Int J Pharm Bio Sci 2015 Oct; 6(4): (P) 119 - 127

**Research Article** **Pharmaceutics**

**International Journal of Pharma and Bio Sciences** **ISSN 0975-6299**

# THE POSSIBLE PATHWAYS OF ANTI-DEPRESSANT ACTIVITY OF NARINGENIN IN ALBINO MICE USING FORCED SWIM TEST MODEL OF DEPRESSION

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## ABSTRACT

Aim To evaluate the possible pathways of the anti-depressant activity of naringenin (5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one) in albino mice using the forced swimming test model. Material and methodsThe mechanism of action involved in the anti-depressant activity of naringenin in various doses (20mg/kg &50mg/kg,p.o.) was investigated by observing the effect of naringenin after pre-treatment with fluoxietine prazosin and haloperidol. para-chlorophenylalanine (p-CPA) was also used as a serotonin synthesis inhibitor .Results The results of forced swim test of naringenin significantly attenuated the duration of immobility induced by fluoxietine(15mg/kg/i.p. selective serotonin reuptake inhibitor) ,Haloperidol (0.2mg/ kg, i.p., a classical D2-like dopamine receptor antagonist) and, Prazosin (1mg/kg, i.p., an α-1-adrenoceptor antagonist) and p-chlorophenylalanine (100 mg/kg, i. p, × 3 days; an inhibitor of serotonin synthesis) .Conclusion It can be concluded that naringenin possess potential antidepressant activity (through dopaminergic, noradrenergic and serotonergic mechanisms) and has therapeutic potential in the treatment of CNS disorders

**KEYWORDS: Depression, Forced swimming test, Naringenin, Serotonin, Nor- adrenalin Dopamine.**

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# INTRODUCTION

Depression is a worldwide devastating and prevalent disease which is very difficult to cure. Depression means low mood, lack of emotion in daily life and loss of interest in favourite activity[1]. It has a negative effect on a person's thoughts, behavior, and feelings. The underlying cause of depression is due to the decrease of extracellular concentration of monoamine neurotransmitters (serotonin, nor adrenaline. dopamine) [2] . Currently available potent synthetic antidepressant drugs produce enormous side effects such as insomnia, excessive sleeping, fatigue, loss of energy, aches and digestive problems[3]. A number of compounds isolated from plants have been claimed to be effective in the treatment of depression and are considered with less side effects and a wider safety margin. Flavonoids are a group of compounds with enormous therapeutic potential[4]. Flavonoids like anthocyanins and quercetin have been implicated in antidepressant activity and anxiolytic activity[5]. Naringenin, a bioflavonoid is reported to possess several medicinal properties such as antioxidant, anti-inflammatory, immune-modulator, hepato protective and neuro-protective effect[6]. An earlier study reported that naringenin has produced antidepressant-like action in tail suspension test and forced swim test[7].The available literature highlights only the role of naringenin as a potent antidepressant. In the present study we decided to delineate the possible mechanisms involved in the anti depressant activity of naringenin by employing forced swim test through different pathways. Naringenin was orally administered to human volunteers at a dose of 500mg with no adverse responses[8,9]and also the present study highlights the advantage of naringenin as compared to the available synthetic antidepressants.

# MATERIALS AND METHODS

### *Animals*

The animal study was carried at the Centre for Toxicology and Development Research (CEFT) Lab a unit of Sri Ramachandra University, Chennai. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC). Only male swiss albino mice were selected for forced swimming test (FST). The male Swiss albino mice (18-23g / 6-8 weeks) were separated and quarantined for a week. All mice were kept under controlled conditions of temperature (22±3°C) and humidity (30-70%). They had free access to food and water ad *libitum.* Behavioral experiment was performed between 9:30 and 14:30 am.

### *Drugs and chemicals*

Prazosin (Sigma-Aldrich, USA), p-CPA (Sigma-Aldrich, USA), Haloperidol (Serenec, Searl, India) Fluoxietin( Eli Lilly and Co ), Venlafaxine(Pfizer. )were used for this study.

### *Drug administration in animals*

Different group of mice, 6 animals per group, were used for the drug treatment for each test. All the standard drugs were administered by i.p route using a suitable solvent. The test drug naringenin and vehicle treated group was administered by oral route; DMSO was used as a solvent.

### *Forced swim test[10].*

Mice were forced to swim individually in a plexi-glass jar (25 X12 X 25 $cm^3$) containing fresh water of 15 cm height and maintained at 25°C. After an initial period (2minutes) of vigorous activity, each animal assume a typical immobile posture. A mouse was considered to be immobile when it remains floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. A decrease in duration of immobility is indicative of an antidepressant effect. The total duration of immobility was recorded during the 4 min test. The change in immobility duration was studied after administering drugs in separate groups of animals.

### *Involvement of neurotransmitters*

***To assess the involvement of the serotonergic system in the antidepressant like effect of naringenin[11].***

In order to investigate the possible contribution of Naringenin on serotonergic system, mice were pretreated with PCPA (100 mg/kg, a serotonin synthesis inhibitor), once daily for four consecutive days. On the fifth day i.e 24 hrs after the last (PCPA)100mg/kg/i.p The animals received naringenin (20mg/kg/p.o, 50mgkg/p.o) in group IV and V and in III group fluoxetine (15mg/kg/i.p) with DMSO (10ml/kg/p.o) and was subjected to forced swimming test 45 min later .The treatment schedule is mentioned below

Group I: Control group received 10ml/kg DMSO p.o
Group II: Group received PCPA 100ml/kg / i .p.
Group III: Group received PCPA 100ml/kg / i .p. + fluoxetine (15mg/kg/i.p.)
Group IV: Group received PCPA 100ml/kg/ i.p.+ Naringenin (20mg/kg/p.o).
Group V: Group received PCPA100ml/kg/ i.p. + Naringenin (50mg/kg/p.o)

***To assess the involvement of the dopaminergic system in the antidepressant like effect of naringenin[12].***

To ascertain the antidepressant-like effect of test drug Naringenin mediated through dopaminergic system, animals were pretreated with haloperidol (0.2 mg/kg, i. p). a nonselective Dopaminergic receptor antagonist,15 min before administration of the drug and then subjected to forced swimming test after 45mins and the treatment schedule is mentioned as below

Group I: Control group received 10ml/kg DMSO p.o
Group II: group received haloperidol, (0.2 mg/kg, i.p)
Group III group received haloperidol (0.2 mg/kg, i.p) + **v**enlafaxine (10mg/kg/i.p).
Group IV: group received haloperidol(0.2 mg/kg, i.p) + Naringenin (20mg/kg./p.o)
Group V: group received haloperidol (0.2 mg/kg, i.p)+ Naringenin (50mg/kg.p.o)

***To assess the involvement of the nor adrenergic system in the antidepressant like effect of naringenin[13]***

To investigate the possible role of Naringenin on noradrenergic system, animals were pretreated with prazosin (1 mg/kg, i.p.), 15 min before administration of naringenin (20mg,50mg/kg//i.p) and venlafaxine (10mg/kg /i.p), and were subjected to forced swimming test 45min later and the treatment protocol is mentioned as follows.

Group I: Control group received 10ml/kg DMSO p.o
Group II: group received Prazosin, 1 mg/kg, i.p.
Group III: group received Prazosin (1 mg/kg, i.p.) + venlafaxine (10mg/kg/i.p).
Group IV: group received Prazosin (1 mg/kg, i.p.) + Naringenin. (20mg/kg/p.o)
Group V: group received Prazosin (1 mg/kg, i.p.)+ + Naringenin (50mg/kg.b.wt)

***Statistical analysis***

The Mean ± SEM values were calculated for each group. The data were analyzed using one-way ANOVA and followed by Dunnet's test. The values were significant at $P < 0.05$ when compared with control group.

## RESULTS

***Effect of compounds pretreated with pCPA on immobility period of mice using Forced Swim Test***

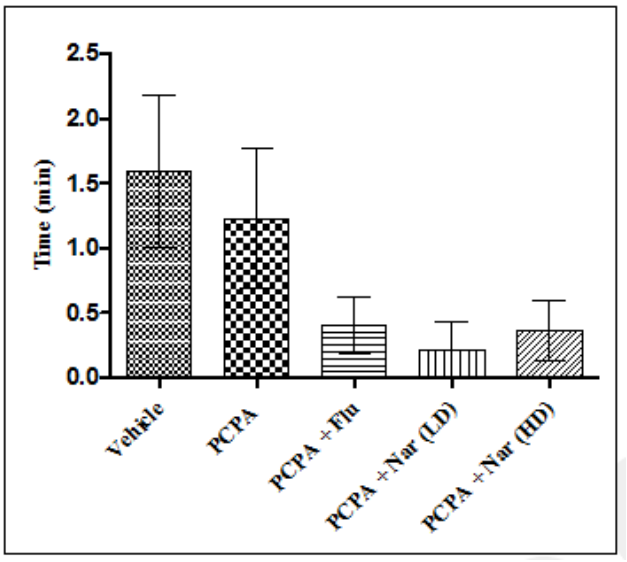
Treatment with p-CPA (100 mg/kg, i.p.) alone significantly ($P < 0.05$) increased the immobility period when compared to the vehicle treated group, whereas, treatment with fluoxetine (15mg/kg/ i. p) and naringenin (20 mg/kg, p.o) significantly reversed the increased immobility

time. However treatment with high dose of naringenin (50mg/kg/i.p) did not significantly reverse the effect after treatment with p-CPA in FST [Table 1].

| ***Group*** | ***Immobility time of Force swimming Test Time (sec)*** |
|---|---|
| | ***Mean±SEM*** |
| *Group I*<br>*Vehicle treatment* | *83±0.54* |
| *Group II*<br>*PCPA, 100 mg/kg, i.p* | *120±0.59* |
| *Group III*<br>*PCPA + Fluoxetine (15mg/kg /i.p.)* | *41±0.22* * |
| *Group IV*<br>*PCPA + naringenin (20mg/kg.i.p)* | *22±0.22** |
| *Group V*<br>*PCPA + naringenin (50mg/kg./i.p)* | *70±0.39* |

***Each value represents the mean ± SEM of six observation***
**** p < 0.05 as compared with vehicle treatment ( one way ANOVA followed by Dunnett's test). p***

**Graph 1**
***Effect of compounds pretreated with pCPA on immobility period of mice using Forced Swim Test***




***2 Effect of compounds pretreated with prazosin on immobility period of mice using Forced Swim Test.***

In Noradrenergic pathway the 5th group showed more significant ($p>0.05$) of naringenin action with Prazosin in high dose(50mg/kg.b.wt) in reduction of immobility duration. The standard error of mean was 0.44±0.05.Which is least compared to all (Table-2 and Graph-2).Third and fourth group are also significant compare to control.

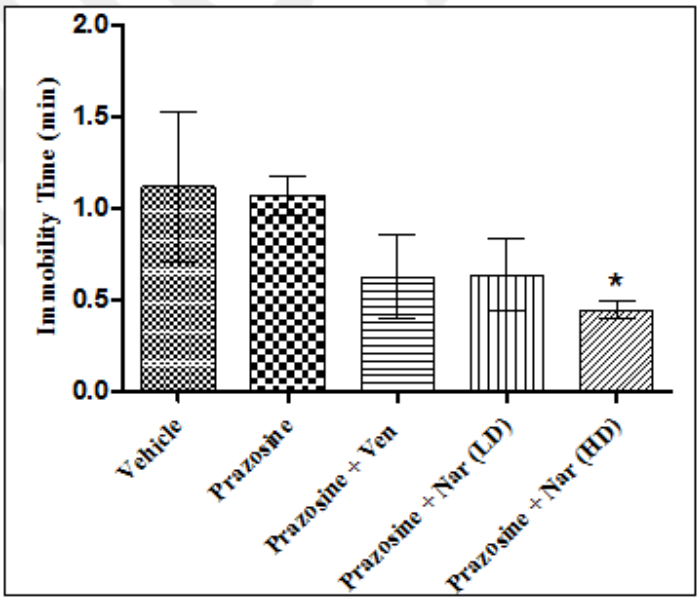
**Table 2**

| Group | Immobility time of Force swimming Test Time ( sec) Mean±SEM |
|---|---|
| **Group I** | **67±0.11** |
| **Group II** | |
| Prazosin, 1 mg/kg, i.p | **103±0.46** |
| **Group III** Prazosin+ Venlafaxine (10mg/kg b.wt) | **63±0.23*** |
| **Group IV** Prazosin+ naringenin (20mg/kg.)) | **64±0.20*** |
| **Group V** *Prazosin + naringenin (50mg/kg.) | **44±0.55*** |

***Each value represents the mean ± SEM of six observation***
**** p < 0.05 as compared with vehicle treatment one way ANOVA followed by Dunnett's test).***

**Graph 2**

***Effects of compounds pretreated with prazoin on immobility period of mice using Forced Swim Test***




***3 Effect of compounds pretreated with Haloperidol on immobility period of mice using Forced Swim Test.***

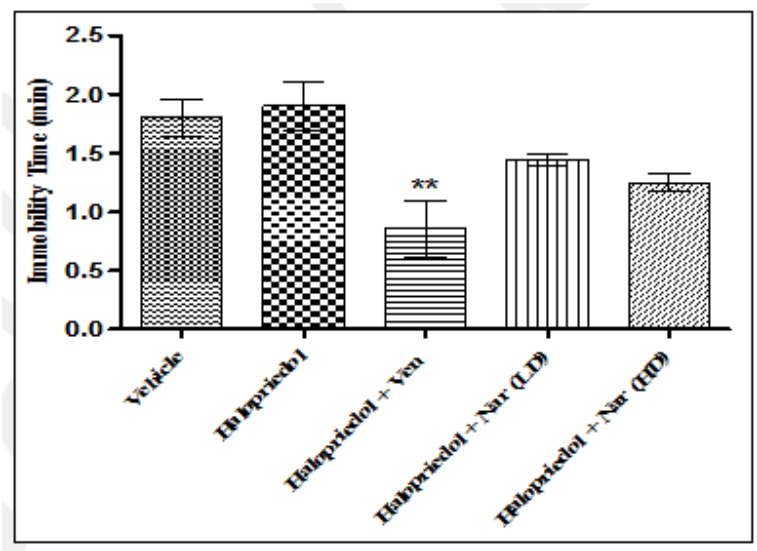
In table-3, Haloperidol and naringenin with low dose and high dose both the groups showed significant reduction of immobility time. But the 5$^{th}$ group showed more significant.

**Table 3**

| Group | Immobility time of Force swimming Test Time ( sec) |
|---|---|
| | Mean±SEM |
| Group I | 143±0.13 |
| Group II | 150±0.21 |
| Group III | 86±0.24* |
| Group IV | 104±0.05* |
| Group V | 85±0.07* |

*Each value represents the mean ± SEM of six observation*
** p < 0.05 as compared with vehicle treatment one way ANOVA followed by Dunnett's test).*

**Graph 3**
***Effect of compounds pretreated with haloperidol immobility period of mice using Forced Swim Test***




## DISCUSSION

The FST remains one of the most common animal models used for screening potential antidepressant agents[14]. In this test a state of immobility in animals facing an inescapable situation (i.e immobility behavior) is induced. This has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorders in humans. Therefore, the antidepressant-like activity of a compound is expressed by a decrease in the immobility of animals submitted to forced swimming[15]. This behavioral change is sensitive to major classes of antidepressant drugs. Depression has been associated with impaired neurotransmission of serotonin, nor- epinephrine and dopamine[16] . The current treatment for major depression is to improve serotonin, dopamine and nor-adrenalin neurotransmission[17]. Three common standard antidepressant drugs involved in different pathways of neurotransmission had been chosen to perform these experiments with the drug of interest naringenin. Selective serotonin

reuptake inhibitors are a group of drugs used to promote an increase in serotonin levels. SSRIS act by directly inhibiting serotonin reuptake, increasing serotonin turnover in the brain, and also interacts with 5-HT receptors. PCPA (an inhibitor of tryptophan hydroxylase) administrated to mice for 4 consecutive days is able to deplete the endogenous stores of 5-HT (by about 60%)[18] In the present study, pretreatment of mice with PCPA significantly increased the duration of immobility which was blocked by drug Fluoxietin and naringenin, suggesting a probable involvement of these receptors in its antidepressant-like activity. The classical antidepressant Fluoxietin is a first line of antidepressant, and third-most-prescribed drug in the United States[19]. The results of pretreatment of low dose of naringenin (20mg/kg) showed a highest significant change in FST when compared to group pretreated with standard drug fluoxietin. Apart from the decrease in serotonin level the state of depression seems to also be associated with a hypo function of the noradrenergic system. Some antidepressants act by increasing the synaptic availability of nor-epinephrine. In this context, the α -adrenoceptors have been shown to underline some of the antidepressant-like responses of drugs in behavioral models of depression[20].Prazosin (an α1-adrenoceptor antagonist, sympatholytic drug) reduces high blood pressure and anxiety21, PTSD, and panic disorder. Receptors were found on vascular smooth muscle and in CNS .It is responsible for the vasoconstrictive action of nor epinephrine. The depressed people with hypertension are treated with Venlafaxine and prazosin, the ideal combination for treating the case in clinic. Venlafaxine**,**serotonin-norepinephrine reuptake inhibitor (SNRI) increases the concentrations of serotonin and norepinephrine in the brain, introduced by Wyeth in 1993. But it elevates supine diastolic blood pressure (SDBP) as side effects. In nor adrenergic pathway, the fifth group treated with prazosin and high dose of naringenin (50mg/kg b.w) showed a significant reduction in immobility time compare to other groups.It is well established that the dopaminergic system, may also be implicated in the regulation of mood[22] Currently, there is evidence from several reports regarding the efficacy of antidepressants related to the potentiation of dopaminergic neurotransmission in the treatment of depression[23]. Our results showed that the dopaminergic system was also involved in the anti-immobility effect of Naringenin through an interaction with Dopamine receptors. In this result both 4$^{th}$ &5$^{th}$ group are significant compared to control, but naringenin in high dose with haloperidol in the 5$^{th}$ group was more significant. Haloperidol (dopamine D2 receptor antagonism) is a typical first generation antipsychotic medicine; it manages delusions, hallucinations or disordered thoughts. It is also used in schizophrenia and bipolar disorder and Psychotic depression but with major side effects like extra pyramidal disorder symptoms. [24] To summarize the result, it may be suggested that the antidepressant like effect of naringenin in the above tested doses may be mediated through serotongenic, dopaminegic and non adrenergic pathway. To understand the action of definite pathway specifically, the study should be extented still more elaborately with different other pathways in future study.

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