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Evaluation of the anxiolytic and Antidepressant Effects of Crocus sativus L (saffron)and Mentha spicata (spearmint) in Wistar Albino Rats

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Abstract

Background

Depression and anxiety are prevalent psychiatric disorders often linked to disturbances in serotonin and other neurotransmitter systems. Although selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed, they may cause side effects, leading to increased interest in alternative herbal therapies. Crocus sativus (saffron) and Mentha spicata (spearmint) are known for their individual anxiolytic and antidepressant properties, but their combined synergistic effects have not been fully explored.

Aim*:To evaluate the synergistic anxiolytic and antidepressant effects of Crocus sativus and Mentha spicata combination extracts in Wistar albino rats using behavioral paradigms.*

Methodology: *Thirty Wistar albino rats were divided into five groups (n=6) and administered varying doses of Crocus sativus + Mentha spicata combinations, diazepam (1 mg/kg), or saline (control). Aqueous extracts were prepared via maceration. Three fixed-dose combinations (25+200, 50+250, and 75+300 mg/kg) were selected. Behavioral assessments included the T-Maze Test, Light-Dark Chamber Test, Tail Suspension Test (TST), and Forced Swim Test (FST). Statistical significance was analyzed using one-way ANOVA and tukey post-hoc.*

Results*:High-dose Crocus+Mentha (75+300 mg/kg) significantly improved performance across all tests, comparable to standard drugs (fluoxetine/diazepam). In the FST and TST, it significantly reduced immobility duration (P = 0.0021 and P = 0.0018 respectively). In the light-dark test, time spent in the light chamber increased significantly (P = 0.0021). T-maze results showed increased open arm entries and time (P = 0.014), indicating reduced anxiety. Lower doses showed trends toward efficacy but were not statistically significant.*

Conclusion*:The combination of Crocus sativus and Mentha spicata exhibited dose-dependent anxiolytic and antidepressant effects, with the highest dose providing results comparable to standard pharmacological treatments. These findings support the potential use of this herbal combination as a natural alternative for managing anxiety and depression, meriting further investigation.*

INTRODUCTION

Major depressive disorder (MDD), known also as depression (lat. depressio 'deepness' from deprimere 'overwhelm'), is a chronic, recurrent, and potentially life-threatening mental disorder characterised by at least two weeks of omnipresent low mood. It is usually accompanied with persistent feeling of sadness, anhedonia, pain without a clear cause, difficulties in thinking and concentration, loss of interest in doing anything, psychomotor retardation, fatigue, spending time sleeping, feelings of worthlessness or inappropriate guilt, and recurrent thoughts of death. These symptoms cause distress or impairment in social life and are not an effect of the influence of other medical conditions (1,2).

Depression refers to a wide range of mental health problems characterised by the absence of a positive effect (a loss of interest and enjoyment in ordinary things and experiences), low mood and a range of associated emotional, cognitive, physical and behavioural symptoms. Distinguishing the mood changes between clinically significant degrees of depression (for example, major depression) and those occurring 'normally' remains problematic and it is best to consider the symptoms of depression as occurring on a continuum of severity (3).

Whereas, Anxiety disorders are characterised by excessive fear and worry and related behavioural disturbances. Symptoms are severe enough to result in significant distress or significant impairment in functioning. There are several different kinds of anxiety disorders, such as: generalised anxiety disorder (characterised by excessive worry), panic disorder (characterised by panic attacks), social anxiety disorder (characterised by excessive fear and worry in social situations), separation anxiety disorder (characterised by excessive fear or anxiety about separation from those individuals to whom the person has a deep emotional bond), and others.(4)

Moreover, anxiety and depression are associated with dysregulation of the serotonergic system in the brain and mood impairments. Therefore, maintaining serotonin at a normal level can improve mood disorders,(5) Serotonin reuptake inhibitors (SSRIs) increase serotonin levels in various regions of the brain and are commonly prescribed as the initial treatment for chronic stress, anxiety, and depression.(6) Escitalopram is one of the newest SSRI antidepressants indicated for improving anxiety and depression.(7) Most individuals may need to take medication after experiencing a period of depression. Whereas, some individuals may prefer to avoid medication and believe that they can enhance their well-being by going through a recovery period after reducing stress.(8) Furthermore, the effect of the recovery period on reducing anxiety and depressive-like behavior is unclear when compared to natural and chemical medications.(9)

On the other hand, certain herbal drugs, such as saffron (Crocus Sativus) have a significant impact on behavior and mood.[7] Various in vitro and in vivo analysis have provided evidence for the use of saffron/or derivative of saffron as a chemo preventive agent. Studies have provided evidence that saffron modulates the expression of various molecules such as dopamine, TNF-α, Wnt /β-catenin pathways, superoxide dismutase, catalase, and several other antioxidant molecules1(8,9).

Nowadays, it is crucial to discover new pharmacological approaches that have minimal side effects on the body's physiological system to effectively manage anxiety and depression in individuals. *Crocus sativus* and *Mentha spicata* are known for their anti-anxiety and anti-depressant properties, but their combined effects remain unexplored. This study examines their potential synergistic role in regulating key neurotransmitters—serotonin, dopamine, and norepinephrine—offering a natural therapeutic approach for anxiety and depression.

Therefore, the present study aimed to investigate the comparative evaluation of the antidepressant and anti-anxiety effects of Of Crocus Sativus and Mentha Spicata Combination Effect in Wistar Albino Rats.

METHODOLOGY

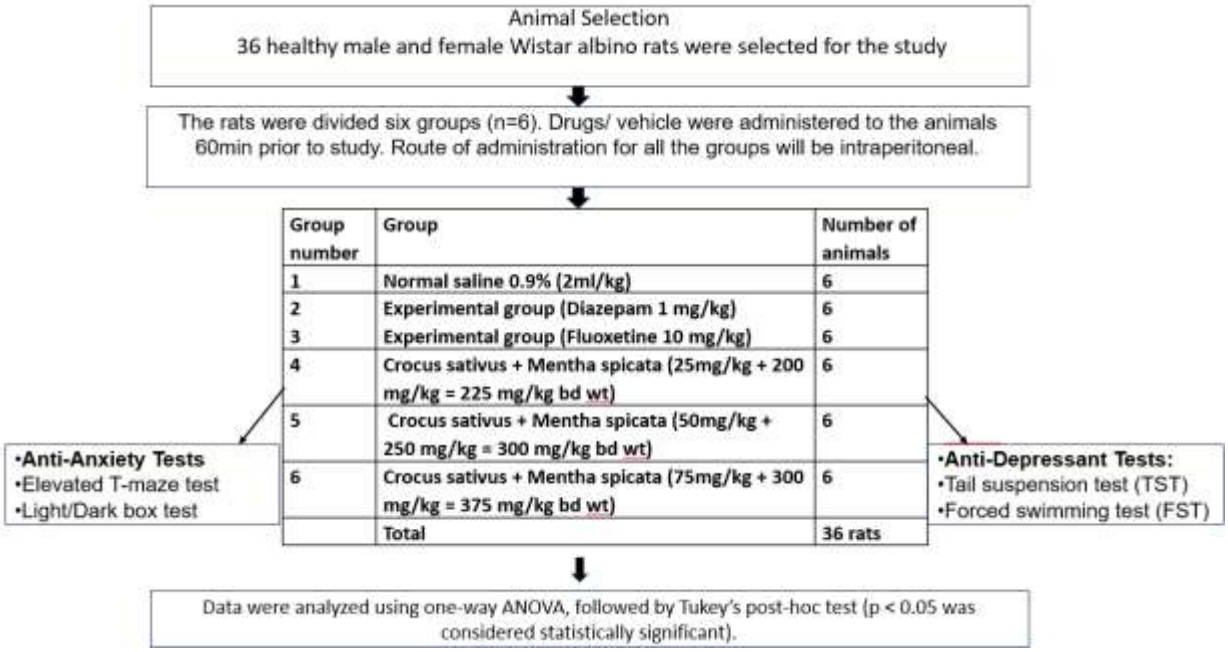
The animal study was conducted with prior approval from the animal ethics committee(REG N0:863/PO/Re/S/04/CPCSEA). The study has five study groups with 6 albino rats in each group(30 albino rats).

Experimental Animals

The adult male and female Wister rats (150-200gm) will be used for the experiments. The rats will be kept in a polyacrylic cage (22.5×37.5 cm) in group of 6 rats per cage and maintained under standard housing conditions (room temperature 24–27 °C and humidity 60–65%) with a 12 h light and dark cycle. Food and water will be available ad libitum, but food will be removed during experimental period, 1 h prior to administration of drug till completion of the trials (approximate 2–3 h). All the behavioral observations will be performed between the 09.30 AM to 13.00 PM to avoid the neuroendocrine associated neurobiological interaction and neurobehavioral abnormalities.

Extraction

Crocus sativus and *Mentha spicata* were collected from verified botanical sources and authenticated at the Research Laboratory of Sree Balaji Medical College and Hospital. The leaves were shade-dried, ground into a fine powder, and subjected to aqueous extraction using the maceration process. The extracts were then filtered, evaporated, and concentrated. Based on the literature, the effective oral dose range is 10–600 mg/kg for *C. sativus* and 100–1000 mg/kg for *M. spicata* in animal models. Toxicity studies conducted according to OECD guidelines showed no adverse effects at a dose of 2000 mg/kg for either extract. For the study, three fixed combination doses were selected by taking approximately half of the effective dose ranges: 225 mg/kg (*C. sativus* 25 mg/kg + *M. spicata* 200 mg/kg), 300 mg/kg (50 mg/kg + 250 mg/kg), and 375 mg/kg (75 mg/kg + 300 mg/kg). These doses were administered simultaneously to evaluate their combined effects (10,11,12)



RESULTS

Diazepam (1 mg/kg) and the higher doses of *Crocus sativus* + *Mentha spicata* significantly increased the time spent in the open arms of the elevated plus maze, indicating reduced anxiety-like behavior ($p < 0.05$). One-way ANOVA analysis further revealed that both Diazepam (1 mg/kg) and the highest combination dose of *Crocus* + *Mentha* (75 mg/kg + 300 mg/kg) significantly increased the time spent in the light chamber in the light/dark box test ($p < 0.05$), whereas the lower doses did not show a significant difference compared to the control group. In the forced swim test, the high-dose combination group (75 mg/kg + 300 mg/kg) significantly increased the latency to immobility and decreased total immobility duration ($P = 0.0021$), comparable to the effects observed with fluoxetine ($P = 0.0008$). While lower doses showed a tendency toward reduced immobility, the results were not statistically significant.

TABLE 1: Light and Dark Chamber Test

Group	Latency to Enter Dark Chamber (s)	Number of Transitions	Time Spent in Light Chamber (s)	Distance Traveled in Light Chamber (cm)	p-value (ANOVA)
Control (Normal saline, 2ml/kg)	5.2 ± 0.8	3.5 ± 0.5	52.4 ± 4.1	105.6 ± 9.3	1.000
Diazepam (1 mg/kg)	12.3 ± 1.1	7.8 ± 0.9	145.6 ± 6.8	178.9 ± 12.4	0.0008*
Crocus+Mentha (25mg/kg + 200mg/kg)	7.8 ± 0.9	5.2 ± 0.7	85.3 ± 5.2	145.4 ± 10.1	0.078
Crocus+Mentha (50mg/kg + 250mg/kg)	9.6 ± 1.0	6.3 ± 0.8	110.2 ± 5.9	160.8 ± 11.3	0.052
Crocus+Mentha (75mg/kg + 300mg/kg)	11.1 ± 1.2	7.1 ± 0.9	130.5 ± 6.4	172.1 ± 12.6	0.0021*

Table 1 shows the light and dark chamber test in which statistical difference was seen in group II and in group V

TABLE 2 : T-Maze Test

Group	Open Arm Time (s)	Closed Arm Time (s)	Open Arm Entries	p-Value
Control (Normal Saline)	25.4 ± 4.8	174.6 ± 13.5	2.3 ± 0.5	0.092
Diazepam (1 mg/kg)	60.7 ± 9.5	118.3 ± 9.7	5.2 ± 0.8	0.002**
Crocus+Mentha (25mg+200mg)	36.2 ± 6.5	146.8 ± 11.2	3.1 ± 0.6	0.092
Crocus+Mentha (50mg+250mg)	46.8 ± 7.4	138.9 ± 10.5	4.3 ± 0.7	0.031**
Crocus+Mentha (75mg+300mg)	51.3 ± 8.1	129.7 ± 9.8	4.7 ± 0.9	0.014**

In elevated T Maze test, three groups found to be statistically significant(group II ,group IV and group V)[table-2 & table 3]

TABLE 3: Tail Suspension Test (TST) Results

Group	Latency (Mean ± SD, sec)	Immobility Duration (Mean ± SD, sec)	P-value (ANOVA)
Control (Normal saline, 2 ml/kg)	48.32 ± 2.85	226.45 ± 8.78	1.000
Fluoxetine (10 mg/kg)	82.14 ± 3.42	115.72 ± 6.45	0.0006*

Crocus+Mentha (25 mg/kg + 200 mg/kg)	55.78 ± 3.91	178.23 ± 7.84	0.080
Crocus+Mentha (50 mg/kg + 250 mg/kg)	67.21 ± 4.62	145.32 ± 5.97	0.049*
Crocus+Mentha (75 mg/kg + 300 mg/kg)	73.56 ± 2.98	128.79 ± 5.12	0.0018*

Table 4: Forced Swimming Test (FST) Results

Group	Latency (Mean ± SD, sec)	Immobility Duration (Mean ± SD, sec)	P-value (ANOVA)
Control (Normal saline, 2 ml/kg)	45.82 ± 2.95	197.34 ± 9.42	1.000
Fluoxetine (10 mg/kg)	78.24 ± 3.12	92.78 ± 7.61	0.0008*
Crocus+Mentha (25 mg/kg + 200 mg/kg)	52.37 ± 4.11	147.92 ± 8.37	0.078
Crocus+Mentha (50 mg/kg + 250 mg/kg)	64.93 ± 5.05	126.58 ± 7.91	0.052
Crocus+Mentha (75 mg/kg + 300 mg/kg)	70.45 ± 2.72	111.34 ± 5.24	0.0021*

DISCUSSION

This study compared the therapeutic effects of different treatments on anxiety and depression in wister rats. The present findings from the FST findings indicated that inducing chronic stress for 14 days resulted in increased despair behavior, suggesting that stress may lead to depression in the experimental subjects. It was demonstrated that depression can be caused by physical, psychological, and neurochemical changes due to chronic stress.(10) Some studies reported that 14 days of restraint stress increased depressive-like behavior in rats through changes in hypothalamic-pituitary-adrenal (HPA) axis activity and elevated corticosterone levels.(11,12) On the other hand, a recovery period after depression (without any medication treatment) also showed persistent depressive-like behavior even after a 14-day rest period. In other words, the recovery period alone could not decrease depression. In contrast, Grippo *et al.* (2003) indicated that chronic stress for four weeks induced depression in rats, and it could be reversed to the baseline levels after a four-week recovery period.(13,14) These conflicting results may be attributed to a longer recovery period in previous studies compared to the recent study. Based on other FST findings, it has been observed that various treatments for depression, such as Fluoxetine (10 mg/kg), and the combination of Crocus+Mentha (75 mg/kg + 300 mg/kg) showed a decrease in depression symptoms. Zhang *et al.* (2022) demonstrated that crocin reduced depressive-like behavior in the FST.(15) In addition, a dose of 30 mg/day of crocin for eight weeks reversed depressive behaviors. The antidepressant effects of crocin may be attributed to several factors, including changes in the reuptake of neurotransmitters such as dopamine, norepinephrine, and serotonin,(16) the reduction of corticosterone levels,[29,31] and the inhibitory effect of crocin on monoamine neurotransmitters.[29] On the other hand, in the present study, both doses of escitalopram, with and without crocin, were found to reverse depressive-like behavior. However, the dose of 20 mg/kg of escitalopram showed a partial decrease in depression-like behavior that was more effective than the dose of 10 mg/kg. Therefore, crocin had no significant effect on the association with either dose of escitalopram in subjects with depression.

Based on the findings of Tail Suspension Test (TST) Results, it has been observed that various treatments for depression, such as Fluoxetine (10 mg/kg), both doses and the combination of Crocus+Mentha (50 mg/kg + 250 mg/kg) and Crocus+Mentha (75 mg/kg + 300 mg/kg) showed a decrease in depression

symptoms. Whereas the Elevated T-Maze Test supported these findings, with reduced escape latency and increased open-arm exploration, similar to Diazepam.

CONCLUSION

The study concludes that the Lower doses were ineffective, highlighting the need for optimal dosage. These results suggest Crocus and Mentha spicata as potential natural alternatives for anxiety and depression, warranting further study for concrete evidence.

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