improved antidepressant effect in the combination group could be attributed to the addition of CLS. Therefore, our study suggests CLS as an effective and safe adjunct to ESC in patients with MDD and provided considerable proof for its efficacy in patients with MDD without other comorbid conditions. This notion was particularly reinforced by previous study, which recommended CLS as an alternative to milnacipran for the treatment of patients with post-stroke depression as it led to a decrease in HDRS after switching from milnacipran to CLS (100 mg/day).\textsuperscript{29} In addition, CLS has been reported to have potential efficacy in geriatric MDD patients with cerebrovascular problems.\textsuperscript{52} These outcomes suggested CLS as a preferred drug for treatment of mild to moderate depression in cardiovascular patients who undergoing angioplasty and requiring adjuvant antplatelet therapy.\textsuperscript{30} In addition, it is consistent with the results of preclinical studies, which indicate that CLS produced antidepressant-like activities when given either alone or in combination with other psychotropic agents.\textsuperscript{23,39}

Despite the promising results regarding the use of CLS in the management of MDD, it is still early to be considered as a primary treatment for MDD due to some study limitations including the short follow-up period and the small sample size. Moreover, measurement of corticosterone level is recommended to evaluate the alteration of hypothalamic-pituitary-gonadal (HPA)-axis dysregulation as both neuroinflammation and neurotrophic activities are directly mediated by HPA-axis. Therefore, more comprehensive, larger scale, multicenter, and longer duration clinical studies are required to confirm the efficacy of CLS in the treatment of MDD.

5 | CONCLUSION

Co-administration of CLS, a selective phosphodiesterase-3 inhibitor, with ESC to patients with MDD enhanced the antidepressant therapeutic effects through its neurotrophic and anti-inflammatory properties. This was reflected clinically by early improvement, better response, and higher remission rate. In addition, the detection of biological markers including CREB/BDNF and FAM19A5 may be clinically useful for the assessment of antidepressant response. Theses outcomes suggest CLS to be promising adjunctive candidate to antidepressants, but further investigations with larger sample size and longer follow-up durations are recommended to overcome the limitations of this study.

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CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES
1. Otte C, Gold SM, Penninx BW, et al. Major depressive disorder. Nat Rev Dis Primers. 2016;2(1):16065.
2. Garcia-Toro M, Medina E, Galan JL, Gonzalez MA, Maurino J. Treatment patterns in major depressive disorder after an inadequate response to first-line antidepressant treatment. BMC Psychiatry. 2012;12(1):1-6.
3. Blier P. Rational site-directed pharmacotherapy for major depressive disorder. Int J Neuropsychopharmacol. 2014;17(7):997-1008.
4. Reierson GW, Guo S, Mastronardi C, Licinio J, Wong ML. cGMP Signaling, phosphodiesterases and major depressive disorder. Curr Neuropharmacol. 2011;9(4):715-727.
5. Fujita M, Richards EM, Nicu MJ, et al. cAMP signaling in brain is decreased in unmedicated depressed patients and increased by treatment with a selective serotonin reuptake inhibitor. Mol Psychiatry. 2017;22(5):754-759.
6. Lee JH, Kim KY, Lee YK, et al. Cilostazol prevents focal cerebral ischemic injury by enhancing casein kinase 2 phosphorylation and suppression of phosphatase and tensin homolog deleted from chromosome 10 phosphorylation in rats. J Pharmacol Exp Ther. 2004;308(3):896-903.
7. Li YJ, Xu M, Gao ZH, et al. Alterations of serum levels of BDNF-related miRNAs in patients with depression. PLoS One. 2013;8(5):e63648.
8. Miyamoto N, Tanaka R, Zhang N, et al. Crucial role for Ser133-phosphorylated form of cyclic AMP-responsive element binding protein signaling in the differentiation and survival of neural progenitors under chronic cerebral hypoperfusion. Neuroscience. 2009;162(2):525-536.
9. Martocchia A, Curto M, Scaccianoce S, et al. Effects of escitalopram on serum BDNF levels in elderly patients with depression: a preliminary report. Aging Clin Exp Res. 2014;26(4):461-464.
10. Haile CN, Murrough JW, Iosifescu DV, et al. Plasma brain derived neurotrophic factor (BDNF) and response to ketamine in treatment-resistant depression. Int J Neuropsychopharmacol. 2014;17(2):331-336.
11. Leonard BE. Inflammation and depression: A causal or incidental link to the pathophysiology? Acta Neuropsychiatr. 2017;30(1):1-16.
12. El-Haggar SM, Eissa MA, Mostafa TM, El-Attar KS, Abdallah MS. The phosphodiesterase inhibitor pentoxifylline as a novel adjunct to antidepressants in major depressive disorder patients: a proof-of-concept, randomized, double-blind, placebo-controlled trial. Psychother Psychosom. 2018;87(6):331-339.
13. Zou W, Feng R, Yang Y. Changes in the serum levels of inflammatory cytokines in antidepressant drug-naive patients with major depression. PLoS One. 2018;13(6):e0197267.

14. Liu JJ, Wei YB, Strawbridge R, et al. Peripheral cytokine levels and response to antidepressant treatment in depression: a systematic review and meta-analysis. Mol Psychiatry. 2019;25(2):339-350.

15. Dantzner R. Role of the kynurenine metabolism pathway in inflammation-induced depression: preclinical approaches. In: Dantzner R, Capuron L, eds. Inflammation-associated depression: evidence, mechanisms and implications. Springer International Publishing; 2017:117-138.

16. Haroon E, Miller AH. Inflammation effects on brain glutamate in depression: mechanistic considerations and treatment implications. In: Dantzner R, Capuron L, eds. Inflammation-associated depression: evidence, mechanisms and implications. Springer International Publishing; 2017:173-198.

17. Shahapal A, Cho EB, Yong HJ, et al. FAM19A5 expression during embryogenesis and in the adult traumatic brain of FAM19A5-LacZ Knock-in Mice. Front Neurosci. 2019;13(917).

18. Han K-M, Tae W-S, Kim A, et al. Serum FAM19A5 levels: A novel biomarker for neuroinflammation and neurodegeneration in major depressive disorder. Brain Behav Immun. 2020;87:852-859.

19. Oyama N, Yagita Y, Kawamura M, et al. Cilostazol, not aspirin, reduces ischemic brain injury via endothelial protection in spontaneously hypertensive rats. Stroke. 2011;42(2):2571-2577.

20. Lee HR, Jo MK, Park KY, Jang YJ, Heo TH. Anti-TNF effect of combined pravastatin and cilostazol treatment in an in vivo mouse model. Immunopharmacol Immunotoxicol. 2019;41(2):179-184.

21. Kwon KJ, Lee EJ, Kim MK, et al. Diabetes augments cognitive dysfunction in chronic cerebral hyperperfusion by increasing neuronal cell death: implication of cilostazol for diabetes mellitus-induced dementia. Neurol Abid. 2015;73:12-23.

22. Kim YR, Kim GN, Hong KW, Shin HK, Choi BT. Anti-depressant effects of phosphodiesterase 3 inhibitor cilostazol in chronic mild stress-treated mice after ischemic stroke. Psychopharmacology. 2016;233(6):1055-1066.

23. Patel DS, Anand IS, Bhatt PA. Evaluation of antidepressant and anxiolytic activity of phosphodiesterase 3 inhibitor - cilostazol. Indian J Psychol Med. 2012;34(2):124-128.

24. Park WS, Jung WK, Lee DY, et al. Cilostazol protects mice against endotoxin shock and attenuates LPS-induced cytokine expression in RAW 264.7 macrophages via MAPK inhibition and NF-κB inactivation: not involved in cAMP mechanisms. Int Immunopharmacol. 2010;10(9):1077-1085.

25. Sheehan DV, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998;59(Suppl 20):22-33;quiz 34-57.

26. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (4th ed.). 5th ed. American Psychiatric Association; 2000.

27. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry. 1960;23:56-62.

28. Teare MD, Dimairo M, Shephard N, Hayman A, Whitehead A, Walters SJ. Sample size requirements to estimate key design parameters from external pilot randomised controlled trials: a simulation study. Trials. 2014;15(1):264.

29. Nishimura K, Ishigooka J, Imamura Y, Ibara S. Cilostazol, a cAMP phosphodiesterase 3 inhibitor, in the treatment of poststroke depression. J Neuropsychiatry Clin Neurosci. 2007;19(4):471-472.

30. Bhatt P, Patel D, Anand I, Shah U, Patel S. Antidepressant activity of phosphodiesterase 3 inhibitor: cilostazol. Hypertension. 2011;10:45.

31. Gougou A, Zareh-Mohammadi N, Raheb S, et al. Simvastatin as an adjuvant therapy to fluoxetine in patients with moderate to severe major depression: A double-blind placebo-controlled trial. J Psychopharmacol. 2015;29(5):575-581.

32. Abdallah MS, Mosalam EM, Zidan A-AA, et al. The antidiabetic metformin as an adjunct to antidepressants in patients with major depressive disorder: a proof-of-concept, randomized, double-blind, placebo-controlled trial. Neurotherapeutics. 2020;17(4):1897-1906.

33. Castillo MFR, Murata S, Schwarz M, et al. Celecoxib augmentation of escitalopram in treatment-resistant bipolar depression and the effects on Quinolinic Acid. Neurology, Psychiatry and Brain Research. 2019;32:22-29.

34. Li G, Shen Y, Luo J, Li H. Efficacy of escitalopram monotherapy in the treatment of major depressive disorder: A pooled analysis of 4 Chinese clinical trials. Medicine. 2017;96(39):e8142.

35. Kennedy SH, Andersen HF, Lam RW. Efficacy of escitalopram in the treatment of major depressive disorder compared with conventional selective serotonin reuptake inhibitors and venlafaxine XR: a meta-analysis. J Psychiatry Neurosci. 2006;31(2):122-131.

36. Esposito K, Reierison GW, Luo HR, Wu GS, Licinio J, Wong ML. Phosphodiesterase genes and antidepressant treatment response: a review. Ann Med. 2009;41(3):177-185.

37. Xiao L, O’Callaghan JP, O’Donnell JM. Effects of repeated treatment with phosphodiesterase-4 inhibitors on cAMP signaling, hippocampal cell proliferation, and behavior in the forced-swim test. J Pharmacol Exp Ther. 2011;338(2):641-647.

38. Sakamoto T, Ohashi W, Tomita K, Hattori K, Matsuda N, Hattori Y. Anti-inflammatory properties of cilostazol: Its interruption of DNA binding activity of NF-κB from the Toll-like receptor signaling pathways. Int Immunopharmacol. 2018;62:120-131.

39. Kim YK, Amidfar M, Won E. A review on inflammatory cytokine-induced alterations of the brain as potential neural biomarkers in post-traumatic stress disorder. Prog Neuropsychopharmacol Biol Psychiatry. 2019:91:103-112.

40. Haroon E, Miller AH, Sanacora G. Inflammation, glutamate, and glia: a trio of trouble in mood disorders. Neuropsychopharmacology. 2017;42(1):193-215.

41. da Motta NA, de Brito FC. Cilostazol exerts antiplatelet and anti-inflammatory effects through AMPK activation and NF-κB inhibition on hypercholesterolemic rats. Fundam Clin Pharmacol. 2016;30(4):327-337.

42. Felger JC, Lotrich FE. Inflammatory cytokines in depression: Neurobiological mechanisms and therapeutic implications. Neuroscience. 2013;246:199-229.

43. Baumeister D, Russell A, Pariente CM, Mondelli V. Inflammatory biomarker profiles of mental disorders and their relation to clinical, social and lifestyle factors. Soc Psychiatry Psychiatr Epidemiol. 2014;49(6):841-849.

44. Wolkowitz OM, Wolf J, Shelly W, et al. Serum BDNF levels before treatment predict SSRI response in depression. Prog Neuropsychopharmacol Biol Psychiatry. 2011;35(7):1623-1630.

45. Alboni S, Benatti C, Capone G, et al. Time-dependent effects of escitalopram on brain-derived neurotrophic factor (BDNF) and neuroplasticity related targets in the central nervous system of rats. Eur J Pharmacol. 2010;643(2):180-187.

46. Ibrahim WW, Abdelkader NF, Ismail HM, Khattab MM. Escitalopram ameliorates cognitive impairment in D-galactose-injected ovariec-tomized rats: modulation of JNK, GSK-3β, and ERK signalling pathways. Sci Rep. 2019;9(1):10056.

47. Abdo SA, Wadie W, Abdelsalam RM, Khattab MM. Potential anti-inflammatory effect of escitalopram in lidoacetamide-induced colitis in depressed ovariec-tomized rats: role of α7-nAChR. Inflammation. 2019;42(6):2056-2064.
50. Kirino E. Escitalopram for the management of major depressive disorder: A review of its efficacy, safety, and patient acceptability. Patient Prefer Adherence. 2012;6:853-861.

51. Gupta BM, Zargar SH, Arora M, Tandon VR. Efficacy and safety of escitalopram versus desvenlafaxine in the treatment of major depression: A preliminary 1-year prospective randomized open label comparative trial. Perspect Clin Res. 2016;7(1):45-50.

52. Takahashi K, Oshima A, Inoue K, Takeyoshi H, Fukuda M, Mikuni M. Novel augmentation therapy with cilostazol for the geriatric major depressive disorder patient with deep white matter hyperintensities on T2-weighted brain MRI: a case report. Pharmacopsychiatry. 2008;41(1):37-39.