Research Article

Antidepressant like activity of Piperine in immobilization induced stress in mice

Harshita Jain[1]*, Prateek Jain[2], Bharti Ahirwar[3], Dheeraj Ahirwar[1]

[1]*School of Pharmacy, Chouksey Engineering College, Bilaspur C.G. India 495004*

[2]*ADINA Institute of Pharmaceutical Sciences, Sagar M.P. India 470002*

[3]*Department of Pharmacy, Guru Ghasidas Central University Bilaspur, C.G India 495009.*

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Abstract

Objective: Present study aimed to evaluate antidepressant like activity of Piperine in unstressed and stressed condition and to explore possible underlying mechanism for this activity. **Methods:** Piperine (5, 10, 20 mg/kg) and fluoxetine *per se* was administered to the unstressed and stressed mice; immobility periods were observed using forced swim test and tail suspension test. Effect of CRF-1 antagonist on antidepressant like activity was also evaluated. The mechanism of action was also explored by measuring plasma corticosterone levels. **Results:** Piperine (10, 20 mg/kg) and fluoxetine *per se* significantly decreased immobility periods in stressed mice indicating significant antidepressant like activity under stress. There was no significant effect on locomotor activity of the mice on treatment with Piperine and fluoxetine *per se.* It significantly decreased plasma corticosterone level. Antalrmin (a CRF-1 receptor antagonist) significantly attenuated pipierine induced antidepressant like effect in both FST and TST. **Conclusion:** Piperine significantly produced antidepressant like activity in mice possibly through inhibiting CRF activity and decreasing plasma corticostreone levels.

Keywords*:* Immobilization, piperine, corticosterone

Introduction

Depression is a mood disorder that affected more than 300 million people worldwide and is most prevalent neuropsychiatric disorder. Investigations have demonstrated that depression encompasses a profound neurocircuitry failure. The brain regions involved in this dysregulation may include the hypothalamus, hippocampus, amygdala, and striatum (Liotti and Mayberg, 2001; Nestler et al., 2002). These nuclei are important regulators in endocrine control of behaviors symptomatic of depression, such as eating, sleeping, circadian rhythm, stress response, learning and memory, and pleasure seeking (Tracy and Wylie, 2003). Depression is characterized by persistent sadness and a loss of interest in activities that people normally enjoy, accompanied by an inability to carry out daily activities.

Depression and Stress has a strong interlinking and has been well established. A key factor in the response to stress is the neuropeptide corticotropin-releasing factor (CRF) (Vale et al., 1981). CRF and its receptors (CRFR1 and CRFR2) are important regulators of the hypothalamic–pituitary–adrenal (HPA) axis. Evidences build up knot bridging CRF to the development of depression (Nemeroff, 1988, 1992; Arborelius et al., 1999; Holsboer, 1999; Reul and Holsboer, 2002). Clinical studies have found increased CRF and decreased CRF receptors in postmortem examination of suicide victims. In addition, excessive activation of the HPA axis has been reported in more than one-half of patients with depression, and these symptoms have been corrected during antidepressant treatment (Holsboer, 1999).

During a stress response, the hypothalamus is activated and releases corticotropin releasing factor (also known as corticotropin releasing hormone, CRH), which stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH induces glucocorticoid synthesis and release from the adrenal/interrenal tissue into the blood (Carrasco and Van De Kar, 2003).

This stress related neuropeptide acts at the level of the pituitary to initiate the hypothalamic pituitary adrenal (HPA) axis response (stress (Bale and Vale, 2004; Owens

**Address for Corresponding Author:*
Harshita Jain
School of Pharmacy,
Chouksey Engineering College, Bilaspur C.G. India 495004
E-mail: harshi.jain1987@gmail.com

and Nemeroff, 1991; Vale et al., 1981). CRF containing neurons are not confined to hypothalamic regions; they are also found in a number of neural circuits that mediate information processing and behavior. CRF has been shown to modulate diverse neurotransmitter systems, including glutamate, dopamine, serotonin and norepinephrine (Lavicky and Dunn, 1993; Price and Lucki, 2001; Valentino and Commons, 2005). Modulation of serotonin and norepinephrine release by CRF supports its effective role in depression (Charney, 2004; Koob, 1999).

Piperine (PP) is a simple and pungent alkaloid found in the seeds of black pepper (*Piper nigrum*). Biological properties of piperine have been extensively studied, and piperine-like derivatives have shown an interesting range of pharmacological activities (Chavarria et al., 2016). Based on modern cell, animal, and human studies PP has been found to have immunomodulatory, anti-oxidant (Srinivasan, 2014), anti-asthmatic (Nilani et al., 2009), anti-carcinogenic (Tak et al., 2011; Selvendiran et al., 2005), anti-inflammatory (Gupta et al., 2015), anti-ulcer (Bai and Xu, 2000), anti-amoebic properties (Ghoshal et al., 1996), anticonvulsant activity (Mishra et al., 2015). Promising role of piperine as antidepressant has been demonstrated in studies where it potentiated activation of monoaminergic system (Huang et al., 2013; Li et al., 2015) as well as brain-derived neurotrophic factor (BDNF) contents in the hippocampus and frontal cortex (Mao et al., 2014). It also exerts neuroprotective effect via inhibiting oxidative stress (Huang et al., 2012; Mao et al., 2014), alters monoaminergic pathways and GABAergic pathways (Pal et al., 2011). Effect of piperine in stress has not yet explored.

This study was designed to investigate potential of piperine as antidepressant under stress and explore involvement of Corticotropin Releasing Factor in mediating its effect.

Materials and methods

Swiss albino mice (20–25 g) were employed in the study. Animals were procured from the DRDE, Gwalior, India. Animals were housed separately in groups of 10/cage under laboratory conditions with alternating light and dark cycles of 12 h each. They had free access to food and water. The animals were acclimatized to the laboratory conditions before behavioral experiments. Animals were kept fasted two hours before 2h before drug administration. The animals were acclimatized for five days before behavioral experiments which were carried between 8:00 and 17:00hrs. The experimental protocol was approved by the Institutional Animal Ethics Committee and care of the animals was taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and chemicals

The following drugs and chemicals were used in this study: Fluoxetine hydrochloride, *p*-nitroso-*N*, *N*-dimethylaniline, Piperine, antalarmin hydrochloride (Sigma-Aldrich, St. Louis, USA), boric acid, hydrochloric acid, potassium hydroxide (CDH Ltd., New Delhi).

Laboratory models employed for evaluation of antidepressant activity

Tail suspension test (TST)

It is a commonly employed behaviors test to evaluate antidepressant like activity. The test was performed according to the method described by Steru et al. for evaluating potential antidepressants. The total duration of immobility induced by tail suspension was measured according to the method described. Mice were suspended on the edge of a table 50 cm above the floor, with the help of a paper adhesive tape placed approximately 1cm from the tip of the tip of the tail. Immobility time was recorded for 6 min period. The mice were considered to be immobile only when they hung passively and were completely motion less (Dhingra and Kumar, 2008).

Forced swim test (FST)

It is another seldomly used behavioral test to evaluate antidepressant like activity. Forced swim test was proposed as a model to test antidepressant activity by Porsolt et al. Mice were forced to swim individually in a glass jar (25 × 15 × 25cm^3) containing fresh water upto 15 cm height and maintained at 25±3^{0}C. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. mice was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs, necessary to keep its head above the water. The total duration of immobility was recorded during the last 4 min of the total test duration of 6 minutes. The changes in immobility duration were studied after administrating the drugs in separate groups of animals (Kulkarni, 2008). Each animal was used only once in this test.

Locomotor activity test

The effect of various treatments on locomotor activity was observed in actophotometer (Inco, Ambala, India). The locomotor activity scores for each animal were recorded for a period of 10 min (Gilhotra and Dhingra, 2009).

Biochemical estimations

Corticotropin Releasing Factor

Measurement of CRF levels was performed by enzyme immunoassay or ELISA using commercially available kit: Mouse/Rat CRF-HS ELISA kit (ALPCO Diagnostics). For determination brains were rapidly removed from mice after

completing behavioural tests and isolated brain were weighed and homogenized in 30–fold volume of extraction buffer 10 mM PBS (pH 7.2) containing 0.2% Nonidet P-40 (NP40) in an ice bath. It was centrifuged (18,360 x g, 20 min) and supernatant was collected and used for measurement. Procedure followed was as described earlier (Nakane et al., 1986; Delawary et al., 2010)

Plasma corticosterone estimation

For cortisterone estimation, blood was withdrawn from tail vein of mice immediately before setting the animal free and subjecting it to behavioral tests in all the groups. The sampling procedure was completed during immobilization to avoid the extra stress incurred upon mice during an altogether a new procedure of mouse immobilization for handling the tail of mice. Plasma was separated using cooling centrifuge at 2500 rpm. (Remi Centrifuge, Mumbai, India) for 10 min. Corticosterone levels were estimated in blood plasma by Bartos and Pesez method (Bartos and Pesez, 1979). To 1.0 mL of plasma sample, 1.0 mL of ethanol, 0.50 mL of 0.10 % solution of p-nitroso-N, N-dimethylaniline in ethanol were added and the tubes were immersed in ice water for 5 min, and then 0.50 mL of 0.1 N sodium hydroxide was added. The tubes were plugged with cotton-wool, and let to stand at 0°C for 5 h, protected from light. To the above solution, 2.0 mL of Clark and Lubs buffer for pH 9.8 (prepared by mixing 50.0 mL of an aqueous solution of both boric acid and potassium chloride with 40.8 mL of 0.20M potassium hydroxide, and diluted to 200 mL with distilled water), 5.0 mL of 0.10 % solution of phenol in ethanol, and 0.50 mL of 1.0 % aqueous solution of potassium ferricyanide were added. The tubes were kept in water bath at 20±2°C for 10 min. The absorbance of the solutions was read at 650 nm using UV Spectrophotometer (Schimadzu-1800).

Experimental design

Sixteen groups of mice were employed in the study. Each group consisted of minimum eight mice. Stress was produced in them by immobilizing for 6h (8 a.m.- 2 p.m.) by taping all its four limbs and trunk on a wooden board (Gilhotra and Dhingra, 2009). Mice subjected to immobilization were called as stressed mice and mice not subjected to immobilization were called as unstressed mice and has been mentioned accordingly. Behavioral testing was performed carefully in a stepwise manner i.e. mice in each group were subjected to three tests (Adrian et al., 2005): (a) Tail Suspension Test; then a 6 min rest in home cage, there after (b) locomotor activity test in actophotometer, again followed by 6 min rest, and then (c) Forced Swim Test. All the drugs were administered intraperitoneally (i.p.) 30 min before the behavioral testing in unstressed group and immediately before immobilization in stressed group. When combinations of the drugs were employed, pretreatments were administered 15 min before the administration of the other drug. For CRF determination brain were rapidly removed from mice after behavioral tests.

Statistical analysis

All the results were expressed as mean±Standard Error Mean (SEM). The data were analyzed by using 1-way ANOVA followed by Tukey's test for multiple comparisons using the software GraphPad Instat. In all tests, the criterion for statistical significance was $P<0.05$.

Results

Study showed that immobilization stress has marked effect on depression and piperine decreased immobility time in stressed condition at 10 mg/kg and 20 mg/kg but in unstressed condition piperine has no significant effect on immobility time. This indicated significant antidepressant effect of piperine under stress.

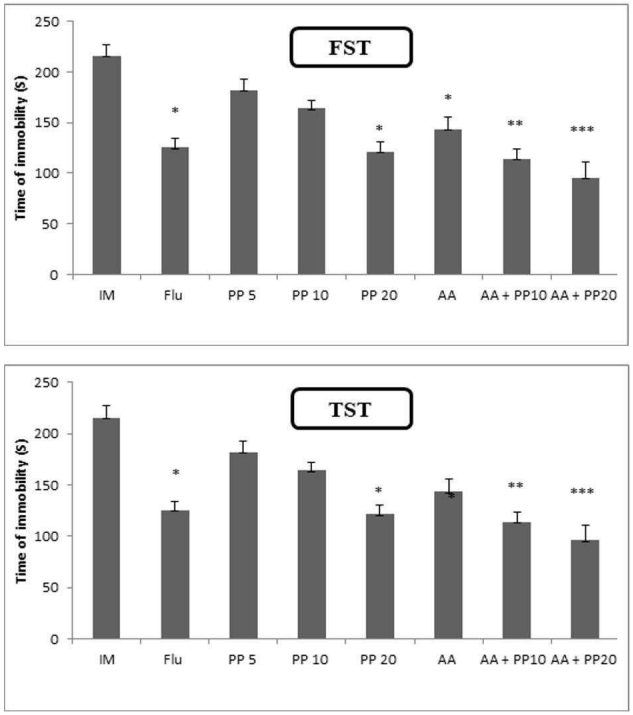

Figure 1. Effect of different treatmens on immobility time on stressed mice in Forced swim test(FST) as well as Tail Suspension Test (TST). n=8 in each group. Values are expressed as mean ± S.E. Data was analyzed by one way ANOVA followed by Tukey's Post Hoc Test, in FST $F(7,40) = 57.06$ TST $F(7,40) = 90.46$; $p < 0.0001$, * = $p < 0.001$ significant difference from immobilized group, **=$p < 0.05$ significant difference from PP (10mg/kg) treated group, ***=$p < 0.05$ significant difference from PP (20mg/kg) treated group, **IM**: Immobilization, **PP**: Piperine, **AA:** Antalarmin. Doses mentioned are in mg/kg.

Different treatments provided to stressed group showed different effects. AA (10 mg/kg i.p.) administered alone to stressed group, significantly decreased immobility time in TST as well FST (figure 1). Where as in unstressed group it

didn't significantly decreased immobility time in both the tests. A significant decrease in immobility time was observed when AA (10mg/kg) was administered in combination with PP (10mg/kg) in stressed group as compared to PP (10mg/kg) group. Similarly, when AA (10mg/kg) and PP (20 mg/kg) was administered together a significant decrease in immobility time was observed in both TST as well as FST. As showm in figure 2 locomotor activity was not effected by treatment with PP alone but AA and its combination with PP (10, 20 mg/kg) increased locomotor activity at a pleatue.

Treatments on unstressed group didn't significantly changed levels of corticotropin releasing factor. As shown in fig 3 coadministration of AA (10mg/kg) and PP (10, 20mg/kg) didn't had any significant changes in brain levels of CRF as compared to AA (10mg/kg) alone. Whereas a significant decrease in Corticosterone levels was found in group treated with AA (10mg/kg) administerd along with PP 10mg/kg as wellas 20 mg/kg as compared to their respective PP treated group.

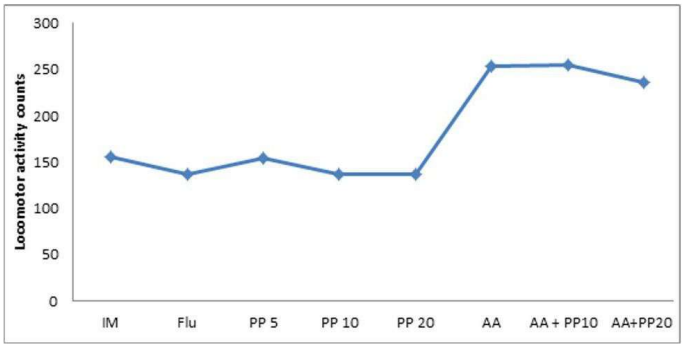

Figure 2. The effect of different treatments on locomotor activity in stressed mice expressed in Locomotor activity counts. n = 8 in each group. **IM**: immobilization, **FLU**: Fluoxetine, **PP**: Piperine, **AA:** Antalarmin. Doses mentioned are in mg/kg.

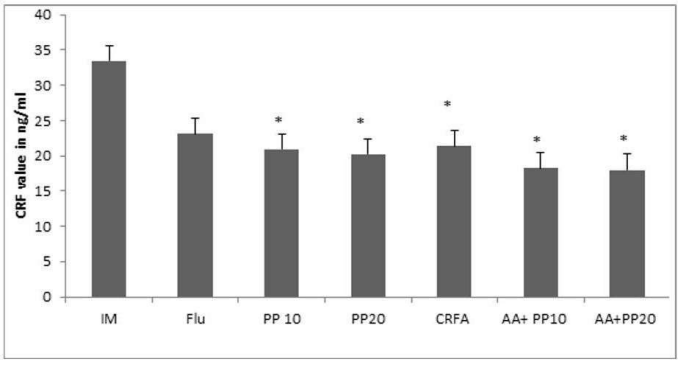

Figure 3. Effect of different treatments on CRF level (ng/ml) in stressed mice. n = 8 in each group. Values are expressed as Mean ± S.E. Data was analysed by one way ANOVA followed by Tukey's Post Hoc Test, $F(7, 40) = 5.2$; $p<0.0001$, * = $p < 0.01$ significant difference from immobilized group. **IM**: immobilization, **FLU**: Fluoxetine, **PP**: Piperine, **AA:** Antalarmin. Doses mentioned are in mg/kg.

Table 1. Effect of treatments on corticosterone levels

Groups	Treatment	Concentration in pg/ml
Group I	**Immo**	621.2
Group IV	PP 10	483.5*
Group V	PP20	438.3*
Group VI	**AA S**	381.0*
Group VII	**AA + PP S 1**	73.6**
Group VIII	**AA + PP S 2**	81.6***

Effect of different treatments on plasma corticosterone levels. Values are expressed as Mean ± S.E. Data was analyzed by one way ANOVA followed by Tukey's Post Hoc Test. $F(7,40)= 40.90$ $p<0.0001$, *=$p<0.001$ significant difference from Immobilised group, **=$p<0.001$ significant difference from PP 10mg/kg treated group group, ***=$p<0.001$ significant difference from PP 20mg/kg treated group. **IM**: immobilization, **FLU**: Fluoxetine, **PP**: Piperine, **AA:** Antalarmin. Doses mentioned are in mg/kg.

Discussion

In present study Piperine (10, 20 mg.kg) to mice under stressed condition produced significant antidepressant like activity in TST and FST. This is the first study showing on antidepressant effect of piperine on immobilization stress for 6 hrs the efficacy of piperine was found to be comparable with fluoxetine. FST and TST are two commonly used behavioral despair models of depression. These models are widely employed in rodents to evaluate antidepressant potential through decreasing immobility period produced by different classes of antidepressant drugs (Steru et al., 1985; Porsolt et al., 1977). Piperine didn't show any effect on locomotor activity as compared to vehicle treated group in unstressed condition as well as immobilized group in stressed condition.

In FST, mice are forced to swim in constrained area which restricts their escape, that attributes immobility in them. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. The TST also induces a state of immobility in animals like that in FST. This immobility, referred as behavioral despair in animals, which is claimed to reproduce a condition similar to human depression (Steru et al., 1985; Thierry et al. 1986; Williner, 1984). Out of three doses of piperine , 20mg/kg PO produced most significant effect in stressed mice whereas none of the dose of pipierine produced significant antidepressant effect in unstressed condition. The most effective dose of piperine was therefore employed in further study elucidating mechanism of antidepressant like activity. Treatment with anatalarmin a CRF antagonist specifically antagonizing CRF1 receptor reversed extended immobility time under stress in both FST and TST. Piperine and antlaramin coadministered together mad the belief stronger by declining immobility time and

exerting direct effect on plasma corticosterone. The most astonishing part was that brain CRf level remained untouched apart from these rapid combinitions. Synergizing effect of piperine and antalrmin suggested that piperine may exert its effect by copperating its coadministered drug and anatagonising CRf receptors.

Major depression has been linked with hyperactivity of the hypothalamic-pituitary-adrenal axis (Barden, 2004). High concentrations of blood glucocorticoid are maintained in patients with depression due to the dysfunction of this feedback mechanism (Johnson et al., 2006). High glucocorticoid levels cause pathological damage to the hippocampal neurons both in vitro and in vivo (Li et al., 2007; Murray et al., 2008) and can induce depression-like behavior in animals (Johnson et al., 2006; Murray et al., 2008). Our results demonstrated pipierine significantly reduced the plasma corticosterone levels, by directly acting on CRF receptors. So, the antidepressant-like action shown by the piperine may be due to inhibition of corticosterone production and CRf antagonism.

Conclusion

In conclusion, our present study shows that Piperine showed significant antidepressant-like activity in stressed mice probably by interaction with CRF- 1 receptor as it reduced plasma corticosterone levels. Therefore pipierine can be explored further for its potential to treat clinical depression.

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