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Piperine protects epilepsy associated depression: a study on role of monoamines

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Abstract. – *Background and Objectives:* **In patients with epilepsy, a common co -morbidity diagnosed is depression. Temporal lobe epilepsy or post status epilepticus (SE) animal model establish and validate the co morbidity and common pathogenesis of depression and epilepsy. Elevation in serotonin concentration gives an inhibitory response to epileptic discharge and stabilizes the depressed mood disorder. Piperine is a potent monoaminooxidase inhibitor and stimulates the synthesis of serotonin. So the present work is undertaken to investigate the effect of piperine on depression associated with by status epilepticus induced by pilocarpine in rats.**

Materials and Methods: **Status epilepticus was induced in the rats by administration of pilocarpine 350 mg/kg i.p.. Behaviour tests like forced swimming test (FST), saccharin consumption test, actophotometer test and rotarod test were conducted. Antidepressant effect and neuroprotective effect of piperine (25 mg/kg, p.o. for 10 days) in post status epilepticus animal model was evaluated. Brain serotonin concentration was also estimated. Fluoxetine (20 mg/kg p.o.) was used as standard.**

Results: **Only piperine but not fluoxetine significantly increased the decrease in number of rotations of wheel in FST, and decrease volume of saccharine consumption caused by pilocarpine. Both fluoxetine and piperine significantly increase the pilocarpine induced decrease in activity score in actophotometer, time taken to fall in rotarod and concentration of serotonin in brain.**

Discussion: **The underlying mechanism behind depression in epilepsy may be alteration in monoaminergic pathways and GABAergic pathways. The antidepressant activity of piperine in post-SE rats may be attributed to its MAO inhibitor activity and neuroprotective activity.**

Key Words:

Pilocarpine, Piperine, Monoamines, Depression, Status epilepticus.

Introduction

Epilepsy is a common neurological disorder affecting almost all ages and both sexes all over the world. The co-relation between epilepsy and psychiatric disorders, more specifically depression has been recognized since the era of Hippocrates. In patients with epilepsy, a common co -morbidity diagnosed is depression[1-3].

A high risk of depression always co-exists with the patient of epilepsy, due to some multifarious factors of both psychological and neurological origin[4]. The prevalence rate of depression in epileptic patients is identified between 19 to 69%, which is higher than that of general population. In pharmacoresistant epilepsy cases, the incidence of depression is most common and may affect the quality of life[5]. From the epidemiologic study the findings enlighten that the epileptic patient have four to five time greater incidence of depression and also five time more incidence of suicide than the general population[6,7].

Some clinical findings show that, hippocampal neurodegeneration or dysfunction is the basis of depression in epileptic patients[8,9]. Temporal lobe epilepsy or post status epilepticus animal model establish and validate the co morbidity and common pathogenesis of depression and epilepsy. The processes like serotonin depletion, neurodegeneration, neuroinflammation by inflammatory mediators can be demonstrated in pilocarpine induced status epilepticus model[10,11,12].

Elevation in serotonin concentration gives an inhibitory response to epileptic discharge and stabilizes the depressed mood disorder[13]. The antidepressant drugs like selective serotonin reuptake inhibitors and mono amino oxidase inhibitors are safe in patient with epilepsy. In addition mono amino oxidase inhibitors are effective in kindling

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model of epilepsy which resembles the status epilepticus[14,15]. Piperine from fruit of piper species is a potent mono amino oxidase (MAO) inhibitor as well as an active antidepressant[16,17] and also possesses anticonvulsant actions[18].

So, this piece of work evaluated the response of piperine to the epileptic seizure induced depression using pilocarpine induced status epilepticus rats. In addition to behavioural study the neurochemical correlation of co morbidity was also studied.

Materials and Methods

Drugs

Piperine: Black peper (*Piper nigrum*) was purchased from local market and seeds were pulverized by using mechanical grinder. The powdered drug was soxheleted with 95% ethanol for three hours. After filtering and concentrating the solution, 10% alcoholic KOH was added and again filtered. The crystals of piperine were separated out after overnight standing of solution[19].

The separated crystals were collected and characterized by TLC and IR spectrophotometry. The piperine in a dose of 25 (mg/kg) was used as test drug. The dosage form of piperine was prepared as a suspension using tween 80 as suspending agent.

The antidepressant drug fluoxetine (Sun Pharma, Baroda, Gugrat, India) 20 mg/kg was used as standard drug.

Animals

Albino rats of both sexes, weighing 150-200 g were used. The animals were housed in group of six under standard light/dark cycle, with food and water ad libitum. The animals were allowed to acclimatize the laboratory conditions before commencement of experimental procedure.

The animals were divided into two groups i.e Naive and SE (pilocarpine). Each group was then divided into three groups (n=6) i.e control, fluoxetine and piperine. So, a total of six groups were there. Gr-I-III were treated by saline (2 ml/kg), fluoxetine (20 mg/kg), and piperine (25 mg/kg) respectively, whereas Gr-IV-VI were treated with pilocarpine (350 mg/kg) followed by saline, fluoxetine, piperine respectively.

Methodology

The design of the study is given in Figure 1. The animals are initially divided into 2 groups i.e. naive and status epilepticus (SE). Then each group is further divided into 3 groups of 6 each for administration of vehicle (saline 2 ml/kg), fluoxetine and piperine for a period of 10 days.

Induction of Status Epilepticus

Status epilepticus (SE) was induced in the animals of SE group by administration of pilo-

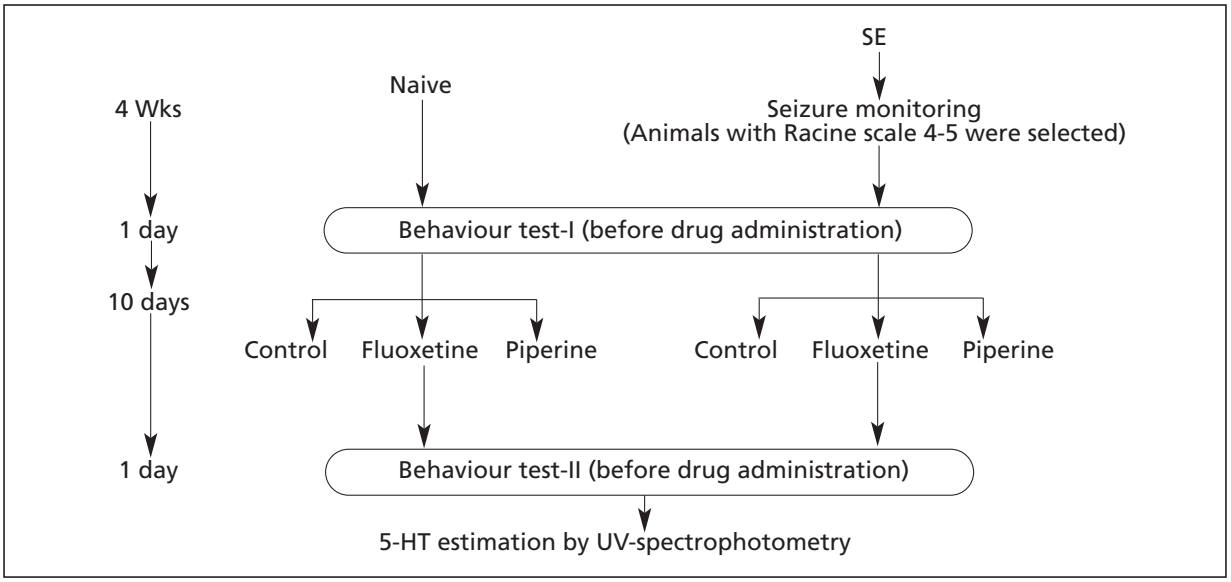

Figure 1. Study design.

carpine (pilocarpine hydrochloride, Himedia, Tocris Bioscience, Eliss, MO, USA) at a dose of 350 mg/kg i.p.. Atropine 1 mg/kg, i.p. was administered 30 min prior to pilocarpine to reduce the peripheral cholinergic effects of pilocarpine. After 3 and 8 hr of seizure onset, rats were injected i.p. with phenytoin (50 mg/kg), in order to alleviate further seizure and to increase survival. Then after four weeks, the rats were assigned a Racine seizure score (RSS) for each 5 min interval over the course of 2 hr session by investigating the behavioural alteration in rats[20,21]. Only the animals which show a Racine seizure score of 4-5 were selected for test in the next week.

Behaviour Test

The following methods were used before (day 0) and after (day 11) administration of vehicle, fluoxetine and piperine for (10 days) in both naive and SE group of animals.

Forced Swimming Test (FST)

The test was performed in the forced swimming test apparatus (INCO). The animal was placed in the apparatus for five minutes. Number of complete rotations made by each animal was noted[22].

Saccharin Consumption Test

The animals had free access to a standard rodent's diet. On the first day, each cage was supplied with two identical graduated water bottles, each containing 250 ml of water. On day two, regular water in one of the bottles was replaced with 0.1% saccharin diluted in water. After 24 hours, test preference was expressed as the volume of saccharin solution of a total volume of fluids (saccharin and regular water) consumed over 24 hrs[23,24].

Actophotometer Test

The animals were placed individually for 10 mins in actophotometer. The activity score on the digital counter were noted[25].

Rotarod Test

Rota rod (INCO) was used in this study. The animal was placed on the rotating rod (20 rpm) and the time taken to fall (in seconds) was noted from the digital counter[26].

Brain Serotonin Estimation

After the behavioural test, the animals were sacrificed and brain homogenate was prepared. The brain serotonin was estimated by the method given by Friend et al. Here, 1-nitroso-2-napthol reacts with many substituted phenols in strong nitric acid to yield coloured derivatives, which can be detected spectrophotometrically (Spectrophotometer, Jasco, Great Dunmow, Essex, UK) at 540 nm and concentration of serotonin, can be estimated from a standard curve[27].

Statistics

Statistical analysis was done by one way ANOVA followed by Dunnet's *t*-test and value of $p < 0.05$ was used as statistical significance. Data are expressed as mean ± SEM. The mean of Group-I (control) was compared with that of Group- IV, to study the effect of pilocarpine before drug administration in each behaviour test. The values obtained in each behaviour test before and after drug administration were compared by Student's *t*-test. Value of $p < 0.05$ was considered statistically significant. Brain serotonin concentration of Gr I was compared with that of Gr II, III and IV and that of Gr IV was compared with that of Gr V and VI.

Results

Forced Swimming Test

The number of rotations of wheel (Mean ± SEM) in forced swimming performance test before and after drug administration are reported in Table I. Pilocarpine (350 mg/kg, i.p.) significantly ($p < 0.05$) reduced the number of rotations as compared to control. Fluoxetine (20 mg/kg, p.o.) and piperine (25 mg/kg, p.o.) both showed significant increase in number of rotation as compared to control. However only piperine (25 mg/kg, p.o.) but not fluoxetine significantly increased the decrease in number of rotation of wheel caused by pilocarpine.

Saccharin Consumption Test

The volume of saccharin consumption (Mean ± SEM) in saccharin consumption test is reported in Table II. Pilocarpine (350 mg/kg, i.p.) significantly ($p < 0.05$) reduced the volume of saccharin consumption as compared to control. Fluoxetine (20 mg/kg, p.o.) and piperine (25 mg/kg, p.o.) both showed significant increase in volume of saccharin consumption as compared to control. However, only piperine (25 mg/kg, p.o.) but not fluoxetine significantly increase the decrease in volume of saccharin consumption caused by pilocarpine.

Table I. Effect of fluoxetine (25 mg/kg) and piperine (20 mg/kg) in normal and pilocarpine (350 mg/kg, i.p.) induced depressed rats using Forced swimming test.

Group			No. of rotation of Wheel (mean ± SEM) before	after
Naive	I	Control	15.34 ± 1.40	15.50 ± 1.23
	II	Fluoxetine	16.16 ± 1.42	20.16 ± 1.56*
	III	Piperine	15.83 ± 1.64	19.33 ± 1.54*
SE (Pilocarpine)	IV	Control	4.83 ± 0.70*	5.83 ± 0.79
	V	Fluoxetine	5.66 ± 0.67	6.83 ± 0.79
	VI	Piperine	6.83 ± 0.70	17.83 ± 1.13*
F			**22.48***	

One way ANOVA followed by Dunnet's *t*-test. Gr-I is compared with Gr-IV. Before and after data of all the groups are compared by Student's *t*-test. *$p < 0.05$. SE: Status Epilepticus.

Table II. Effect of fluoxetine (25 mg/kg) and piperine (20 mg/kg) in normal and pilocarpine (350 mg/kg, i.p.) induced depressed rats using saccharin consumption test.

Group			Volume of saccharin consumption (Mean ± SEM) before	after
Naive	I	Control	52.33 ± 1.47	52.16 ± 1.56
	II	Fluoxetine	53.16 ± 1.55	59.83 ± 2.17*
	III	Piperine	54.33 ± 2.60	58.83 ± 2.48*
SE (Pilocarpine)	IV	Control	19.83 ± 2.16*	18.33 ± 1.72
	V	Fluoxetine	21.16 ± 1.64	21.33 ± 1.25
	VI	Piperine	20.83 ± 1.10	42.83 ± 2.42*
F			**96.21***	

One way ANOVA followed by Dunnet's *t*-test. Gr-I is compared with G-IV. Before and after data of all the groups are compared by Student's *t*-test. *$p < 0.05$.

Table III. Effect of fluoxetine (25 mg/kg) and piperine (20 mg/kg) on locomotor activity in normal and pilocarpine (350 mg/kg, i.p.) induced depressed rats using actophotometer test.

Group			Activity score (Mean ± SEM) before	after
Naive	I	Control	193.33 ± 3.63	191.16 ± 1.83
	II	Fluoxetine	191.67 ± 3.87	249.16 ± 3.40*
	III	Piperine	195.66 ± 3.40	248.33 ± 1.86*
SE (Pilocarpine)	IV	Control	63.16 ± 2.25*	61.83 ± 1.86
	V	Fluoxetine	65.33 ± 2.45	131.66 ± 2.27*
	VI	Piperine	67.16 ± 1.79	124.33 ± 3.48*
F			**30.61***	

One way ANOVA followed by Dunnet's *t*-test. Gr-I is compared with G-IV. Before and after data of all the groups are compared by Student's *t*-test. *$p < 0.05$.

Actophotometer Test

The locomotor activity score (mean ± SEM) in actophotometer are reported in Table III. Pilocarpine (350 mg/kg, i.p.) significantly ($p < 0.05$) reduced the activity score as compared to control. When fluoxetine (20 mg/kg, p.o.) and piperine (25 mg/kg, p.o.) were administered there was significant increase in activity score as compared to control. Both fluoxetine (20 mg/kg, p.o.) and piperine (25 mg/kg, p.o.) significantly increase the decrease in activity score caused by pilocarpine.

Table IV. Effect of fluoxetine (25 mg/kg) and piperine (20 mg/kg) on time to fall on rotarod in normal and pilocarpine (350 mg/kg, i.p.) induced depressed rats.

Group			Time on rota rod (Mean ± SEM) before	after
Naive	I	Control	36.16 ± 2.10	35.33 ± 2.56
	II	Fluoxetine	35.83 ± 2.13	47.16 ± 2.44*
	III	Piperine	37.33 ± 2.02	46.83 ± 2.34*
SE (Pilocarpine)	IV	Control	8.83 ± 1.22*	9.50 ± 0.95
	V	Fluoxetine	9.83 ± 0.94	26.16 ± 2.48*
	VI	Piperine	8.33 ± 0.71	24.83 ± 2.13*
F			**84.91***	

One way ANOVA followed by Dunnet's *t*-test. Gr-I is compared with G-IV. Before and after data of all the groups are compared by Student's *t*-test. *$p < 0.05$.

Rotarod Test

The time taken to fall (mean ± SEM) in rota rod are reported in Table IV. Pilocarpine (350 mg/kg, i.p.) significantly ($p < 0.05$) reduced the time taken to fall as compared to control. When fluoxetine (20 mg/kg, p.o.) and piperine (25 mg/kg, p.o.) were administered to naive animals there was no significant increase in time taken to fall as compared to control. They also significantly reversed the decrease in time taken to fall caused by pilocarpine.

Brain Serotonin Estimation

The concentration of serotonin (mean±SEM) in rat brain is reported in Table V. Pilocarpine (350 mg/kg, i.p.) significantly ($p < 0.05$) reduced the concentration of serotonin as compared to control. When fluoxetine (20 mg/kg, p.o.) and piperine (25 mg/kg, p.o.) were administered to naive animals there was significant increase in concentration of serotonin as compared to control. Again fluoxetine (20 mg/kg, p.o.) and piperine (25 mg/kg, p.o.) significantly reversed the decrease in concentration of serotonin caused by pilocarpine.

Discussion

Depression represents one of the most common co-morbidities in patients with epilepsy. Major depression was significantly more frequent in epileptics with suicide. Major depression might be associated with late age onset of epilepsy, longer treatment duration, polytherapy. Post-Status Epilepticus (SE) model of temporal lobe epilepsy (pilocarpine induced) serves as a model of the co-morbidity of epilepsy and depression[10,28].

The behavioural and biochemical impairments in pilocarpine induced status epilepticus are congruent with depression. The behavioural impairments included increase immobility time under conditions of FST, which represented a model

Table V. Effect of fluoxetine (25 mg/kg) and piperine (20 mg/kg) on brain serotonin concentration in normal and pilocarpine (350 mg/kg, i.p.) induced depressed rats.

Group			Serotonin concentration (µg/gm tissue) (Mean ± SEM)
Naive	I	Control	38.66 ± 2.45
	II	Fluoxetine	49.67 ± 1.20*
	III	Piperine	52.33 ± 1.67*
SE (Pilocarpine)	IV	Control	14.83 ±1.90*
	V	Fluoxetine	30.83 ± 1.42*
	VI	Piperine	28.50 ± 1.58*
F			**64.52***

One way ANOVA followed by Dunnet's *t*-test. Gr-I is compared with Gr-IV. Before and after data of all the groups are compared by Student's *t*-test. *$p < 0.05$.

equivalent of despair and the loss of test preference under conditions of saccharin consumption test, which suggested the development of anhedonia like symptomatology. The biochemical impairements included compromised serotonergic transmission in brain[28].

Decreased monoaminergic (serotonergic, dopaminergic and noradrenergic) and GABAergic functions have been identified as pivotal mechanisms of depression and have been basis for antidepressant drugs. Again, decreased activity of same neurotransmitters has been shown to facilitate the kindling process of seizure foci to exacerbate seizure severity and to intensify seizure predisposition in same animal models of epilepsy. So, epilepsy and depression may share common pathogenic mechanisms and, hence, the occurrence of one may facilitate the development of the other and viceversa[29-33].

The novel highly selective and reversible MAO-A inhibitors proved to be an effective anticonvulsant in the kindling model of temporal lobe epilepsy[15]. The widely used antidepressant Specific Serotonin Reuptake Inhibitors (SSRI) has been tried with success as anticonvulsants in cases of nonsymptomatic epilepsy. Antidepressant drugs of the SSRI and MAO inhibitors are safe in patients with epilepsy. MAOIs are efficacious, well tolerated and associated with a low incidence of seizures in patient with depression[34,35].

Piperine possesses potent antidepressant like properties that are mediated in part through the inhibition of MAO activity and, therefore, represents a promising pharmacotherapeutic candidate as antidepressant agent. In our investigation piperine like fluoxetine is found to be an effective antidepressant. Our study shows that pilocarpine significantly reduced the number of rotation in FST. Fluoxetine is resistant to such effect of pilocarpine. However, piperine was able to significantly increase the number of rotations decreased by pilocarpine in FST[36].

Loss of taste preference observed in post-SE rats represents a behavioural symptom of depression in animal models during chronic epileptic state. Fluoxetine has no effect on loss of taste preference on post SE rats[1,25], whereas piperine reversed the loss of taste preference caused by pilocarpine.

Deficits in motor co-ordination and increase in activity could invalidate conclusion drawn from the forced swimming test. Therefore, the effect of various treatments on motor co-ordination was assessed using rota rod apparatus. Piperine as well as fluoxetine improved the compromised motor activity and co-ordination caused by pilocarpine[37]. Again rotarod test is a basis of study of neurotoxicity of a drug. Pilocarpine significantly reduced the duration for which animal stayed on the rotarod, suggesting it's neurotoxic effect[38]. Co-administration of piperine (like fluoxetine) prevents this effect of pilocarpine as shown by an increase in duration, so piperine may possess neuroprotective effect.

Again, pilocarpine decreased the locomotor activity score which was prevented by co-administration of fluoxetine as well as piperine. Piperine like fluoxetine increases locomotor activity as compared to control[39,40]. This suggests that pilocarpine depress the central nervous system and piperine may possess excitatory action on central nervous system.

Fluoxetine is a selective serotonin reuptake inhibitor. The finding that the behavioural equivalents of depression were resistant to fluoxetine suggested that depression in epilepsy might have other mechanisms beyond alterations in serotonergic pathways[28]. However, piperine shows significant antidepressant activity in post SE rats.

Piperine is a MAO inhibitor and increases the monoaminergic transmission like noradrenergic, dopaminergic in addition to serotonergic transmission[41]. Increase in serotonergic transmission was observed in our study. Piperine and two of its derivatives, antiepilepsirine and compound 7448 are very effective in stimulating serotonin synthesis[42]. Again, piperine is effective against maximal electroshock seizure in mice[17]. It was shown to significantly block convulsions induced by intracerebroventricular injection of threshold doses of kainate. However, although piperine did block convulsions, induced by kainate, the compound does not appear to act as a kainate receptor antagonist[16].

So the underlying mechanism behind depression in epilepsy may be alteration in monoaminergic pathways and GABAergic pathways. The antidepressant activity of piperine in post-SE rats may be attributed to its MAO inhibitor activity and neuroprotective activity.

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