

# Pentoxifylline as a Novel Add-on Therapy for Major Depressive Disorder in Adult Patients: A Randomized, Double-Blind, Placebo-Controlled Trial

## Authors

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## ABSTRACT

**Background** Evidence indicates an association between immune dysregulation and major depressive disorder (MDD). Pentoxifylline (PTX), a phosphodiesterase inhibitor, has been shown to reduce pro-inflammatory activities. The aim of this study was to evaluate changes in depressive symptoms and pro-inflammatory markers after administration of PTX as an adjunctive agent to citalopram in patients with MDD.

**Methods** One hundred patients were randomly assigned to either citalopram (20 mg/day) plus placebo (twice daily) (n = 50) or citalopram (20 mg/day) plus PTX (400 mg) (twice daily) (n = 50). The Hamilton Depression Rating Scale-17 (HAM-D-17) scores at baseline, weeks 2, 4, 6, 8, 10, and 12 and serum levels of interleukin-1-β (IL-1-β), tumor necrosis factor-α, C-reactive protein, IL-6, serotonin, IL-10, and brain-derived neurotrophic factor (BDNF) at baseline and week 12 were evaluated.

**Results** HAM-D-17 score in the PTX group significantly reduced in comparison to the control group after weeks 4, 6, 8, 10, and 12 ((LSMD): -2.193, p = 0.021; -2.597, p = 0.036; -2.916, p = 0.019; -4.336, p = 0.005; and -4.087, p = 0.008, respectively). Patients who received PTX had a better response (83%) and remission rate (79%) compared to the placebo group (49% and 40%, p = 0.006 and p = 0.01, respectively). Moreover, the reduction in serum concentrations of pro-inflammatory factors and increase in serotonin and BDNF in the PTX group was significantly greater than in the placebo group (p < 0.001).

**Conclusion** These findings support the safety and efficacy of PTX as an adjunctive antidepressant agent with anti-inflammatory effects in patients with MDD.

## Introduction

In 2008, the World Health Organization rated major depressive disorder (MDD) as one of the primary causes of disease burden, and it is suggested that it will rise to the first cause of disease burden by 2030 [1]. MDD is a debilitating mental disorder that is more prevalent in women than in men [1, 2]. Depressed mood, low self-esteem, sleep disturbances (insomnia or hypersomnia), changes in appetite, feelings of guilt, social isolation, anhedonia, and suicidal thoughts are among the symptoms that patients may experience [3–5].

The three most common therapeutic approaches for MDD are pharmacotherapy, psychotherapy, and electroconvulsive therapy. The primary course of treatment for patients with MDD comprises antidepressants that mainly target the monoaminergic system. However, one-third of patients fail to recover [6]. Furthermore, many patients experience unpleasant side effects, which contribute to poor treatment compliance [7–9]. These limitations highlight the need for safe, novel adjunctive treatments for MDD.

Recently, mounting preclinical and clinical evidence has highlighted the role of immune dysregulation and inflammation as underlying

ing mechanisms of mood disorders [10]. Increased inflammatory biomarkers have been found to have cross-sectional and longitudinal associations with depression [11–14]. Inflammatory cytokines have also been shown to disrupt and bypass the therapeutic mechanisms of conventional antidepressants and elevate the likelihood of treatment failure [15–17]. One possible mechanism is the regulation of monoamine metabolism [18] by increasing the activity of monoamine transporters (an increase of uptake activities) and/or reducing monoamine precursors [19–21]. For example, in cancer patients receiving immunotherapy, hyperinduction of indoleamine 2,3-dioxygenase decreases tryptophan bioavailability, the main amino acid precursor for serotonin synthesis [22]. Inflammation can also reduce serotonin, norepinephrine, and dopamine synthesis rates by decreasing the bioavailability of essential enzyme co-factors necessary for tryptophan hydroxylase and dopamine hydroxylase activity [23, 24]. Additionally, acute and chronic inflammatory markers may directly impact the function of the central nervous system. For example, inflammatory cytokines may activate nuclear factor-kappa B (NF- $\kappa$ B) and consequently inhibit neurogenesis [25]. Furthermore, inflammatory cytokines can affect astrocytes by reducing the expression of glutamate transporters and elevating their release, subsequently downregulating the brain-derived neurotrophic factor (BDNF) and affecting neurogenesis [26].

The serum concentrations of several pro-inflammatory markers, including tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin (IL)-1- $\beta$ , and IL-6, are elevated in patients with MDD [27, 28]. Given the link between inflammatory cytokines and MDD, researchers have been investigating whether suppressing inflammatory cytokines could be a therapy option for MDD. Several clinical studies have found that suppressing cytokines in patients with MDD improves mood and increases antidepressant responsiveness [29, 30].

Pentoxifylline (PTX) is a methylated xanthine derivative with the phosphodiesterase (PDE) inhibitory effect [31]. It is FDA-approved for the treatment of intermittent claudication [32]. It has been shown that PTX directly correlates with an increased serum level of BDNF and enhanced cerebral blood flow (CBF), alongside antioxidant and anti-inflammatory activities [28, 33–35]. PTX, through the PDE-inhibitory effect, increases the generation of cyclic adenosine monophosphate (cAMP) within the cell and activates downstream targets, including protein kinase A (PKA), which, in turn, downregulates TNF- $\alpha$  mRNA, and pro-inflammatory markers, namely IL-1- $\beta$  [36, 37]. Some studies have also found that PTX may exert anti-inflammatory effects by increasing the level of immune suppression cytokines [38]. Recent evidence has found that anti-inflammatory mechanisms of PTX are associated with monoamines in MDD patients [28].

The aim of this study was to assess the efficiency of PTX as an adjunctive agent with citalopram for the treatment of patients with MDD. PTX antidepressant effects were assessed by evaluating changes in the Hamilton Depression Rating Scale-17 items (HAM-D-17) score and various blood-based inflammatory markers.

## Methods

### Study design

This 12-week, randomized, double-blind, placebo-controlled, parallel-group, fixed-dose clinical trial was conducted in Hawler Psychiatric Hospital and Private Clinic, Erbil, Iraq. The study protocol was approved by the institutional review board (IRB) and research ethics committee of Hawler Medical University, Erbil, Iraq (approval: HMU PH-EC 16112021/382). This study is registered on ClinicalTrials.gov (identifier: NCT05271084). Before enrolling in the study, all participants signed written informed consent forms.

### Participants

One hundred and seventy-four patients who were admitted to Hawler Psychiatric Hospital and Private Clinic for routine clinical care were assessed for eligibility. Among these, 100 adult patients with MDD (21–65 years) were finally enrolled in this study. The eligibility criteria were diagnosis confirmation by the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) and Mini-International Neuropsychiatric Interview (MINI) [39, 40], a total 17-item HAM-D score of  $\geq 18$ , and a score of  $\geq 2$  for item 1 (depressed mood). Data were collected and evaluated from November 2021 to February 2022.

The exclusion criteria were as follows: patients with a concurrent medical disorder, bipolar disease, history of seizures, renal impairment, drug dependency or abuse, cardiovascular diseases, personality disorders, active inflammatory disorders, allergy to the used medications, at risk of suicide or effectively suicidal, patients who had been under treatment with psychotropic agents, including antidepressants (4 weeks before the study), and electroconvulsive therapy in the last 2 months, and pregnant females.

### Intervention

The participants were randomly allocated to receive either citalopram 20 mg/day plus two placebo tablets (control group) twice daily or citalopram 20 mg/day plus PTX (400 mg) twice daily for 12 weeks.

### Outcomes

The HAM-D-17 score was measured at the starting point and at weeks 2, 4, 6, 8, 10, and 12 after starting the intervention, serving as the primary outcome. Treatment response ( $\geq 50\%$  drop in HAM-D-17 score) and remission rate (HAM-D-17 score  $\leq 7$ ) were also measured. Side effects were also systematically monitored weekly using a side-effect checklist.

As secondary outcome measures, serum concentrations of IL-6, IL-10, IL-1- $\beta$ , C-reactive protein (CRP), TNF- $\alpha$ , BDNF, and serotonin were assessed at baseline and week 12 using enzyme-linked immunosorbent assay. Peripheral blood (10 mL) was collected for each participant, and sera were separated by centrifuging at a speed of 250 g for 15 min. Finally, the sera were stored at  $-30^{\circ}\text{C}$  until use. Samples were tested in similar runs, including duplicate measurements for a set of standards.

### Sample size

By considering an effect size of 0.81, 80% power, and a two-sided significance of 0.05, the study sample size was calculated as 35 subjects per group. Based on a 15% attrition rate, a sample size of 45

was achieved. A final sample size of 50 for each group was considered [28, 41, 42].

## Randomization and blinding

A computer-generated code was used to randomly divide patients into five unit blocks in a 1: 1 ratio for enrollment in the placebo or PTX groups. For allocation concealment, sequentially numbered opaque envelopes were used. All allocation, randomization, and grading processes were performed by separate investigators. The allocation was hidden from the patients, physicians, psychiatrists, and statisticians.

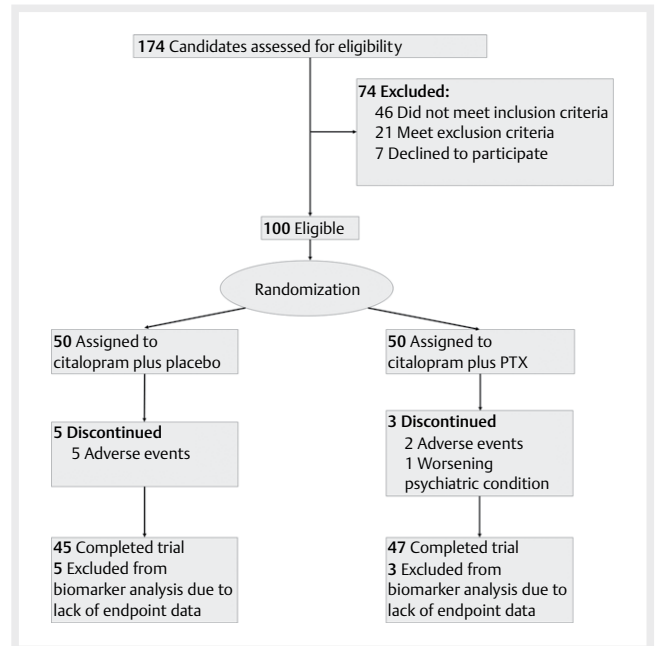
## Statistical analysis

For all treatment efficacy tests,  $p < 0.05$  was considered significant. Categorical variables were reported as numbers (percentage), whereas continuous variables were reported as mean  $\pm$  standard deviation. Comparison of baseline HAM-D-17 scores with each time point was analyzed using mixed-effects model repeated measures (MMRM) analysis of covariance. Between groups, changes in HAM-D-17 score were analyzed by two-factor repeated measure analysis of variance. The same analysis was used to examine the effect of therapy and the time-treatment interaction. Throughout the study, time interval measurements were considered a within-subject component, and the study groups were considered a between-subject component. A paired t-test was employed for comparisons of biological markers at baseline and endpoint in each group. Pearson's correlation coefficient was used to perform the correlation analysis of biological markers with HAM-D-17 scores. Qualitative variables were evaluated using Fisher's exact test. Analyses were performed using SPSS Statistic 26 (IBM Corp., NY, USA), and all graphs were generated using GraphPad Prism software version 8.3.0 (GraphPad Software, La Jolla, CA, USA).

## Result

### Participants

A total of 174 individuals were assessed for eligibility, 74 of whom were excluded. Ultimately, 100 participants were recruited and randomized to either citalopram plus placebo ( $n = 50$ ) or citalopram plus PTX ( $n = 50$ ) (► Fig. 1). Baseline sociodemographic data for the participants are presented in ► Table 1. In terms of demographic data, no statistically significant difference was observed between the two groups. Five subjects in the citalopram plus placebo group



► Fig. 1 A flow diagram of study participants.

► Table 1 Sociodemographic and clinical characteristics of the study sample.

Participant Characteristic	Control group ( $n = 50$ )	Pentoxifylline group ( $n = 50$ )	<i>P</i> Value
<b>Age (yrs.) – mean (SD)</b>	30.7 (7.12)	32.47 (8.31)	0.77
No. of lifetime psychiatric hospitalizations	1.1 (2)	1.3 (1.8)	0.71
Lifetime episodes of MDD – no. (SD)	7.1 (17.2)	6.8 (16)	0.80
Duration of Current MDD Episode (mos.) – mean (SD)	13.7 (18.3)	12.4 (20.3)	0.65
Sex (female) – no. (%)	23 (46%)	23 (46%)	0.42
BMI (kg/m <sup>2</sup> ) – mean (SD)	27.56 (3.1)	26.29 (4)	0.73
<b>Education (Highest Degree) – no. (%)</b>			0.25
High school	11 (22%)	16 (32%)	
College or university	35 (70%)	31 (62%)	
Graduate school	4 (8%)	3 (6%)	
<b>Marital status</b>			0.31
Single	12 (24%)	9 (18%)	
Married	28 (56%)	33 (66%)	
Divorced	10 (20%)	8 (16%)	
Smoking	37	29	
Baseline HAM-D 17 – mean (SD)	26.1 (1.9)	27.6 (9)	0.39

Ham-D-17, Hamilton Depression Rating Scale-17; BMI, Body Mass Index; SD, Standard Deviation.

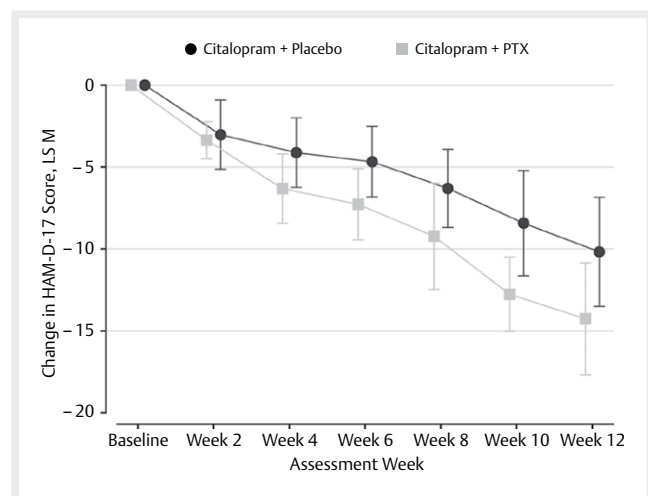
dropped out (one at week 4, two at week 6, two at week 8) due to medical/psychiatric complications.

Three subjects in the citalopram plus PTX group discontinued participation (one each at weeks 4, 6, and 8), two of which were due to medical complications (nausea), and one was due to worsening psychiatric status. HAM-D-17 analysis was performed on data from these patients, but cytokine marker analysis was not applicable to them due to a lack of endpoint data. Overall, 92% of the included patients finished the 12 weeks of the trial ((45/50 (90%) in the citalopram plus placebo group and 47/50 (94%) in the citalopram plus PTX group,  $p = 0.31$ )).

## Antidepressant efficacy

A significant outcome in terms of time-treatment interaction was observed, signifying a difference in the performance of the two treatment groups throughout the trial ( $F(1, 78) = 16.232$ ,  $P = 0.003$ ). Also, the outcome was significant for time ( $F(1, 78) = 114.467$ ,  $p = 0.001$ ), and for treatment ( $F(1, 49) = 18.21$ ,  $p = 0.007$ ). Furthermore, based on the primary MMRM analysis, after 4, 6, 8, and 12 weeks, the drop in the scores of the PTX group from baseline was significantly greater than that of the placebo group (► **Fig. 2**, ► **Table 2**).

Treatment response rates were significantly different between groups, with 83% (39/47) responding to the PTX and 49% (22/45)



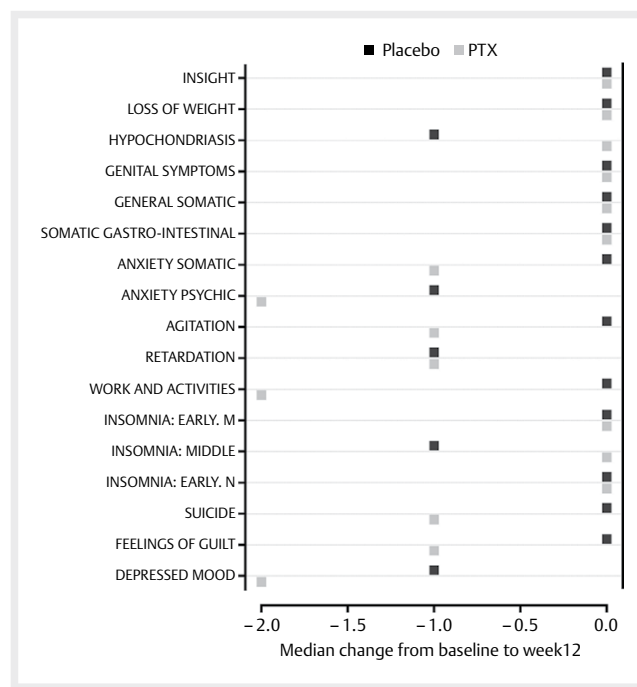
► **Fig. 2** Change in HAM-D-17 Scores in MDD patients randomly assigned to PTX or placebo group. PTX, Pentoxifylline; HAM-D-17, Hamilton Depression Rating Scale-17 items; MDD, major depressive disorder.

responding to the placebo after 12 weeks ( $p = 0.006$ ; number needed to treat (NNT), for one additional patient to have the study outcome = 3). In terms of the remission rate, 79% of the PTX group patients and 40% of the placebo group patients were remitted ( $p = 0.01$ ).

Median change in specific parameters of the HAM-D-17 from the start date to week 12 was examined to determine which symptoms improved. Depressed mood, feeling of guilt, suicidal thoughts, work and activities, psychic and somatic anxiety, and agitation were more responsive symptoms among the PTX group participants in comparison to the placebo group (► **Fig. 3**).

## Effect on serum markers

A paired t-test showed that serum concentrations of IL-1- $\beta$ , TNF- $\alpha$ , IL-10, and IL-6 were significantly reduced at week 12 in either group. CRP level indicated a non-significant reduction in the placebo group, although its level reduced significantly in the PTX group. However, the PTX group demonstrated a significantly greater

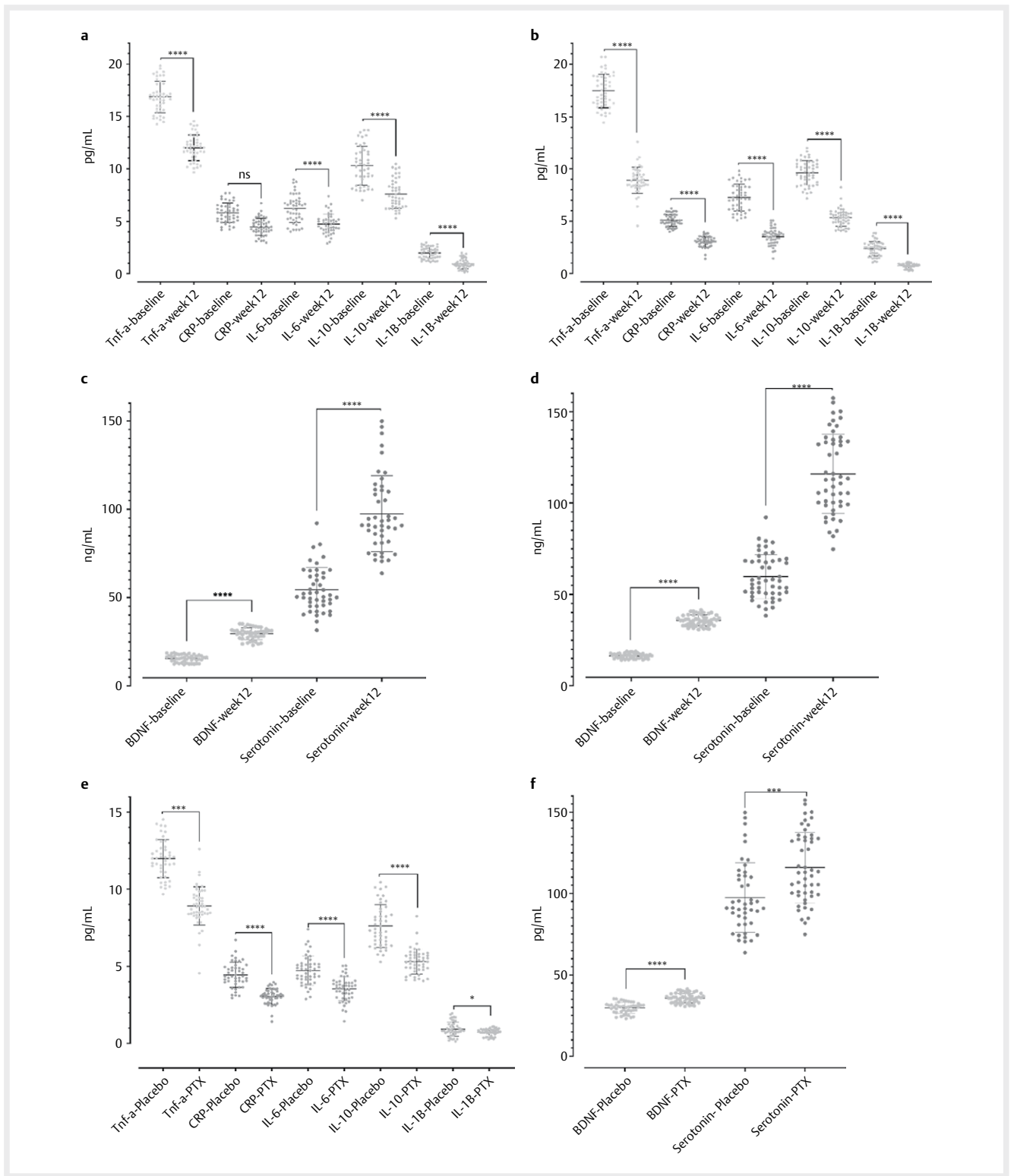


► **Fig. 3** Median change in individual HAM-D-17 items from baseline to week 12 in PTX group versus placebo-treated MDD patients. PTX, Pentoxifylline; HAM-D-17, Hamilton Depression Rating Scale-17 items; MDD, major depressive disorder.

► **Table 2** Hamilton Depression Rating Scale score changes from baseline to week 12.

Weeks after treatment	Citalopram + Placebo (LSM (SE))	Citalopram + PTX (LSM (SE))	LSMD vs. control (95% CI)	P value
Week 2	-3.023 (2.112)	-3.361 (1.33)	-0.338 (-0.129 to -2.11)	0.13
Week 4	-4.117 (2.122)	-6.31 (2.11)	-2.193 (-0.61 to -3.841)	0.021
Week 6	-4.675 (2.149)	-7.272 (2.166)	-2.597 (-0.238 to -3.246)	0.036
Week 8	-6.301 (2.381)	-9.217 (3.249)	-2.916 (-1.503 to -4.018)	0.019
Week 10	-8.425 (3.210)	-12.761 (2.262)	-4.336 (-2.011 to -5.565)	0.005
Week 12	-10.172 (3.327)	-14.259 (3.417)	-4.087 (-2.965 to -5.73)	0.008

SE, standard error; LSM, least squares mean; LSMD, least squares mean difference; CI, confidence interval, PTX, Pentoxifylline.



► **Fig. 4** Change in the serum concentration of the selected inflammatory markers. **a.** Change in TNF- $\alpha$ , CRP, IL-6, IL-10, and IL-1 $\beta$  serum levels in the placebo group at baseline and week 12 **b.** Change in TNF- $\alpha$ , CRP, IL-6, IL-10, and IL-1 $\beta$  serum levels in PTX group at baseline and week 12 **c.** Change in BDNF and serotonin serum levels in the placebo group at baseline and week 12 **d.** Change in BDNF and serotonin serum levels in PTX group at baseline and week 12 **e.** Change in TNF- $\alpha$ , CRP, IL-6, IL-10, and IL-1 $\beta$  serum levels in placebo group vs. PTX group at baseline and week 12 **f.** Change in BDNF and serotonin serum levels in placebo group vs. PTX group at baseline and week 12. TNF- $\alpha$ , tumor necrosis factor-alpha; CRP, C-reactive protein; IL-6, interleukin-6; IL-6, IL-10, interleukin-10; IL-1 $\beta$ , interleukin-1 beta; BDNF, brain-derived neurotrophic factor; PTX, Pentoxifylline; \*\*\*\*,  $P < 0.0001$ , \*\*\*,  $p < 0.001$ ; \*\*,  $p < 0.01$ ; ns, non-significant; Data are presented as mean  $\pm$  standard deviation.

► **Table 3** Changes in the serum concentration of selected biological markers of the patients at baseline and after 12 weeks in PTX and Placebo group.

	Placebo group (n = 45)			PTX group (n = 47)			Placebo vs PTX		
	Base line	Week 12 <sup>th</sup>	P value	Base line	Week 12 <sup>th</sup>	P value	Week 12 <sup>th</sup>	Week 12 <sup>th</sup>	P value
<b>TNF-α</b>	16.86 ± 1.51	11.99 ± 1.23	<0.0001	17.45 ± 1.59	8.91 ± 1.25	<0.0001	11.99 ± 1.23	8.91 ± 1.25	<0.0001
<b>CRP</b>	5.82 ± 0.94	4.96 ± 0.82	= 0.067	5.09 ± 0.58	3.7 ± 0.5	<0.0001	4.96 ± 0.82	3.7 ± 0.5	<0.0001
<b>IL-6</b>	6.23 ± 1.23	4.74 ± 0.94	<0.0001	7.26 ± 1.29	3.55 ± 0.77	<0.0001	4.74 ± 0.94	3.55 ± 0.77	<0.0001
<b>IL-10</b>	10.30 ± 1.85	7.62 ± 1.38	<0.0001	9.64 ± 1.18	5.33 ± 0.81	<0.0001	7.62 ± 1.38	5.33 ± 0.81	<0.0001
<b>IL-1-β</b>	1.97 ± 0.55	0.94 ± 0.47	<0.0001	2.38 ± 0.69	0.74 ± 0.21	<0.0001	0.94 ± 0.47	0.74 ± 0.21	= 0.03
<b>BDNF</b>	15.63 ± 2.09	29.74 ± 3.32	<0.0001	16.32 ± 1.33	35.86 ± 2.97	<0.0001	29.74 ± 3.32	35.86 ± 2.97	<0.0001
<b>Serotonin</b>	54.44 ± 12.62	97.42 ± 21.44	<0.0001	58.36 ± 10.96	114.27 ± 21.35	<0.0001	97.42 ± 21.44	114.27 ± 21.35	<0.0001

TNF-α, tumor necrosis factor alpha; CRP, C-reactive protein; IL-6, interleukin-6; IL-10, IL-10; interleukin-10; IL-1-β, interleukin-1 beta; BDNF, brain-derived neurotrophic factor; PTX, Pentoxifylline; Data presented as mean ± standard deviation.

reduction in these cytokines in comparison to the placebo group. In contrast, BDNF and serotonin levels significantly increased in both groups, with a significantly greater increase in the PTX group (► **Fig. 4**, ► **Table 3**).

Correlation analysis was performed between HAM-D-17 scores and serum concentrations of IL-1-β, TNF-α, IL-10, CRP, IL-6, BDNF, and serotonin in both groups at baseline and endpoint of the trial. A significant positive correlation between IL-1-β, TNF-α, IL-10, CRP, IL-6, and HAM-D-17 scores was observed in both groups, either before or after treatment. BDNF and serotonin serum levels were negatively correlated with HAM-D-17 scores in both groups before and after treatment (► **Table 4**).

No severe side effects and no significant difference in the frequency of side effects between the two groups were observed. The frequency of reported adverse events is presented in ► **Table 5**.

## Discussion

Given the novel findings regarding the involvement of multiple pathophysiological pathways in mood disorders and the inefficiency of targeting single pathological pathways (i. e., monoaminergic system), recent approaches in drug development have changed. Decreased neuroplasticity, immune dysfunction, CBF, oxidative stress, and neuronal apoptosis are among the altered pathophysiological pathways to target mood disorders [43–45]. PTX is a pleiotropic agent with the potential to affect several pathways involved in the dysregulated mechanisms of MDD, particularly immune dysfunction. As a result, this study aimed to assess the adjunctive antidepressant efficacy of PTX with a focus on its effect on inflammatory markers.

These results showed that regardless of being well-tolerated, PTX can be a potential adjunctive treatment to improve the efficacy of common antidepressants. A statistically meaningful difference in HAM-D-17 score reduction between the control group (citalopram + placebo) and the treatment group (citalopram + PTX) highlights the potential synergistic effect of PTX with SSRIs. Among specific parameters of the HAM-D-17, depressed mood, the feeling of guilt, suicidal thoughts, works and activities, psychic and somatic anxiety, and agitation were more responsive symptoms in the PTX group, in accordance with those identified by Bech et al. in his development of the HDRS6 [46]. This effect can be preliminarily attributed to the significant increase in serotonin levels in the treatment cohort compared with the control cohort. Furthermore, the same effect has been observed for the neurotrophin BDNF, which has long been recognized as a vital part of the synaptic regulation and plasticity pathway [47]. A significant negative correlation between BDNF and depression scores in MDD patients was observed, which is well documented, and it is thought that it plays an important role in controlling depressive symptoms (i. e., suicidal thoughts) [28, 48]. Mechanistically, BDNF can exert its action by protecting against stress-induced neuronal damage and improving neurogenesis in the hippocampus, which provides a potential explanation for the observed antidepressant effect of PTX [49]. PTX inhibits PDEs, which in turn increases the cAMP level, which may raise the BDNF level as a downstream element [50]. In addition, a correlation between increased levels of pro-inflammatory cytokines and attenuation of BDNF has been reported, although this effect is

► **Table 4** The correlation between serum levels of the selected biological markers and HAM-D-17 score at baseline and week 12 in PTX and Placebo groups.

	Placebo group (n = 45)				PTX group (n = 47)			
	Baseline		Week 12 <sup>th</sup>		Baseline		Week 12 <sup>th</sup>	
	r	P value	r	P value	r	P value	r	P value
<b>TNF-α</b>	0.5185	<0.0001	0.5326	<0.0001	0.5997	<0.0001	0.606	<0.0001
<b>CRP</b>	0.3346	= 0.0246	0.5995	<0.0001	0.5341	<0.0001	0.3094	= 0.0345
<b>IL-6</b>	0.4935	<0.0001	0.4807	<0.0001	0.3923	= 0.0076	0.4346	= 0.0022
<b>IL-10</b>	0.4737	<0.001	0.4327	<0.001	0.4583	= 0.0015	0.2987	= 0.0414
<b>IL-1-β</b>	0.5669	<0.0001	0.3577	= 0.0158	0.405	= 0.005	0.4437	= 0.0017
<b>BDNF</b>	- 0.6086	<0.0001	- 0.5666	<0.0001	- 0.5313	<0.0001	- 0.5215	<0.0001
<b>Serotonin</b>	- 0.7736	<0.0001	- 0.7051	<0.0001	- 0.5223	<0.0001	- 0.5263	<0.0001

TNF-α, tumor necrosis factor alpha; CRP, C-reactive protein; IL-6, interleukin-6; IL-6, IL-10, interleukin-10; IL-1β, interleukin-1 beta; BDNF, brain-derived neurotrophic factor; PTX, Pentoxifylline; r: sample correlation coefficient; Data presented as mean ± standard deviation.

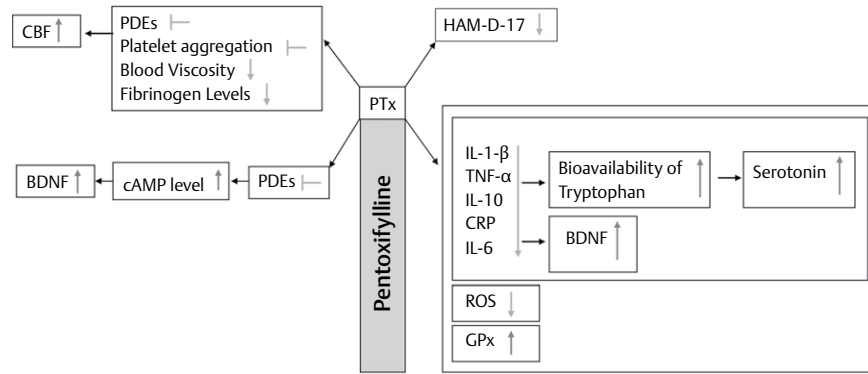
► **Table 5** Adverse events.

Adverse events	Citalopram + Placebo	Citalopram + PTX
Headaches	12 (24%)	10 (20%)
Insomnia	3 (6%)	5 (10%)
Abdominal pain	4 (8%)	7 (14%)
Increased appetite	4 (8%)	6 (12%)
Decreased appetite	8 (16%)	5 (10%)
Nausea	7 (14%)	5 (10%)
Vomiting	6 (12%)	4 (8%)
Diarrhea	6 (12%)	6 (12%)
Sexual dysfunction	7 (14%)	6 (12%)
Fever	7 (14%)	9 (18%)
Fatigue	4 (8%)	8 (16%)
Anxiety	1 (2%)	0 (0.0%)

yet to be clarified [51]. In this regard, there was a significant reduction in serum levels of IL-1-β, TNF-α, IL-10, CRP, and IL-6 in the patients who received PTX compared to those who received placebo. This phenomenon not only increases the serum level of BDNF but also increases the bioavailability of serotonin [52, 53]. In line with this, reduced concentrations of pro-inflammatory cytokines have been shown to increase the bioavailability of tryptophan, the main amino acid precursor for serotonin synthesis [54], and tryptophan hydroxylase co-factors [55]. Therefore, the decreased pro-inflammatory profile may be the underlying cause of the elevation in serotonin and BDNF levels as positive mood regulators. However, the effect of citalopram should not be neglected as SSRIs, which have been shown to increase the BDNF level in MDD patients, can also negatively regulate the inflammatory profile [28, 56, 57]. The above-mentioned findings are well-documented, and several clinical and preclinical studies support these results [36, 58–62]. Moreover, our result showed the decreased levels of the anti-inflammatory mediator, IL-10 in patients treated with PTX. This phenomenon is in contrast with previous studies and may be due to the activation of some transcription factors that have dual roles in inflammation and should be further investigated in future studies.

As mentioned earlier, one globally accepted notion about the antidepressant effect of PTX is its PDE-inhibitory function, which mimics the antidepressant effect by increasing intracellular cAMP [28]. Given the various roles of PDEs and other functions of PTX within the cell, it has a pleiotropic impact not limited to the immune system. Evidence shows that improving CBF may be associated with improved mood disorders. Therefore, CBF might be a therapeutic target that is not addressed by conventional monoaminergic agents [45, 63]. PTX improves CBF by inhibiting membrane-bound PDEs, suppressing platelet aggregation, and reducing blood viscosity and fibrinogen levels [50]. PTX is also an anti-oxidative agent that exerts its action by reducing the production of reactive oxygen species (ROS), increasing levels of hippocampal glutathione peroxidase (GPx), and reducing TNF-α and IL-6 levels as producers of oxygen and nitrogen free radicals [33, 35, 64]. Therefore, suppressing oxidative damage as a pathophysiological pathway of MDD may be beneficial for positive mood regulation [65]. ► **Fig. 5** shows a diagram explaining various mechanisms by which PTX may affect on MDD patients (► **Fig. 5**).

These results provide further clinical evidence regarding the antidepressant efficacy of PTX, which as an adjunctive agent, can potentially improve the positive mood regulatory effect of citalopram and enhance treatment response and remitting rate. Several similar studies have tested the effect of PTX as an adjunctive agent to different antidepressants. Farajollahi-Moghadam et al. [66] evaluated the efficacy of PTX combined with sertraline in the treatment of MDD. They reported that sertraline plus PTX improves HAM-D scores and response to treatment rate, supporting our results and implying the efficacy and safety of PTX combination therapy in patients with MDD. The adjunct role of PTX in combination with escitalopram, in the treatment of patients with MDD was evaluated by El-Haggar et al. [28]. They reported a significant improvement in HAM-D score and reduction in the serum levels of TNF-α, IL-6, IL-10, and 8-OHdG while significant increase in the levels of BDNF and serotonin in comparison with the control group. Yasrebi et al. [67] investigated the efficacy of PTX for treating MDD in CAD patients undergoing percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) reporting an improvement in HAM-D scores compared with patients receiving placebo. Nassar et al. [68] examined the effect of dexamethasone (DEX) and PTX in rat models



► **Fig. 5** Various mechanisms demonstrating the effects of PTX in MDD patients. TNF- $\alpha$ , tumor necrosis factor-alpha; CRP, C-reactive protein; IL-6, interleukin-6; IL-10, interleukin-10; IL-1 $\beta$ , interleukin-1 beta; BDNF, brain-derived neurotrophic factor; PDE, phosphodiesterase; CBF, cerebral blood flow; ROS, reactive oxygen species; GPx, glutathione peroxidase; PTX, Pentoxifylline.

of depression, mania, and aggression. They observed antidepressant-like and anti-manic-like effects of PTX, but not dexamethasone in rat models accompanied by a significant reduction in pro-inflammatory mediator levels in the brain. These studies are in line with The results of the present study align with the findings of the above-mentioned studies. Moreover, the current study highlights the positive correlation between pro-inflammatory cytokines and higher HAM-D-17 scores, which could be counteracted by the significant anti-inflammatory function of PTX as an adjunctive agent to citalopram. However, as a limitation, the mechanistic effect of PTX related to its anti-inflammatory function could not be addressed in this study. Another limitation is the lack of further analyses to explore other functions of PTX related to its antidepressant activity. Future studies need to assess the effect of PTX alone in patients with MDD in a more comprehensive context associated with the pleiotropic functions of PTX.

## Conclusions

This study suggests that the PTX add-on, a well-tolerated adjunct for treating adult patients with MDD, improves the antidepressant efficacy of citalopram, which is clinically reflected by a significant decrease in HAM-D-17 scores (improved treatment response as well as enhanced remission rates). Therefore, the findings of this study support the efficacy of PTX as a promising agent for treating MDD patients.

## Ethics Statement

The study protocol was approved by the institutional review board (IRB) and research ethics committee of Hawler Medical University, Erbil, Iraq (approval: HMU PE-EC 16112021/382).

## Data Availability Statement

The data that support the findings of this study are available upon request from the author.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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