

**Neuropharmacological Evaluation of *Ocimum Sanctum* With *Coleus Amboinicus*
For Antidepressant Activity On Mice**

ABSTRACT

Objective: This study aimed to evaluate the antidepressant effects of a combination of *Ocimum sanctum* and *Coleus amboinicus* leaf extracts compared to amitriptyline using the tail suspension test (TST) and forced swim test (FST) in mice.

Background: Depression is a common mental health disorder marked by persistent sadness and loss of interest. While effective, current antidepressants like amitriptyline often cause side effects, leading to interest in natural alternatives. *Ocimum sanctum* and *Coleus amboinicus* have traditionally been used for their therapeutic, potentially antidepressant, properties.

Methods: The study involved male mice, divided into groups receiving either a combination of *Ocimum sanctum* and *Coleus amboinicus* extracts at varying doses, amitriptyline, or a control. Antidepressant activity was assessed using the TST and FST, with decreased immobility time indicating antidepressant effects.

Results: The combination of *Ocimum sanctum* and *Coleus amboinicus* extracts significantly reduced immobility in the TST and FST, showing a dose-dependent antidepressant effect. At higher doses, it was comparable to amitriptyline, suggesting the extracts act through monoaminergic systems in the brain.

Conclusion: The combination of *Ocimum sanctum* and *Coleus amboinicus* extracts showed significant antidepressant activity, comparable to amitriptyline, in preclinical models. These findings suggest potential for use as alternative or complementary therapies for depression, with further research needed to confirm efficacy and safety in humans.

Keywords: Antidepressant, *Coleus amboinicus*, Monoaminergic system, *Ocimum sanctum*

INTRODUCTION:

Depression is a common mental illness that is characterized by symptoms like mood swings, hopelessness, diminished cognitive function, and decreased activity level. In severe situations, there may be sleep disturbances, appetite loss, reduced focus, and suicidal thoughts. According to a September 13, 2021, World Health Organization estimate, 280 million people worldwide or 3.8% of the population struggle with depression. The prevalence is 50% greater in adult females than in males. Approximately 15% of depressed people choose suicide each year, making depression a significant contributing factor to both ischemic heart disease and suicide rates. Depression ranks second globally. Depression thus has a major impact on the total burden of disease worldwide (1). Up to two thirds of patients do not achieve remission even after an acceptable therapeutic trial, and up to 33% will not react even after several interventions (2).

The pathophysiology of depression theories are primarily based on the following: (i) the monoaminergic hypothesis, which suggests that monoamine neurotransmitters are not active enough; (ii) abnormalities in the limbic cortical model and cortico-striatal model; (iii) dysfunction of the hypothalamic-pituitary-adrenal axis; and (iv) over-activation of pro inflammatory cytokines (3).

Ocimum sanctum (OS) Linn. (*Labiatae*), commonly referred to as Holy Basil in English and Tulsi in Hindi, is a native of India. The plant is grown and distributed all over India. It is a 30-to 60-cm-tall, erect, heavily branched, softly pubescent under shrub with sub-quadrangular branches that are either purple or scarlet. Simple, opposite, oblong leaves have thin, hairy petioles, minute gland spotted edges, and whole or dentate margins (4).

About 71% of the volatile oil in OS leaves is eugenol, while 20% is methyl eugenol. Carvacrol, luteolin, caryophyllene, sesquiterpine hydrocarbon, ocimumosides A and B, orientin, olluadin, apigenin-7-O-glucuronide, ocimarin, and ursolic acid (5) are other components.

Previous research on *Ocimum sanctum* has shown anti-stress activity (6,7) and been shown to influence central monoamines such as dopamine, noradrenaline, and 5-hydroxytryptamine. Antidepressants are known to raise the levels of certain monoamines, and these neurotransmitters are known to have a significant part in the pathophysiology of depression. Our investigation into antidepressant action was driven by the *Ocimum sanctum*'s antistress, anticataleptic, and monoamine modulation properties.

Coleus amboinicus Loruri, also known as Indian Borage, is a medicinal plant that contains flavanoids (quercetin, agapigenin, luteolin, salvigenin, and genkwanin) and phytochemicals (carvacrol, a monoterpenoid, and caryophyllene, a bicyclic sesquiterpene). This plant's leaves have long been used to treat fever, renal and vesicle calculi, severe bronchitis, asthma, diarrhea, and epilepsy. According to reports, it possesses antioxidant, chemo preventive, and antilithiatic qualities (8). The aim of this study was to examine how oral administration of *ocimum sanctum* and *coleus amboinicus* enhances antidepressant effects in mice on depression-model animals.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Ocimum sanctum* and *Coleus amboinicus* has been procured from the Chalapathi Institute of Pharmaceutical Sciences, Guntur. Plant-based matter was taxonomically identified and authenticated by Dr. P. Satyanarayana Raju Garu M.Sc., M.Phil., Ph.D from Department of Botany and Microbiology, Acharya Nagarjuna Nagar, AP on 21st November 2023. The leaves that had fully dried was ground into a coarse powder using a mechanical grinder. It was then evenly powdered and kept in an air tight container after passing through 40 mesh sieves.

Preparation of the plant extract:

The fifty grams of botanical matter were extracted using the hot continuous Soxhlet extraction technique. The resulting substance was dried, concentrated, and filtered using a rotary evaporator. The extract was gathered, kept at a comfortable temperature in a container, and utilized in additional research projects.

Selection of animals

We purchased 25–30 g of Swiss albino mice of both sexes from Mahaveer Enterprises, Hyderabad, an animal supplier recognized by the CPCSEA. Before the trial began, the animals were housed at 24±02°C and given 14 days to get acquainted to our animal facility. A standard pellet diet and unlimited access to clean drinking water were provided to the animals. In all of the experiment sets, 5 rats per group were utilized. After receiving approval from the Institutional Animal Ethics Committee (IAEC) of the Chalapathi Institute of Pharmaceutical Sciences, Guntur, all studies were carried out. The protocol of the experiment was approved by the Institutional animal ethics committee (03/IAEC/CLPT/2023-24). All animal experimental procedures were in compliance with the rules of the committee for the supervision and regulation of animal experiments (CCSEA), ministry of social justice and empowerment, Government of India.

DRUGS:

The drug used within this research is amitriptyline (Dr. Reddy's Laboratories, Uttarakhand)

VEHICLE:

Plant extract was suspended in distilled water and administered orally to mice. Amitriptyline was dissolved in normal saline and administered orally.

DRUG TREATMENT:

The mice in this investigation were divided into four groups in order to test their antidepressant activity. Every group has five animals in it. Group 1 was given only saline treatments and served as the control group. Group 2 is given standard medication amitriptyline (10 mg/kg) representing the standard. As test groups, groups 3 and 4 are given *Ocimum sanctum* and *Coleus amboinicus* in varying dosages (100 mg and 150 mg/kg) of the extract.

Results

Data of Automated Motion tracking.

Forced Swim Test (FST)

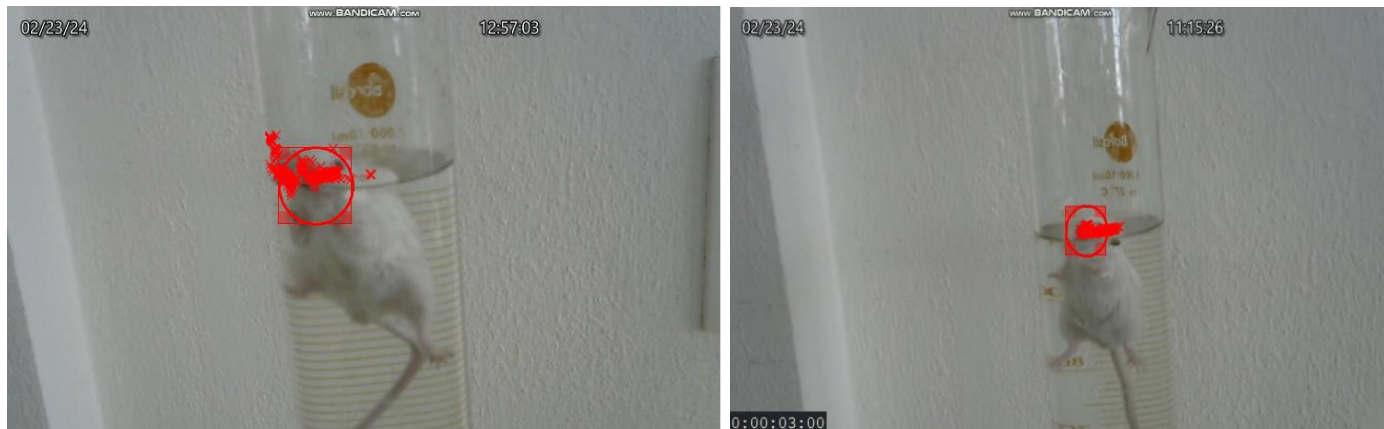


Figure 1 : Evaluation of **control group** using forced swim test

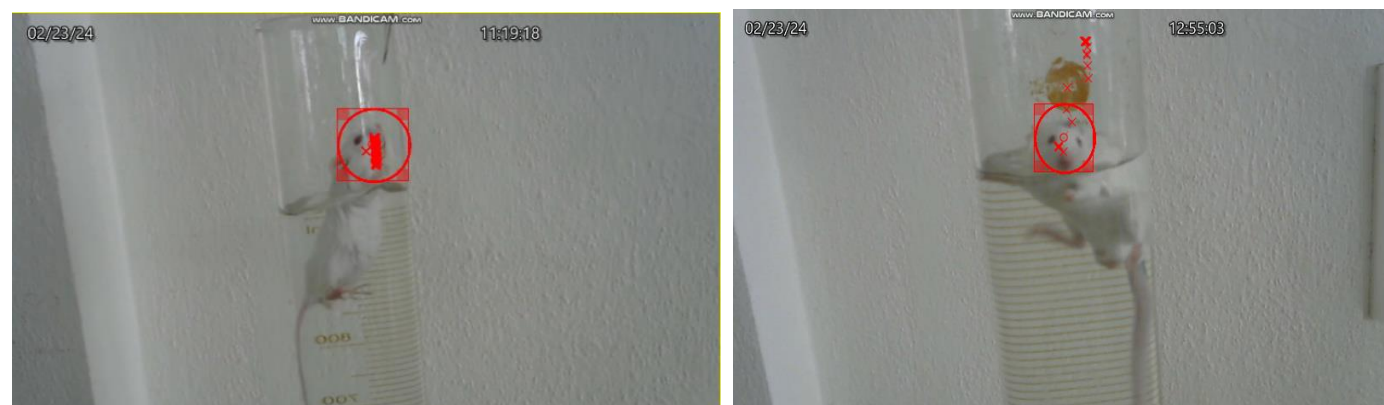


Figure 2 : Evaluation of **standard group** using forced swim test



Figure 3 : Evaluation of *Coleus Amboinicus*

Figure 4: Evaluation of *Ocimum Sanctum*

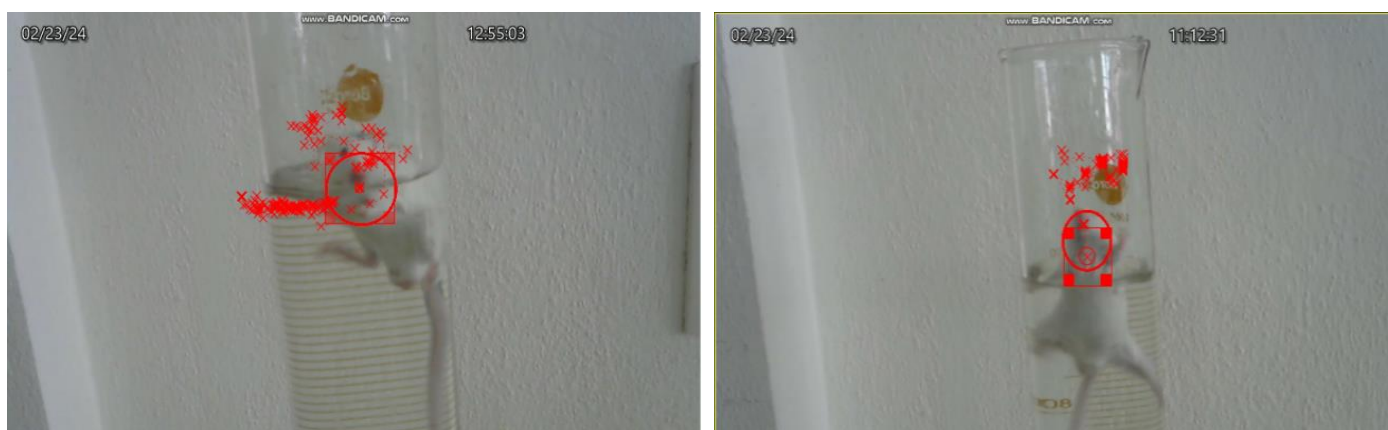
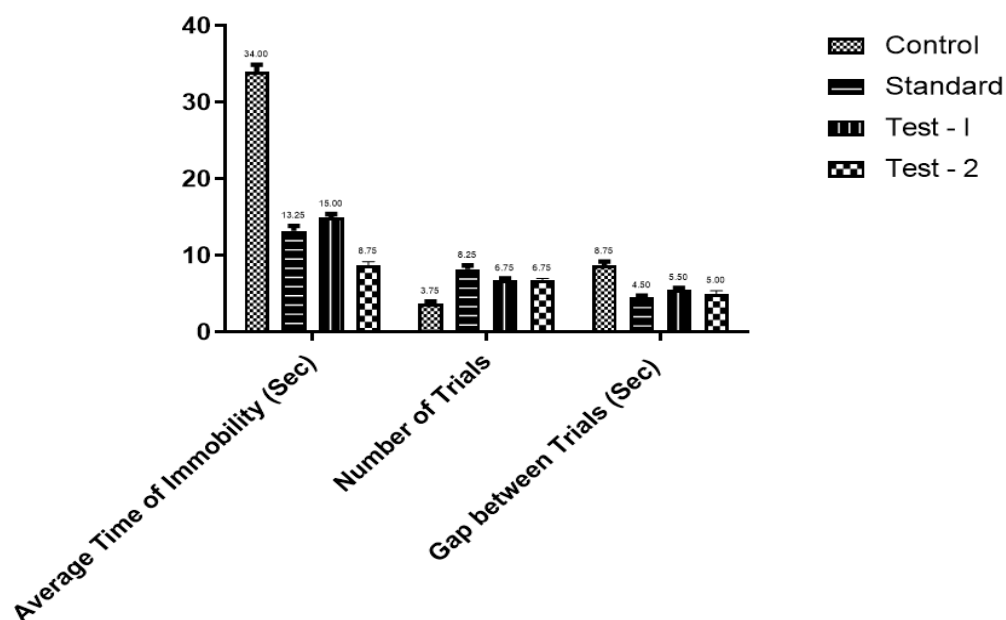


Figure 5: Evaluation of **Test – I and II treatments group** using forced swim test

Table 1: Evaluation data using Forced swim test

S.No	Treatment Groups	Average Time of Immobility (Sec)	Number of Trials	Gap between the trials (Sec)
1	Control	34.00 ± 0.91	3.75 ± 0.25	8.75 ± 0.47
2	Standard (Amitriptyline 10mg/kg)	13.25 ± 0.62	8.25 ± 0.47	4.50 ± 0.28
3	Test-I(<i>ocimum sanctum</i> with <i>coleus amboinicus</i> - dose 1)	15.00 ± 0.40	6.75 ± 0.25	5.50 ± 0.28
4	Test- II (<i>ocimum sanctum</i> with <i>coleus amboinicus</i> -dose 2)	8.75 ± 0.47	6.75 ± 0.25	5.0 ± 0.40

Forced Swim test



Graph 1: Evaluation of **all treatments group** using forced swim test

Tail Suspension Test (TST)



Figure 6: Evaluation of **control group** using Tail suspension Test

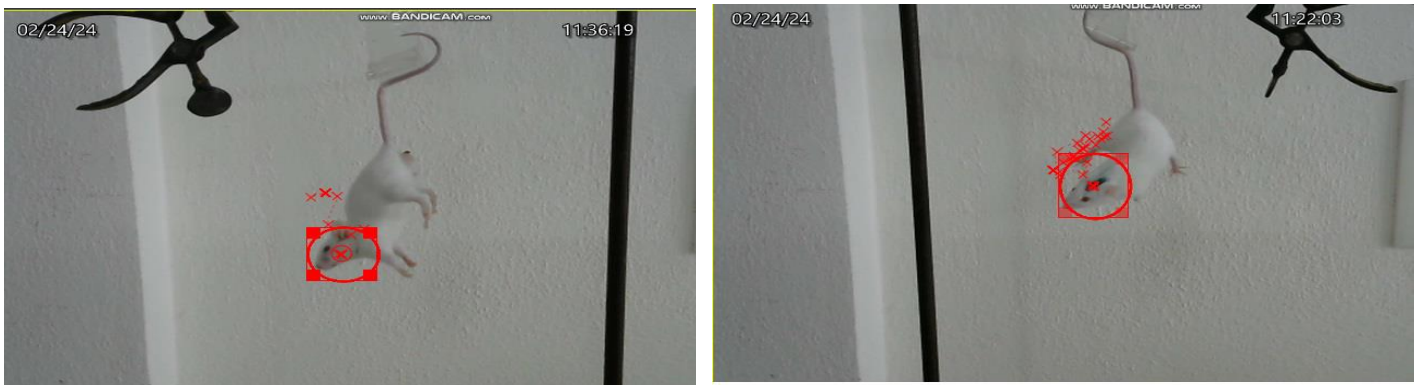


Figure 7: Evaluation of **Standard group** using Tail suspension Test



Figure 8: Evaluation of ***Coleus Amboinicus***

Figure 9 : Evaluation of ***Ocimum Sanctum***



Figure 10 : Evaluation of **Test – I and II treatments group** using Tail suspension test

Table 2 Evaluation data using Tail suspension test

S.No	Treatment Groups	Average Time of Immobility (Sec)	Number of Trials	Gap between the trials (Sec)
1	Control	45.50 ± 2.39	3.75 ± 0.47	10.00 ± 1.29
2	Standard (Amitriptyline 10mg/kg)	13.75 ± 1.37	8.75 ± 0.47	3.00 ± 0.40
3	Test – I (<i>Ocimum sanctum</i> with <i>Coleus amboinicus</i> - Dose 1)	16.75 ± 0.75	6.75 ± 0.47	4.25 ± 0.47
4	Test – II (<i>Ocimum sanctum</i> with <i>Coleus amboinicus</i> Dose 2)	11.25 ± 0.47	7.00 ± 0.40	4.0 ± 0.40

Tail Suspension Test

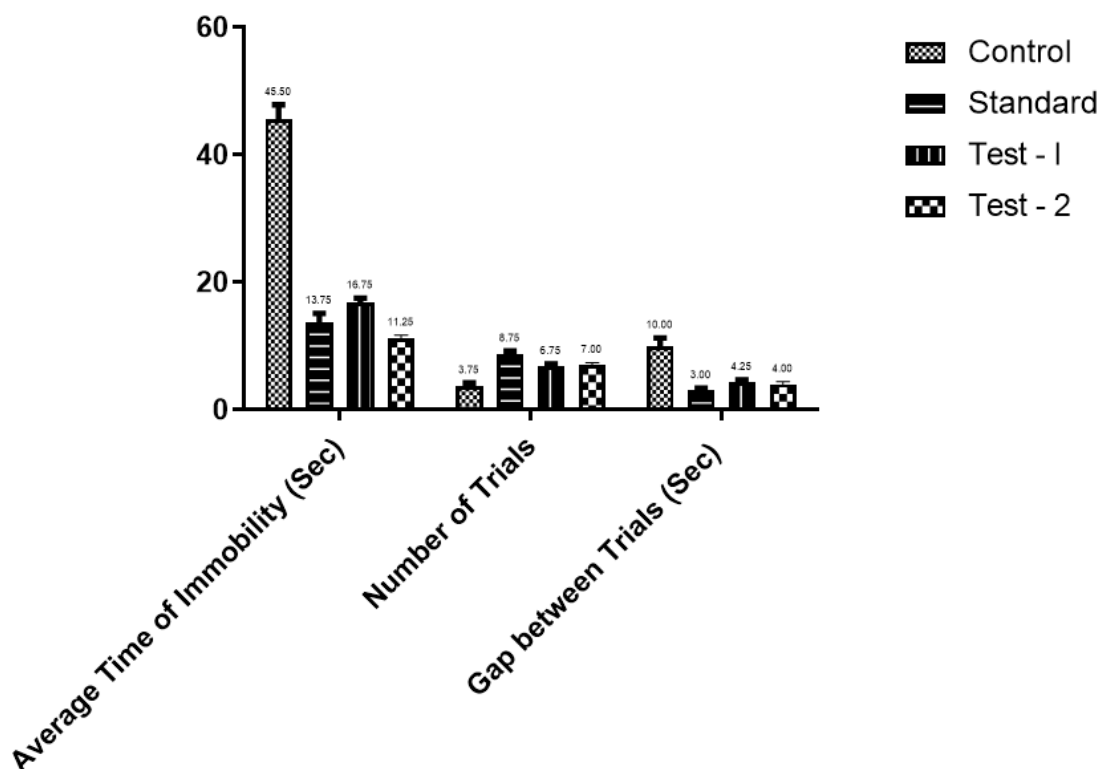


Figure 11: Evaluation of **all treatments group** using Tail suspension Test

Discussion

The forced swim test (FST) is a widely used behavioral assay for assessing depressive-like states and the efficacy of antidepressant treatments in animal models. In this test, animals are placed in an inescapable container filled with water, and their behavior is observed, particularly the time spent immobile, which is considered indicative of behavioral despair.

In the current study, the FST results revealed notable differences between the test groups and the control group. The control group, which did not receive any treatment, exhibited a typical level of immobility, as expected in this model. In contrast, the test groups, which were subjected to a specific intervention or treatment, demonstrated significantly reduced immobility time. This suggests that the animals in these groups were more active and exhibited less depressive-like behavior, indicating that the treatment had a positive effect on their behavior under stress.

The similarity in activity levels between the test groups and the control group suggests that the treatment was effective in reducing behavioral despair, bringing the activity of the treated animals closer to normal levels. This outcome implies that the treatment may have potential antidepressant properties, as it successfully mitigated the symptoms of immobility typically observed in the FST. The observed improvements in the test groups highlight the potential for further investigation into the mechanisms underlying these effects and their applicability in therapeutic contexts.

The tail suspension test (TST) is another commonly used behavioral assay to assess depressive-like behavior and the efficacy of antidepressant treatments in animal models, particularly mice. In this test, animals are suspended by their tails, and their movements are monitored, with immobility time being the primary measure of despair-like behavior.

In this study, the TST results revealed important distinctions between the test groups and the control group. The control group, which did not receive any experimental treatment, exhibited a standard duration of immobility, consistent with expected outcomes in this type of assay. The test groups, however, showed a marked reduction in immobility time compared to the control group. This reduction suggests that the animals in the test groups were less prone to behavioral despair, indicating an increased level of motivation or decreased depressive-like symptoms.

The reduced immobility observed in the test groups, similar to the control group's active behavior, suggests that the intervention or treatment applied to these groups had a significant antidepressant-like effect. The results support the hypothesis that the treatment is effective in improving the animals' coping behavior under stress, as evidenced by their enhanced struggle to escape, which is reflected in the decreased immobility time.

These findings align with the idea that the treatment used in the test groups may have therapeutic potential for

alleviating depressive symptoms. The consistency of the activity levels between the test and control groups further underscores the treatment's efficacy, warranting additional research to explore the underlying mechanisms and potential clinical applications of these findings.

STATISTICAL ANALYSIS

The mean standard error (SEM) accustomed to represent all of the above results. Two-way ANOVA was used to analyse the data, and Tukey's multiple comparison test was then performed. A P value of less than 0.0001 was deemed statistically significant.

CONCLUSION

The combination of *Ocimum sanctum* and *Coleus amboinicus* extracts demonstrates effective antidepressant activity, as indicated by the results from the Forced Swim Test and Tail Suspension Test in mice. The combination treatment group showed significant reductions in immobility time compared to the control group, suggesting enhanced antidepressant effects. Furthermore, the efficacy of the combined extracts was comparable to that of the standard treatment groups, indicating their potential as a natural and synergistic alternative for managing depressive disorders. These findings warrant further exploration in clinical settings to confirm the antidepressant benefits and safety profile of this herbal combination.

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