

To Evaluate Synergetic Potential of Piperine with Citalopram on Chronic Unpredictable Mild Stress-Induced Depression in Wistar Rats

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ABSTRACT

Depression is a neurological and mood disorder identified by a discouraged state of mind and low confidence, influencing the cognitive and social wellness of an individual. An imbalance in the monoamine neurotransmitters leads to depression. Selective serotonin reuptake inhibitors drugs are the generally recommended anti-depressants as they enhance the levels of serotonin in the brain. The efficiency of selective serotonin reuptake inhibitors (SSRIs) in mild or moderate cases of depression has been disputed. In this study, co-administration of piperine, along with citalopram, was evaluated. Citalopram, in combination with piperine, possesses effective potency to improve the behavioral imperfections through forced swim test (FST), tail suspension test (TST), and actophotometer behavioral tests. Thus, the results revealed that a combination of piperine with citalopram has excellent beneficial effects than an individual in the treatment of depression. This synergetic effect of piperine with citalopram may attribute to their inhibitory activity of the CYP450 enzymes.

Keywords: Chronic unpredictable mild stress, Citalopram, Depression, Piperine, Wistar rats.

1. INTRODUCTION

Depression is a neurological disorder which is characterized by a discouraged state of mind, low confidence, feelings of hopelessness, extreme sadness, altered appetite, and weight, diminished enthusiasm for pleasurable stimuli, and intermittent thoughts of death and suicide (Farmer et al., 2005). Depression has become a typical reason for premature death or disability. As indicated by the World Health Organization (WHO), consistently around 120 million individuals all through the world experience the ill-effects of depression. The statistics show that 15% of us experience the ill effects of depression eventually during our lifetime. Depression is liable for 8.5 lakh deaths every year (WHO, 2007; Grover et al., 2010).

The “monoamine hypothesis” has been the central theme for research in the field of depression. It recommends that inadequacy or imbalance in the monoamine neurotransmitters, for example, serotonin, dopamine, and norepinephrine, can lead to depression. This theory

also shows that anti-depressants drugs like monoamine oxidase (MOA) and SSRIs, will improve monoamine performance (Cavus & Duman, 2003; Lee et al., 2010; Donnell et al., 2011).

SSRI raises the availability of the serotonin neurotransmitter by increasing its concentration in the synaptic cleft and constraining its reabsorption. Citalopram, escitalopram, and duloxetine are widely known as SSRI medications. The efficiency of SSRIs in mild or moderate cases of depression has been disputed (Celexa, 2019).

Piperine is an alkaloid found in the flowering vines of the black pepper plant, *Piper nigrum* (Fig. 1). The chief importance of piperine as a health supplement is its ability to enhance the bioavailability of some other nutrients and minerals (Kumoro et al., 2009). Piperine enhances the bioavailability of a variety of drugs and therapeutic agents (Begum et al., 2015). Piperine inhibits the enzymes that are involved in the metabolism and transport of various metabolites such as P-glycoprotein, CYP3A4, and many CYP450 enzymes in humans that metabolize many drugs

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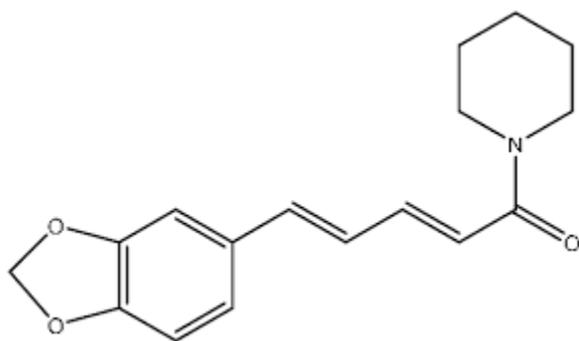


Fig. 1: Structure of piperine

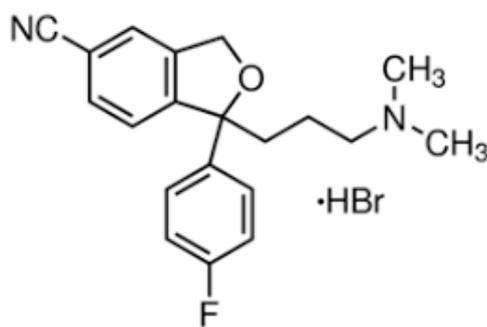


Fig. 2: Structure of citalopram hydrobromide

Table 1: CUMS schedule

S. No.	Day	CUMS
1	Day 1	24 h food deprivation
2	Day 2	Inversion of day/ night light cycle
3	Day 3	24 h water deprivation
4	Day 4	Wet bedding
5	Day 5	Clipping tail with forceps
6	Day 6	Forced swimming test 4 degree cold water for 6 min
7	Day 7	Electrical stimulation 30–60 V current of 1 mA and 2 Hz

(Wang et al., 2010; Huang et al., 2013). Previously piperine has already been studied for anti-depressant activity (Li et al., 2007), but co-administration of piperine with citalopram inhibits the CYP450 enzymes that eventually inhibit the metabolism of citalopram improving the bio-availability of drugs. Therefore, this investigation was attempted to assess the synergetic potential of piperine with major anti-depressants on chronic and unpredictable mild stress-induced depression in Wistar rats.

Citalopram hydrobromide is a drug of class of SSRI. Its chemical structure is different from those of other SSRIs, tricyclic, and any other anti-depressant agents (Fig. 2). Therefore, there is some different therapeutic strength of citalopram when compared to other classes of drugs and even among the SSRIs (Cipriani et al., 2012).

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Piperine was obtained from the Sami Labs, Hyderabad. Piperine and citalopram (Celexa) were dissolved in Polyethylene Glycols (PEG) 1% 0.1 mL individually and administered to rats through i.p. route.

2.2 Experimental Animals

Male Wistar albino rats weighing 180 to 200 grams (colony inbred strains) were used for the pharmacological studies. The animals were kept under standard environment at a temperature of 23 to 25°C and 35 to 60% humidity, 12

hours light/ dark cycle and given standard pellet diet, Provimi Ltd. (India), *ad libitum*. For a week before the experiment, the animals were acclimatized to the laboratory conditions and then divided randomly into six groups.

Principles of animal handling were strictly adhered to the supervision of animal ethics committee of the institute. The experimental protocol was duly approved by the Institutional Animal Ethics Committee (IAEC), School of Pharmacy, Anurag Group of Institution (Protocol No: I/IAEC/AGI/018/2018 WR ♂).

2.3 Experimental Design

For depression study, 30 animals are divided into five groups with each group having six rats as follows-

Group 1: Control group, received only vehicle (PEG 1%, 0.1 mL, i.p.)

Group 2: Depression controlled [chronic unpredictable mild stress (CUMS)]

Group 3: CUMS + piperine (20 mg/kg, i.p.)

Group 4: CUMS + citalopram (7.5 mg/kg, i.p.)

Group 5: CUMS + piperine (10 mg/kg, i.p.) + citalopram (5 mg/kg, i.p.)

Animals were exposed to a schedule of CUMS for 28 days period (Table 1), while the animals of unstressed groups remained in their home cages. After induction of stress, animals were assessed for depression by various behavioral parameters to understand the synergetic effect of piperine on various anti-depressants.

2.4 Experimental Procedure

2.4.1 FST

The FST was performed according to the process illustrated by Yankelevitch et al., 2015. Each individual animal was deliberately forced to swim in water maintained at 25°C inside a vertical Plexiglas cylinder (height: 60 cm; diameter: 20 cm). After strong swimming activity, each animal supposed to be in a characteristic immobile posture, which is characterized by only minimum movements of limbs (required to keep its head higher than water level). On the day of the experiment, each animal was forced to swim for 6 minutes. The total time taken for immobility was recorded during the last 4 minutes of the test. After removing the rats from the water, they are allowed to dry for 15 minutes and returned into the cage. The water was changed before testing each animal.

2.4.2 TST

In TST, the total time taken by the animal for immobility was determined by the method described by Shinde et al., 2015. In this test, acoustically and visually isolated rats were suspended 50 cm from above the floor by adhesive tape placed just about 1-cm from the tip of the tail. Immobility time was recorded during a 6 minutes period.

2.4.3 Actophotometer Test

The locomotor activity was measured using an actophotometer. This device works on the photoelectrical cells that are connected in the circuit with a counter. In this test, a count was recorded when the animal cut off the beam of the light falling on the photocells. The locomotor activity of each animal was evaluated for 10 minutes by placing them into actophotometer (Yu et al., 2013).

2.5 Statistical Significance

The data obtained from the observations were statistically treated and analyzed using one-way ANOVA followed by Tukey's multiple comparison test and expressed

as mean \pm SEM using Graph Pad Prism 5.0 software. $p < 0.05$ was considered to be statistically significant.

3. RESULTS

3.1 Effect of Synergetic Potential on FST

The results from the forced swim test indicated an increase in the immobility time in CUMS animals when compared to the vehicle-treated animals ($P < 0.001$). Groups treated with piperine (20 mg/kg), citalopram (7.5 mg/kg), and combination of piperine (10 mg/kg) and citalopram (5 mg/kg), showed a significant decrease in the immobility time ($P < 0.001$) when compared to the CUMS group. The results are shown in Table 2.

3.2 Effect of Synergetic Potential on TST

The results from the forced swim test indicated a significant increase in the immobility time in CUMS animals when compared to the vehicle-treated animals ($P < 0.001$). Groups treated with piperine (20 mg/kg), citalopram (7.5 mg/kg), and combination of piperine (10 mg/kg) and citalopram (5 mg/kg) showed significant decrease in the immobility time ($P < 0.001$) when compared to the CUMS group. The results are shown in Table 2.

3.3 Effect of Synergetic Potential on Locomotor Activity using Actophotometer

Actophotometer was used to evaluate locomotor activity in rats. In CUMS animals, there was decreased locomotion compared with the control group ($P < 0.001$). Treatment with piperine (20 mg/kg), citalopram (7.5 mg/kg), and combination of piperine (10 mg/kg) + citalopram (5 mg/kg) showed improved locomotor activity. The results are shown in Table 2.

4. DISCUSSION

Depression is a neurotropic illness that influences a person's life, distressing mood, thinking, conduct, behavior, feelings, etc. The main reason behind depression was

Table 2: Effect on various behavioral parameters

Groups	FST	TST	Locomotor activity
Group I	171 \pm 7.197	155.5 \pm 5.948	152.8 \pm 6.718
Group II	248.5 \pm 9.756 ^{###}	270.7 \pm 5.719 ^{###}	84.76 \pm 4.205 ^{###}
Group III	206.2 \pm 1.138 ^{**}	229.5 \pm 4.787 ^{***}	141 \pm 4.830 ^{***}
Group IV	198 \pm 3.651 ^{***}	219.2 \pm 4.183 ^{***}	122.5 \pm 4.212 ^{***}
Group V	193.3 \pm 6.586 ^{***}	220 \pm 6.465 ^{***}	119 \pm 3.89 ^{***}

Values are expressed as mean \pm SEM; symbols represent the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicates the significant difference on comparison of treatment groups with negative control group; ### $P < 0.001$ indicates the significant difference on comparison of negative control group with control group

thought to be decreased by mono-aminergic signaling (Badhe et al., 2010). Among all the monoamines, a decrease in serotonin can be an important cause of depression. Anti-depressants like citalopram increase synaptic concentrations of serotonin by inhibiting the reuptake mechanism. Metabolites of citalopram and duloxetine are polar and discharged out. CYP450 enzymes are significantly involved in the metabolism of these drugs. To enhance drug availability, inhibition of CYP450 enzymes plays a major role (Tabassum et al., 2010).

The course of the study was 38 days. After the treatment, the improvement in behavioral parameters was compared to CUMS rats. Exposure of the animals to CUMS caused alterations in various behavioral parameters; this was in accordance with a previous study conducted by Jun-Shen et al., 2016. The behavioral parameters are assessed by the forced swim test, tail suspension test, and locomotor activity.

In the present study, treatment with piperine alone has shown an improvement in behavioral parameters, which is in agreement with the previous results of Song Li et al., 2016.

The results also revealed that the animals treated with a combination of piperine and citalopram showed significant anti-depressant activity. The time of immobility in FST and TST behavioral tests was significantly less in treatment groups when compared to that of CUMS group.

Actophotometer was used to evaluate locomotor activity in rats. CUMS rats showed decreased locomotion, i.e., less number of beam splits, while animals treated with the combination of piperine and citalopram increased the locomotion of animals. The number of beam splits per animal into test drug groups is significantly more than that of CUMS group.

5. CONCLUSION

In the current study, CUMS was used for the induction of depression in experimental animals. Treatment with piperine alone and combination with citalopram restored the activities of behavioral parameters.

Hence, it can be concluded that the combination of piperine with citalopram possess good beneficial effects than individual in the treatment of depression. This synergetic effect of piperine with citalopram may feature to their inhibitory activity of the CYP450 enzymes.

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