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## Evaluation of the effects of silymarin on behavioural models of depression in albino mice

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### Abstract

**Objectives:** To evaluate the antidepressant activity of silymarin compared to desipramine and fluoxetine in albino mice. **Methods:** Total of 72 albino mice (N=72) were divided into 6 treatment group (n=6) for each model of depression and were treated with normal saline 0.1ml/kg (control), desipramine 30mg/kg(standard), fluoxetine 20mg/kg(standard), silymarin 200 mg/kg, 250 mg/kg and 300 mg/kg (test drug) fed orally to each group respectively. Duration of immobility was observed for 6 minutes in tail suspension test and for 6 minutes in forced swimming test in the differently grouped animals. Results were compared among the different groups using (ANOVA) followed by post hoc Tukey's test. **Results:** In the forced swimming model the immobility time (mean  $\pm$  standard deviation) was 108.6  $\pm$  19.3 sec, 16.5  $\pm$  4.4 sec, 86.6  $\pm$  6.2 sec, 99.8  $\pm$  15.3 sec, 76.8  $\pm$  10.8 sec, 56  $\pm$  6.5 sec in the control, desipramine, fluoxetine, silymarin at 200 mg/Kg, 250 mg/Kg and 300 mg/Kg respectively. In the tail suspension test the immobility time (mean  $\pm$  standard deviation) was 170.8  $\pm$  28.8 sec, 153.1  $\pm$  12.0 sec, 128.8  $\pm$  9.0 sec, 159.5  $\pm$  24.0 sec, 140.5  $\pm$  16.4 sec, 125.6  $\pm$  38.2 sec in the control, desipramine, fluoxetine, silymarin at 200 mg/Kg, 250 mg/Kg and 300 mg/Kg respectively. Silymarin at 300 mg/Kg showed significant reduction in the immobility time in these models of acute depression compared to control and fluoxetine. (p <0.05). **Conclusion:** Silymarin has significant antidepressant activity in animal models.

### 1. INTRODUCTION

Mood disorders encompass a large group of psychiatric disorders. Major Depressive disorders (MDD) is the most common mood disorder.<sup>1</sup> According to Diagnostic and statistical manual of mental disorder (DSM) IV, a major depressive disorder occurs without history of mania, mixed or hypomanic episode. A MDD must last atleast 2 weeks and typically a person with a diagnosis of MDD also experiences at least 4 symptoms from a list that includes changes in appetite and weight, changes in sleep and activity, lack of energy, feelings of guilt, problem thinking and decision making and recurring thoughts of suicide or death.<sup>2</sup>

Individuals with major mood disorders are at an increased risk of having one or more additional comorbid disorders. The most frequent disorders are alcohol abuse or dependence, panic disorder, obsessive-compulsive disorder (OCD), and social anxiety disorder.<sup>1</sup> Conversely individuals with substance use disorders and anxiety disorders also have an elevated lifetime risk of comorbid mood disorders.<sup>3</sup>

Investigations into the neurobiology of major depressive disorder have traditionally focused on the monoamine neurotransmitters serotonin and norepinephrine. The monoamine hypothesis states that depressed individuals are likely to have low levels of these neurotransmitters because various antidepressant drugs acutely increase their levels.<sup>4</sup> Few studies also suggest that oxidative damage to macromolecules such as lipid, protein and nucleic acids as a result of excessive reactive oxygen species lead to neuronal dysfunction that is associated with the development of depression disorder<sup>5</sup>

Although, several classes of antidepressants like selective serotonin reuptake inhibitors, tricyclic antidepressants, atypical antidepressants, MAO-A inhibitors are currently being used, due to clinical limitations and adverse effects and delayed onset of action there is critical interest in development of efficient and safe drugs for treatment of depression.<sup>6</sup>

Silymarin is a flavonolignan, a mixture of silybin (major constituent), isosilybin, silydianin and silychristin. *Silybum marianum*, seeds have been used for over 2000 years as remedy for several diseases especially for liver. Silymarin and its constituents (mainly silibinin) act as antioxidant and hepatoprotective; effective in treating toxin poisoning. In some animal models silymarin was found to be useful in prevention and treatment of neurodegenerative and neurotoxic processes due to its antioxidant effects.<sup>7</sup>

The objective of this study is to make attempt to evaluate the antidepressant activity of silymarin in standard models for depression in albino mice using desipramine and fluoxetine as standards.

## 2. MATERIALS AND METHODS

**Animals:** Male swiss albino mice weighing 20-30g aged between 3-4 months which are healthy with normal behaviour and activity were procured from central animal house of Department of Pharmacology, JJM Medical College, Davangere. Mice were kept under suitable conditions of housing, temperature (26-28°C), ventilation and nutrition. However food was withdrawn 1hr before and 2hr after the administration of the drugs. The animals were housed in an animal house with alternatively light-dark cycle of 12 hr each.

The animals were acclimatized to the laboratory conditions for at least five days prior to the behavioral experiments. The experiments were carried out between 0900 h and 1800 h. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol. Care of laboratory animals was in adherence with the guidelines specified by the CPCSEA, Ministry of Forests and Environment, Government of India. (Registration number 0436).

A total of 72 animals (N=72) were used. Different set of animals were used for the two models. Each model consisted 6 groups of 6 mice each (n=6). Each group were housed in different cages.

### 2.1 Drugs and chemicals

Normal saline used as a control in the dose of 0.1ml/kg. Desipramine at a dose of 30 mg/kg and fluoxetine at 20mg/kg were taken as a standard drugs and test drug silymarin at different doses of 200mg/kg, 250mg/kg and 300mg/kg. Both the test drug and standard drugs were dissolved in distilled water to make them suitable for administration.

All the drugs were fed orally one hour before testing for immobility. Animals were subjected to two laboratory models employed for testing antidepressant activity:

**Forced-swim test (FST):** Forced swim test was proposed as a model to test antidepressant activity by Porsolt et al. The method was the same as described by Dhingra and Sharma. Mice were forced to swim individually in a glass jar (25 x 12 x 25 cm<sup>3</sup>) containing fresh water up to 15 cm height and maintained at 25°C ( $\pm 3^\circ\text{C}$ ). After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture.

A mouse 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 next 4 min of the total test duration of six minutes. The changes in immobility duration were studied after administering the drugs in separate groups of animals. Antidepressants significantly reduce the immobility duration. Each animal was used only once.<sup>8</sup>

**Tail-suspension test (TST):** The total duration of immobility induced by tail suspension was measured according to the method described as a means of evaluating potential antidepressants. Mice were suspended on the edge of a table, 50 cm above the floor, with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. The animal was considered to be immobile when it did not show any movement of the body and hanged passively.<sup>8</sup>

## 3. RESULTS

The mean duration of immobility after silymarin administration orally in both the models was comparing to the control and standards (desipramine and fluoxetine). The summarized results are given in **Table 1**. The data were analyzed by means of one-way ANOVA. Statistically significant results were followed by post-hoc tukey's test to know the intergroup significance.  $P < 0.05$  was considered to be statistically significant.

### 3.1 Forced Swim model

In our study, there was a highly significant reduction in immobility period with standard, desipramine ( $16.5 \pm 4.4$ ),  $p < 0.01$  and fluoxetine ( $86.6 \pm 6.2$ ),  $p < 0.05$  when compared to control ( $108.6 \pm 19.3$  sec). All the three groups of test drug i.e., silymarin 200mg/kg group ( $99.8 \pm 15.3$  sec), silymarin 250 mg/kg group ( $76.8 \pm 10.8$  sec), silymarin 300 mg/kg group ( $56 \pm 6.5$  sec), also showed reduction in the immobility time. Compared to the control group except silymarin 200mg/kg which was insignificant, the other two test groups i.e., silymarin 250 mg/kg group and silymarin 300 mg/kg group showed highly significant ( $p < 0.01$ ) reduction in immobility duration. The desipramine group showed greater reduction in the immobility than all the test groups. Silymarin only in the highest dose (300mg/kg) was comparable to the fluoxetine group. (Figure 1).

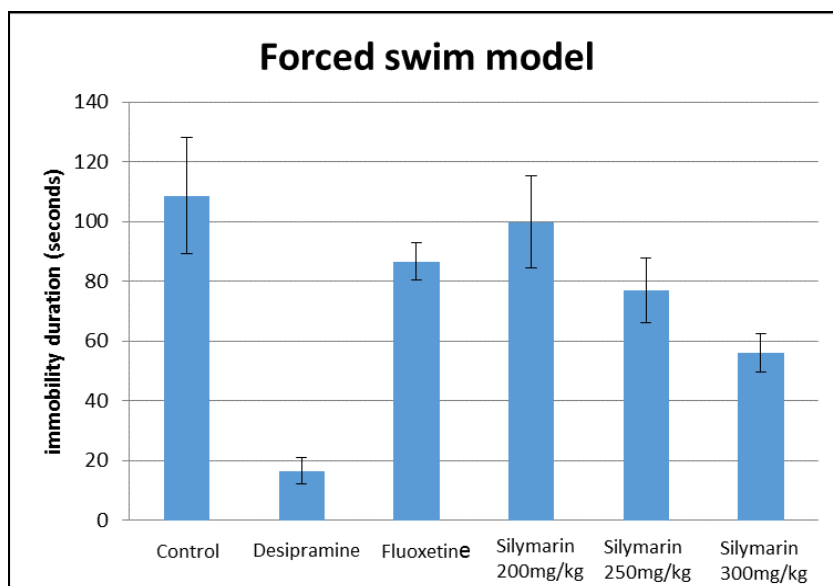
### 3.2 Tail suspension model

In this model, there was no significant reduction in immobility period with standard drug, desipramine ( $153.1 \pm 12.0$  sec) when compared to control ( $170.8 \pm 28.8$  sec) whereas fluoxetine ( $128.8 \pm 9.0$ ) showed significant reduction in immobility duration. With silymarin 200 mg/kg, the duration of immobility was  $159.5 \pm 24.0$  sec, with a dose of 250 mg/kg it was  $140.5 \pm 16.4$  sec and with 300 mg/kg it was  $125.6 \pm 38.2$  sec. When the test groups were compared with the control group only silymarin 300mg/kg showed significant reduction ( $p < 0.05$ ) in immobility time. The results of all the three test groups were found to be insignificant when compared to the two standard groups i.e., desipramine and fluoxetine. (Figure 2).

**Table 1: The results of both the models summarized**

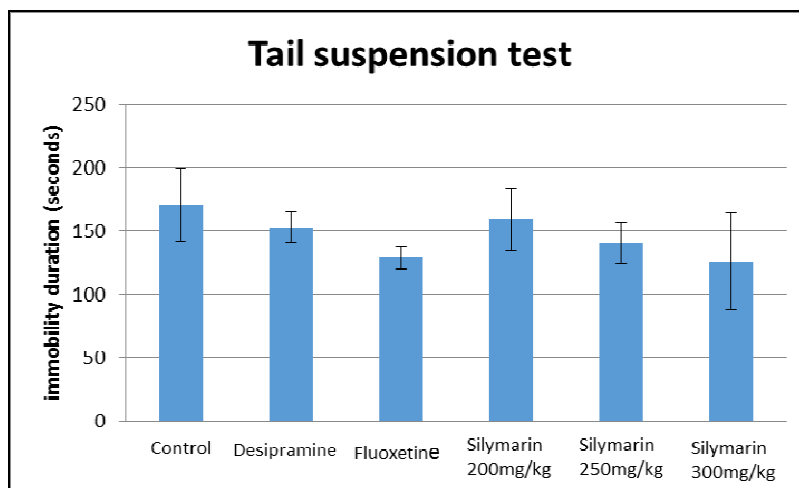
Group		Forced swimming model (in seconds $\pm$ SD )	Tail suspension model (in seconds $\pm$ SD )
1. Control		108.6 $\pm$ 19.3	170.8 $\pm$ 28.8
2. Desipramine 30 mg/kg		16.5 $\pm$ 4.4	153.1 $\pm$ 12.0
3. Fluoxetine 20 mg/kg		86.6 $\pm$ 6.2	128.8 $\pm$ 9.0
4. Silymarin 200 mg/kg		99.8 $\pm$ 15.3	159.5 $\pm$ 24.0
5. Silymarin 250 mg/kg		76.8 $\pm$ 10.8	140.5 $\pm$ 16.4
6. Silymarin 300 mg/kg		56 $\pm$ 6.5	125.6 $\pm$ 38.2
ANOVA	F	49.2160	3.3967
	P	< 0.05	0.015
Difference between groups (p value)	1 and 2	** p<0.01	insignificant
	1 and 3	* p<0.05	* p<0.05
	1 and 4	insignificant	insignificant
	1 and 5	** p<0.01	insignificant
	1 and 6	** p<0.01	* p<0.05
	2 and 3	** p<0.01	insignificant
	2 and 4	** p<0.01	insignificant
	2 and 5	** p<0.01	insignificant
	2 and 6	** p<0.01	insignificant
	3 and 4	insignificant	insignificant
	3 and 5	insignificant	insignificant
	3 and 6	** p<0.01	insignificant
	4 and 5	* p<0.05	insignificant
	4 and 6	** p<0.01	insignificant
5 and 6	* p<0.05	insignificant	

The p value of < 0.05 was considered significant (\*) and a p < 0.001 (\*\*) was considered highly significant; a value of p > 0.05 (NS) was considered insignificant.



**Fig 1: Results of Forced Swimming Model**

Each histogram represents mean duration of immobility in seconds (n=6). Vertical line on top represents SD. The standards showed significant reduction in immobility compared to control group (desipramine > fluoxetine). The test drugs showed dose dependant reduction in immobility.



**Fig 2: Results of Tail Suspension Model**

Each histogram represents mean duration of immobility in seconds (n=6). Vertical line on top represents SD. Immobility time of fluoxetine showed significant reduction compared to control. Among test drug, only silymarin 300mg/kg group showing significant reduction compared to control group.

#### 4. DISCUSSION

In this study two widely used models to screen antidepressant activity i.e., forced swim model and tail suspension model are used to evaluate antidepressant activity of silymarin. TST and FST represents the behavioural despair model, claimed to reproduce a condition similar to human depression. FST behaviour despair test is most frequently used as an authentic animal model of depression to screen antidepressants as well as to explore the underlying mechanism of action of antidepressant.<sup>9</sup>

Studies done on animals (mice) with silymarin have shown that they increase the level of monoamines like serotonin, norepinephrine and dopamine in the brain<sup>10</sup>. It also has cytoprotective activities due to its antioxidant activity and radical scavenging properties.<sup>11,12</sup>

According to pharmacological studies, silymarin has been considered as a safe herbal product when adequate therapeutic dosages are used. Silymarin acceptability is good and mild gastrointestinal disturbance and mild allergic reactions, nausea, headache, joint pain, and mild laxative symptoms have been reported.<sup>11</sup>

In previous similar studies evaluating antidepressant activity of silymarin by Upama Sharma et al., serotonergic effect of silymarin was studied by comparing with fluoxetine. In our study both the serotonergic and its noradrenergic activity is studied comparing with fluoxetine and desipramine respectively.

Silymarin showed reduction in immobility duration suggesting its antidepressant like activity in these acute models of depression. It produced significant decrease in immobility time at all three doses in forced swim model but not better than that of standard desipramine whereas results with silymarin 300mg/kg showed significant results than standard fluoxetine. In antidepressant activity using tail suspension model, only standard fluoxetine and silymarin at 300mg/kg showed significant reduction in immobility duration compared to control.

Desipramine acts predominantly by inhibiting the norepinephrine reuptake and fluoxetine by inhibiting the serotonin reuptake.<sup>13</sup> In our study silymarin showed reduction in immobility duration in the forced swim model when compared with desipramine also suggesting, silymarin shows some increase in norepinephrine levels also.

The reduction in immobility in forced swim model when compared with both standards, desipramine and fluoxetine suggesting silymarin's antidepressant like activity. However results of tail suspension model require investigating using more models supporting its antidepressant activity.

## 5. CONCLUSION

Silymarin showed good antidepressant activity as shown in forced swim model. This suggests the silymarin probably acts by increasing monoamine levels and also due to its antioxidant activity it provides protection against depression caused by oxidative stress. The antioxidant property can also provide protection against various organ damage due to substance abuse in depressed individuals. Also as silymarin is clinically used widely for its hepatoprotective property it can be beneficial in depressed individuals who are alcoholics. Further evaluation with a bigger sample size can substantiate its antidepressant activity.

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