



## Evaluation of Antidepressant Like Activity of *Crocus sativus* Linn Stigmas In Mice

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Accepted on: 19-09-2013; Finalized on: 30-11-2013.

### ABSTRACT

*Crocus sativus* linn stigmas (Saffron) is believed to show antidepressant like activity. The present study was taken up to evaluate its antidepressant like activity, to compare it with standard imipramine and to investigate its combined effect with submaximal dose of imipramine in mice. Two validated behavioral animal models of anti depressant activity viz, forced swimming test (FST) and tail suspension test (TST) were used. Mice were divided into 8 groups of 6 animals each. Saffron was given at the doses of 200, 400 and 800mg/kg i.p and potentiation was investigated with concomitant administration of submaximal dose of imipramine (7.5mg/kg i.p) and was compared with standard dose of imipramine (15mg/kg). Immobility time (IT) was calculated in both the models. Saffron at various doses showed significant reduction in immobility time when compared to control. Immobility time of combination of saffron with submaximal dose (7.5 mg/kg) of imipramine was significantly reduced on comparison with control and it was comparable to standard dose of imipramine (15mg/kg) in both FST and TST. *Crocus sativus* linn stigmas showed significant antidepressant like activity on its own and also added to the action of submaximal dose of imipramine. Hence it can be considered as potential antidepressant and can be further investigated for the same.

**Keywords:** Antidepressant, Imipramine, Saffron, Submaximal Imipramine.

### INTRODUCTION

Major depression is defined as depressed mood on a daily basis for a minimum duration of 2 weeks.<sup>1</sup> Depression and anxiety disorders are the most common mental illnesses, each affecting in excess of 10-15% of the population at some time in their lives.<sup>2</sup> At present 121 million people in the world are estimated to suffer from depression.<sup>3</sup>

It is responsible for the largest proportion of disease burden attributable to non-fatal health outcomes, accounting for almost 12% of total years lived with disability worldwide. It has been estimated that 15% of patients with severe depressive episodes commit suicide.<sup>4</sup> In India the prevalence of depression is 31.2 per 1000 population.<sup>5</sup>

An episode may be characterized by sadness, indifference, apathy, or irritability and is usually associated with changes in sleep patterns, appetite, and weight; fatigue; impaired concentration and decision making; feelings of shame or guilt and thoughts of death.<sup>1</sup>

Depression is often undiagnosed, and even more frequently, it is treated inadequately.<sup>1</sup> Although many antidepressant drugs are being used currently, patients are often reluctant to take these drugs in appropriate doses due to their anticipated adverse effects.<sup>6</sup> Moreover 30-40% of patients fail to show improvement with drug treatment<sup>7</sup> and risk of relapse and recurrence remains high.<sup>8</sup>

Plant extracts are one of the attractive sources of new drugs. Some of these have shown encouraging results in the treatment of depression.<sup>9</sup>

*Crocus sativus* linn commonly known as saffron is an herbaceous perennial-cormous plant belonging to the family Iridaceae, which is believed to show many pharmacological actions. The chemical studies have shown that plant contains important constituents like crocin, crocetin, safranal and picrocrocin.<sup>10</sup> Pharmacological activities demonstrated by the chemical constituents of *Crocus sativus* include antinociceptive, anti-alzheimer's, anti-parkinsonian, learning or memory improving properties, anti oxidant activity, anxiolytic activity etc.<sup>10-12</sup>

Several studies have shown saffron and its active constituents like crocin and safranal to have antidepressant activity.<sup>13-15</sup> This activity is probably due to uptake inhibition of dopamine, norepinephrine and serotonin.<sup>16</sup> Hence it was decided to study the antidepressant like action of saffron with imipramine as standard and also to study the combined effect of both since the latter also acts by inhibition of monoamine reuptake.

### MATERIALS AND METHODS

Male swiss albino mice weighing between 20-30g, aged 3-4 months, healthy and with normal behavior and activity were taken for the study. The mice were inbred in the central animal house of the Department of Pharmacology, J.J.M Medical College, Davangere, under suitable

conditions of housing, temperature, ventilation and nutrition.

### Drugs and chemicals

Normal saline: Control- 5ml/kg

Imipramine: Standard-15mg/kg obtained from Sun pharma.

Crocus sativus linn stigmas: Test drug 200-800mg/kg received from Natural remedies Pvt Ltd.

Institutional animal ethical committee approval was taken for the study.

A total of 48 animals (n=48) were used for each test. They were divided into 8 groups of 6 animals each. They were housed in cages containing wooden shavings with 6 animals per cage. Animals were randomly housed at a controlled temperature of  $21 \pm 3^\circ\text{C}$ , with a 12 hour light: 12 hour dark cycle. The animals had free access to standard pellet and water.

Mice were evaluated for antidepressant activity using two models – Tail suspension test (TST) and Forced swimming test (FST). The experiment was conducted in Post Graduate Experiment Laboratory of the Department of Pharmacology, J.J.M. Medical College between 8:00 A.M. to 2:00 P.M. Prior to the test, animals are allowed to acclimatize. The food and water was removed for the duration of test. All drugs were given intraperitoneally. The experiment was conducted 30 minutes after injecting the drug.

### Grouping of animals for each test

Group I: Control-5ml/kg of Normal saline.

Group II: Standard-15 mg/kg imipramine.<sup>17</sup>

Group III: 200mg/kg of Crocus sativus linn stigmas.<sup>15</sup>

Group IV: 400mg/kg of Crocus sativus linn stigmas.<sup>15</sup>

Group V: 800mg/kg of Crocus sativus linn stigmas.<sup>15</sup>

Group VI: 200mg/kg of Crocus sativus linn stigmas+ 7.5mg/kg imipramine.

Group VII: 400mg/kg of Crocus sativus linn stigmas+ 7.5mg/kg imipramine.

Group VIII: 800mg/kg of Crocus sativus linn stigmas+ 7.5mg/kg imipramine.

### Forced swim test

The forced swimming model to test for antidepressant activity was developed by Porsolt et al.<sup>18,19,20</sup> The model used in the present study was similar to the original method described. The animals were forced to swim in a plastic cylinder measuring 30 X 30 cm containing water at room temperature to a depth of 20 cm. After an initial 2 minute period of vigorous activity, each animal assumed a typical immobile posture. The mouse was considered immobile when it remained floating in the water without struggling, making only minimum movements of its limbs

necessary to keep its head above water. The total duration of immobility was recorded during next 4 minutes of total 6 minute test. Following swimming sessions, the mice were dried with towel and placed in a cylinder heated under 60 W bulb for 15 minutes before returning to the home cages.<sup>21</sup>

### Tail suspension test

The method was similar to that described by Steru et al.<sup>22</sup> Animals were suspended upside down on a metal rod at a height of 55 cm from the ground with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Initially the animals tried to escape by making vigorous movements but when unable to escape became immobile. The animal was considered immobile when it did not show any movement of body and hanged passively. The immobility displayed by rodents when subjected to this kind of unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. The total duration of immobility was noted during 6 minute period.<sup>21</sup>

### Statistical Analysis

Results are presented as Mean  $\pm$  SEM. Data was analyzed using Graphpad Prism 5. One way analysis of variance (ANOVA) was used followed by Tukey's post hoc test for multiple group comparisons.  $P < 0.05$  was considered as statistically significant.

## RESULTS

### Forced swim test

There was a significant reduction of immobility time ( $29.83 \pm 7.25\text{s}$ ) in imipramine group compared to control group ( $160.5 \pm 8.96\text{s}$ ). Saffron 200mg, 400mg and 800mg/kg groups also showed significant reduction in immobility time (IT) compared to control ( $85 \pm 6.33$ ,  $52.17 \pm 14.35$ ,  $88.5 \pm 22.27\text{s}$  respectively). So also the combination groups ( $36 \pm 12.71$ ,  $70.5 \pm 17.20$ ,  $79.34 \pm 9.56\text{s}$  respectively) (Table 1, Figure 1).

There was no significant difference in IT when saffron with submaximal dose of imipramine was compared with standard dose of imipramine. All three doses of saffron also produced comparable IT. (Table 1, Figure 1).

### Tail suspension test

Imipramine group showed significant reduction of IT ( $31.5 \pm 7.07\text{s}$ ) compared to control ( $157.6 \pm 16.12\text{s}$ ). Saffron at 400mg also showed a significant reduction of IT ( $85.16 \pm 8.99\text{s}$ ). But with 200 and 800mg/kg, there was no significant difference ( $147.83 \pm 23.05$ ,  $112.83 \pm 22.23\text{s}$  respectively). Combination of submaximal dose of imipramine with saffron 400mg and 800mg/kg produced significant decrease in IT but not the 200mg/kg dose ( $79.5 \pm 8.89$ ,  $82.67 \pm 10.71$ ,  $115.67 \pm 13.66\text{s}$  respectively) when compared with control. (Table 2, Figure 2).

IT of saffron 400mg/kg group was comparable to that of imipramine group but not the 200 mg and 800mg/kg.

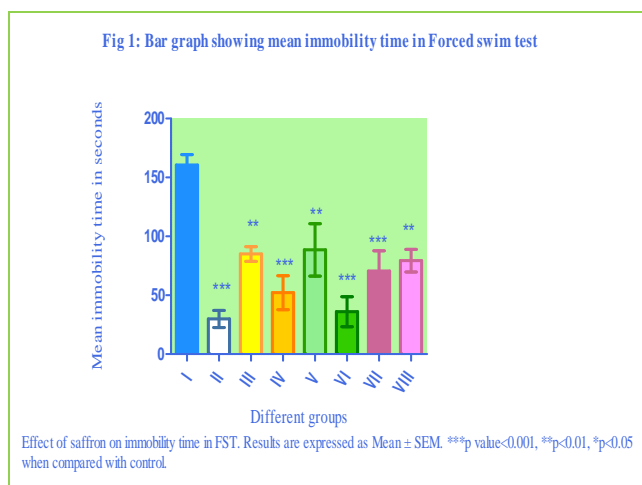


Combinations of submaximal imipramine with saffron 400mg and 800mg/kg produced IT comparable to that of standard imipramine. IT of all three doses of saffron was not significantly different, hence they were comparable. (Table 2, Figure 2).

**Table 1:** Mean immobility time in Forced swim test

Group	Treatment Groups	Mean immobility time in seconds
I	Normal saline (5ml/kg)	160.5± 8.96
II	Imipramine 15 mg/kg	29.83±7.25 <sup>***</sup>
III	Saffron 200mg/kg	85±6.33 <sup>**</sup>
IV	Saffron 400mg/kg	52.17±14.35 <sup>***</sup>
V	Saffron 800mg/kg	88.5±22.27 <sup>**</sup>
VI	Saffron 200mg/kg + Imipramine 7.5mg/kg	36±12.71 <sup>***</sup>
VII	Saffron 400mg/kg + Imipramine 7.5mg/kg	70.5±17.20 <sup>***</sup>
VIII	Saffron 800mg/kg + Imipramine 7.5mg/kg	79.34 ±9.56 <sup>**</sup>

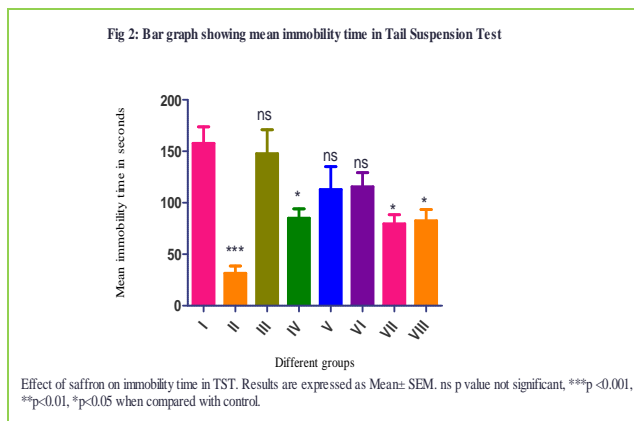
Effect of saffron on immobility duration in FST. Results are expressed as Mean ± SEM. <sup>\*\*\*</sup> p value<0.001, <sup>\*\*</sup>p<0.01, <sup>\*</sup>p<0.05 when compared with control.



**Table 2:** Mean immobility time in tail suspension test

Group	Treatment Groups	Mean Duration of Immobility in seconds
I	Normal saline (5ml/kg)	157.6±16.12
II	Imipramine 15 mg/kg	31.5±7.07 <sup>***</sup>
III	Saffron 200mg/kg	147.83 ±23.05 <sup>ns</sup>
IV	Saffron 400mg/kg	85.16±8.99 <sup>*</sup>
V	Saffron 800mg/kg	112.83±22.23 <sup>ns</sup>
VI	Saffron 200mg/kg + Imipramine 7.5mg/kg	115.67 ±13.66 <sup>ns</sup>
VII	Saffron 400mg/kg + Imipramine 7.5mg/kg	79.5±8.89 <sup>*</sup>
VIII	Saffron 800mg/kg + Imipramine 7.5mg/kg	82.67± 10.71 <sup>*</sup>

Effect of saffron on immobility time in tail suspension test. Results are expressed as Mean ± SEM. <sup>ns</sup> p value not significant, <sup>\*\*\*</sup> p <0.001, <sup>\*\*</sup>p<0.01, <sup>\*</sup>p<0.05 when compared with control.



**DISCUSSION**

In our study immobility time was reduced significantly with standard antidepressant drug imipramine in both forced swimming test and tail suspension test (p<0.001) when compared to control.

Saffron (C.S) also showed significant reduction in immobility duration with all doses and with combination of submaximal dose of imipramine when compared to control (p<0.05) in FST. But with TST, though there was reduction in immobility duration in all groups when compared to control, it was significant (p<0.05) with 400mg/kg dose and with 400 and 800mg/kg with imipramine 7.5 mg/kg.

The characteristic immobility seen in the test animals is indicative of 'behavioral despair' which reflects a depressive state in humans.<sup>23</sup> Antidepressant drugs decrease the immobility time.<sup>24</sup> Hence we can consider saffron to have antidepressant like activity.

Saffron has been used in traditional medicine for treatment of depression.<sup>25</sup> Previous studies on animals have shown saffron to have antidepressant like activity between 200-800mg/kg dose.<sup>13,15,16</sup> Our results are in line with these studies. More over the immobility duration with 400mg/kg alone and its combination with submaximal imipramine is comparable to that of standard imipramine. Previous animal study and small double blind clinical trials in patients suffering from mild to moderate depression have shown saffron to be comparable to imipramine in antidepressant effect.<sup>8,16</sup>

Saffron has several active constituents like crocin and saffranal. Data indicate that antidepressant like activity of saffron may be due to these two ingredients, particularly crocin-1.<sup>13,15,26</sup> It has been suggested that crocin and saffranal inhibit the reuptake of dopamine, nor epinephrine and serotonin.<sup>8,15,16</sup> This mechanism is partially similar to that of imipramine which inhibits the reuptake of monoamines.<sup>2</sup> In addition saffron has other properties like antioxidant<sup>11,27</sup> and antianxiety.<sup>12</sup> These properties could also contribute to its antidepressant like action.<sup>23,24</sup> Further studies are needed to determine the exact mechanism of action.

The combined effect of sub maximal dose of imipramine with saffron could be beneficial considering the adverse effects of tricyclic antidepressants especially when given in higher doses.<sup>2</sup>

The present work is an acute study and further chronic studies are needed in this direction.

## CONCLUSION

Saffron between 200 and 800mg/kg dose has antidepressant like activity which is comparable to that of imipramine. In addition, saffron has added to action of submaximal dose of imipramine. Hence saffron can be considered as potential antidepressant and can be further evaluated.

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Source of Support: Nil, Conflict of Interest: None.

