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BEHAVIOURAL AND BIOCHEMICAL INDICATIONS OF THE ANTIDEPRESSANT ACTIVITIES OF ESSENTIAL OIL FROM NIGELLA SATIVA LEAF IN RATS

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Abstract

Background: Nigella sativa (Ranunculaceae) is a medicinal plant traditionally used to treat neurological disorders such as depression, epilepsy, pain, neurotoxicity, and memory impairment.
Objective: This study aimed to evaluate the antidepressant potential of Nigella sativa leaf essential oil using in vitro enzyme-based analysis and in vivo experiments on animal models.
Materials and Methods: The chemical constituents of NSEO were identified by Gas Chromatography–Mass Spectrometry (GC–MS) using a Thermo Trace1300–TSQ 8000 system equipped with a 30 m × 0.25 mm BP-5MS column (0.25 μm film thickness). Antidepressant activity was initially assessed by evaluating inhibition of total MAO and MAO-A. For the in vivo study, fifty-four Wistar rats were randomly divided into six groups (n = 6): normal control, UCMS control, fluoxetine-treated, and NSEO-treated groups (100, 200, and 400 mg/kg, p.o.). Following 42 days of UCMS exposure, antidepressant activity was assessed using the tail suspension test. Brain oxidative stress markers, including thiobarbituric acid-reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and nitrite levels, were also determined.
Results: GC–MS analysis identified 84 volatile constituents, with thymoquinone, o-cymene, and α-pinene as the predominant compounds. NSEO selectively inhibited total MAO and MAO-A at 25–800 μg/mL, although reduced selectivity at 800 μg/mL suggested additional MAO-B inhibition. NSEO exhibited antidepressant-like effects by reducing immobility time, inhibiting MAO, highlighting its potential as a natural therapy for depression.
Conclusion: The volatile oil of Nigella sativa shows strong antidepressant effects by reducing depression and protecting key organ functions.

Keywords: Depression, Nigella sativa, Monoamine oxidase, Unpredictable Chronic Mild Stress, Tail suspension test

INTRODUCTION:

Depression is associated with several distinct behavioral or motor signs, which is a complex condition, such as suicide attempts, crying, facial expressions of despondency, sadness, and hostility toward oneself; cognitive symptoms, such as negative self-environment; social symptoms, such as increased dependence on others and withdrawal from social situations; as well as biological signs, such as changes in weight, insomnia, fatigue, appetite, altered sexual functioning and restlessness [1]. There are several risk factors for depression, which can be hypertension, smoking, hyperlipidemia, cardiovascular disease, diabetes, musculoskeletal disorder, and hemorrhage [2,3]. One of the prevalent disorders of modern living is depression. Over 350 million individuals globally, spanning all age groups, experience depression. According to estimates, depression causes one million suicides annually [4]. Even though antidepressant medications, including tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase-A, and selective serotonin reuptake inhibitors (SSRIs), are readily available, about 50% of depressed individuals do not receive any kind of treatment. While synthetic medications make up the great bulk of depression treatments, they have a variety of side effects, from benzodiazepines' drowsiness and ataxia to selective SSRIs' insomnia and libido [5]. Natural drugs appear to have the same curative potential as their synthetic counterparts, but with fewer side effects. The hunt for new medications made from medicinal plants to treat mental illnesses has advanced recently, revealing encouraging pharmacological efficacy of some plants used in complementary and alternative therapies to treat mood disorders [6]. Considering the

aforementioned, the polar essential oil from Nigella sativa seeds contains possible antidepressant components, attributing this effect to the presence of triterpenoid and flavonoid phytoconstituents. Numerous phytoconstituents, such as volatile and fatty oils, monoterpenes, alkaloids, triterpenes, and saponins, have been found to be abundant in N. sativa. Apart from the diverse range of activities, Nigella sativa shows activity such as cytotoxic, melanogenic, immunostimulant, smooth muscle relaxant, anticancer, anti-inflammatory, analgesic, antiaflatoxin, and actions that prevent diabetes [7]. More than 35 active substances, including the monoterpene derivatives thymoquinone (TMQ), dithymoquinone, thymol, and carvacrol, have been found through phytochemical investigation of Nigella sativa oil. This Mediterranean native plant has a long history of use for a variety of medical purposes, in addition to being a popular culinary herb [8]. Previous studies on the essential oil of Nigella sativa exhibited complete inhibition zones against B. cereus, B. subtilis, S. aureus, and Pseudomonas aeruginosa at concentrations of 2000 and 3000 ppm. At 1000 ppm, the essential oil also completely inhibited the growth of B. subtilis and S. aureus. Overall, the antibacterial efficacy of Nigella sativa essential oil was comparable to that of standard antibiotics, including ampicillin and cloxacillin. The antioxidant potential of Nigella sativa essential oil was assessed by determining the peroxide, thiobarbituric acid (TBA), and total carbonyl values of rapeseed oil fortified with the essential oil at different time intervals. The essential oil demonstrated potent antioxidant activity, outperforming the synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Its antioxidant efficacy was further confirmed in the linoleic acid system by inhibiting peroxide formation. Moreover, Nigella sativa essential oil exhibited remarkable free radical scavenging activity against the DPPH radical and showed a strong reducing power, highlighting its substantial antioxidant capacity [9]. This spice plant is thought to be crucial in the management and treatment of several stress-related illnesses, such as depression, because it is known to contain components with strong antioxidant activity. There is no recent report on the antidepressant effect of the essential oil from these spices. This study aimed to explore and compare the biochemical effects and antidepressant potentials of the essential oil from N. Sativa leaf in experimental rats.

2. MATERIALS AND METHODS

2.1 Plant materials

The fresh leaves of the Nigella sativa plant were collected in May and November of 2024, respectively, from Khairthal district, Swaroop Sarai village, Karni Kot post, Mundawar tehsil, Rajasthan, India. The plant N. sativa (Herbarium Voucher number: 2011) was successfully identified and verified by Dr. S.S. Yadav, an associate professor in the botany department of Maharshi Dayanand University in Rohtak, Haryana.

2.2 Extraction of essential oil

The leaves of Nigella sativa were air dried and coarsely powdered. Then extraction was carried out by using hydro distillation method. Using a measuring cylinder, distilled water was added to a conical flask containing a finely powdered sample of plants. The plant sample and water were mixed properly. Then that round-bottom flask was kept on a heater, which was connected directly to a condenser (Clevenger-type apparatus). Also, the water pipe was connected to the Clavenger apparatus, and the inlet and outlet of the pipe were maintained. After the boiling of the sample, the temperature was turned to medium, and the vapours went directly to the condenser. Hence, the drops of essential oil came down and were collected in a bottle placed downwards in the Clavenger apparatus [10,11].

2.3 Analysis of essential oil constituents using GC-MS

Nigella sativa essential oil (NSEO) was profiled by means of GC–MS on Thermo's Trace 1300 system coupled with a TSQ 8000 detector, equipped with a BP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film). The analysis was performed using helium as the carrier gas at 1 mL/min, with a split injection ratio of 1:10. The oven temperature was configured to begin at 60 °C for 1 minute, then ramp up to 250 °C at 4 °C/min and hold for 15 minutes. Mass spectra were recorded at 70 eV. A 1 μL injection was made from a solution prepared by diluting 30 μL of pure essential oil in 970 μL of hexane. Compound identification was performed by matching spectra with the NIST 2.0 and WILEY libraries, which confirmed thymoquinone as the major volatile constituent [12].

2.4 In vitro antidepressant activity

2.4.1 Monoamine oxidase analysis

The essential oil in vitro monoamine oxidase (MAO) inhibitory action will be tested using the brain homogenate of untreated rats made in phosphate buffer solution (pH 7.4).

The Biuret assay will be used to measure the total protein concentration in accordance with the procedure outlined by Cain et al. (1987) and standardized to a final value of 1 mg/mL.

Monoamine oxidase (MAO) inhibition will be assessed by incubating the tissue homogenate with varying concentrations of the essential oil (25, 100, 200, 400, and 800 mg/mL) or phosphate buffer as the control at 37 °C

in a water bath with continuous stirring for 5 min. Subsequently, 90 mM Kynuramine, a non-selective MAO substrate, will be incorporated, and the mixture will be left to incubate for an additional 30 min under identical conditions.
To assess Monoamine oxidase-A inhibitory activity, the homogenate will first be pre-incubated with 250 nM selegiline, an MAO-B–specific inhibitory agent, for 5 min. The essential oil will then be introduced at the previously mentioned concentrations. 90 mM Kynuramine will be added after 5 minutes of incubation, and the combination will then be incubated for 30 minutes under the same conditions as before. In all experimental assays, the reactions will be stopped by adding 10% trichloroacetic acid. After centrifuging the samples, 800 mL of the supernatant will be put into new microtubes together with 1000 mL of 1 M NaOH. Three microplate wells will then be filled with 200 mL aliquots of each sample. A spectrophotofluorometer operating with excitation and emission wavelengths set at 320 nm and 460 nm, respectively, will be used to measure the concentration of 4-hydroxyquinoline, the fluorescent product produced by the MAO-mediated oxidation of kynuramine.
The inhibitory effect on total MAO activity will be expressed using the equation below:

$$\% \text{ of inhibition} = \frac{\text{Fluorescence of control} - \text{Fluorescence of sample}}{\text{fluorescence of control}} \times 100$$

For the assessment of MAO-A inhibition, the same calculation formula will be applied; however, the control fluorescence values will be substituted with the fluorescence values obtained from samples treated with selegiline alone, without the extract. MAO-B inhibitory activity will be determined indirectly by calculating the difference between total MAO activity and MAO-A activity. Linear regression analysis of percentage inhibition as a function of final concentration, using the average data from three independent experiments, will be employed to determine the IC_{50} values for each MAO isoform [13].

2.5 Invivo Antidepressant activity

2.5.1 Approval of the experimental protocol

The Institutional Animal Ethics Committee (caf/mm(DU)/26/IAEC-77) reviewed and approved the study protocol for the Antidepressant activity model, the UCMS model of depression in rats, which was implemented under the ethical and husbandry standards prescribed by CPCSEA.

2.5.2 Unpredictable chronic mild stress induced Depression

Before the study began, the animals were moved from the housing facility to the experimental room in their home cages and given two to three hours to get used to their new environment. Over the course of each week, the rats were randomly assigned to experience seven distinct unexpected chronic moderate stressors; one stressor was given each day, and no stressor was repeated on consecutive days. The UCMS procedure was maintained for six weeks, while pharmacological intervention was initiated two weeks after the onset of the UCMS regimen and continued throughout the remaining study period. The study was conducted over a period of six weeks. In summary, the animals were exposed to a single stressor daily between 11:00 a.m. and 11:30 a.m., except for stress procedures extending over 24 h. The stress regimen comprised the following mild stressors:
• **Day I-** Animals were subjected to forced swimming in 37 °C water for 5 min.
• **Day II**-The animals were subjected to a 24-h wet bedding condition using a mixture of 100 mL water and 50 g litter.
• **Day III-** Twenty-four hours of feed deprivation
•**Day IV**- A 90-second period of tail pinch stimulation
• **Day V-** Water deprivation for 24 hours.
• **Day VI-** The rats underwent 5 min of forced swimming in 4 °C water and were subsequently dried using a towel.
• **Day VII**- The cages were tilted 45° from the horizontal plane and maintained in this position for 24 hours [14-16].

Table 1- Chronic unpredictable mild stress-induced depression in rats.

Sr. No	Model	Treatment group	No of animals
1.	Acute Toxicity Study	Already reported [17, 18]	-----

2.	Antidepressant activity of essential oil in UCMS-induced depression was studied individually	**Acclimization (1 week)** **Group I**: C**ontrol** group will receive normal saline orally (1 ml/kg BW) for 6 weeks. **Group II**: UCMS group (Unpredictable chronic mild stressor for 4 weeks)	6 rats 6 rats
		Group III: Standard drug group, diseased animals of this group will receive Fluoxetine 10mg/kg p.o. for 6 week. **Group IV ,V and VI** : Diseased animals will be given 100,200 and 400 mg/kg,p.o. essential oil from leaves of N. sativa for 6 weeks. **Group VII ,VIII and IX** : Diseased animals will be given 200, 300, and 400 mg/kg,p.o essential oil from leaves of S.indicum for 6 weeks.	6 rats 18 rats 18 rats
		Total	54 rats

2.6 Behavioural depression models

2.6.1 Sucrose preference consumption test

The sucrose preference test was conducted both prior to and following the 30-day exposure to UCMS. Along with social avoidance or isolation, anhedonia, which is defined as a decreased taste for sweet solutions, is a characteristic that is closely associated with an animal's feeling of defeat. By creating this test both before and after the UCMS, it is feasible to determine that the animals are in an anhedonic state at the conclusion of the stress phase, which is indicated by a notable reduction in their sucrose intake. Wilmer et al.'s instructions were followed for conducting the test. Rats were initially trained to experience and consume sucrose solution (1%) for a full day by being given two identical bottles at the same time: one containing sucrose solution (1%) and the other tap water. Rats were allowed to choose between the two bottles for a whole day as part of the testing protocol. In order to avoid any potential impacts of side preference on drinking behavior, the bottles were moved halfway through the testing. Weighing bottles were used to quantify the amount of water and sucrose solution consumed simultaneously by the experimental and control groups. It was determined how their initial and ultimate weights differed from one another. The following formula was used to determine the sucrose preference [19]:

$$\%\text{sucrose preference} = \frac{\text{Sucrose intake (g)}}{\text{Sucrose intake (g) + Water intake (g)}} \times 100$$

2.6.2 Tail suspension test

According to Can et al. (2012), the tail suspension test model of depressive-like behavior was employed to assess behavior despair, a characteristic of depression. Using an adhesive tape positioned about 1 cm from the tail tip, rats were suspended upside down on a metal rod in a tail suspension box at a height of 23.5 cm from the ground. Direct observation and video capture were used to document the whole duration of immobilization during the 6-minute test. The rat was deemed immobile when it did not attempt to grasp the sticky tape, twist its body, or jolt for a minimum of five seconds. This procedure will involve incubating 42 Albino rats with a standard and a plant essential oil [18].

2.7 Biochemical Parameters

1) Brain Homogenate Preparation

Ice-cold saline of about 0.9% sodium chloride was used to properly clean the brain tissues before being prepared as a homogenate in chilled phosphate buffer (pH 7.4). To remove nuclear debris, the homogenized sample was centrifuged at 800 × g for 5 minutes while maintained at 4°C. Subsequently, to obtain the postmitochondrial supernatant, the supernatant was centrifuged at 10,500 × g for 20 minutes at 4°C, which served as the sample for biochemical analyses of catalase, lipid peroxidation, nitrite, reduced glutathione, and superoxide dismutase [20].

2) Determination of lipid peroxidation levels

Using Wills' procedure (1965), Thiobarbituric acid-reactive chemicals were used to assess the quantity of MDA, a marker of lipid peroxidation. In summary, two hours at 37 degrees Celsius were spent incubating 0.5 milliliters of Tris and postmitochondrial supernatant, respectively. At the end of the incubation period, one milliliter of 10% trichloroacetic acid was added to the mixture, and the samples were centrifuged for ten minutes at 1,000 × g. One milliliter of the supernatant and one milliliter of 0.67% thiobarbituric acid were then boiled in boiling water for ten minutes. After chilling and adding one milliliter of double-distilled water, absorbance was measured at 532 nm. An extinction coefficient of $1.56 \times 10^2\ M^{-1}\ cm^{-1}$ was used to compute the concentration of TBARS, which were subsequently represented as milligrams of protein per nanomole of MDA. The quantity of brain MDA was expressed as nmol/mg protein using the Biuret technique to measure the protein content [20].

3)Measurement of GSH activity

Reduced glutathione (GSH) levels were measured using the Jollow et al. (1974) technique. To precipitate proteins, one milliliter of 4% sulfosalicylic acid and one milliliter of 10% postmitochondrial supernatant were mixed together. The mixture was centrifuged at 1,200 g for 15 minutes at 4°C after being kept at that temperature for at least an hour. The assay mixture had a final volume of three millilitre and was composed of 0.1 mL of the supernatant, 2.7 mL of phosphate buffer (0.1 M, pH 7.4), and 0.2 mL of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB; Ellman's reagent, 0.1 mM, pH 8.0). Using a molar extinction coefficient of $1.36 \times 10^2\ M^{-1}\ cm^{-1}$, reduced glutathione concentrations were computed and expressed as μmol/mg protein. At 412 nm, the yellow tint that appeared was measured right away [20].

1) Assessment of Superoxide dismutase

The Kono (1978) method was used to quantify the superoxide dismutase effect. The assay combination was 96 μM NBT, 0.1 mM EDTA, and 50 mM Na2CO3. After transferring 2 mL of this reaction mixture to a cuvette, 0.05 mL of the supernatant from the mitochondrial separation and 0.05 mL of hydroxylamine HCl that had been pH-adjusted with NaOH were added. Over the course of two minutes, the extent of hydroxylamine auto-oxidation was determined from changes in absorbance measured at 560 nm, with readings taken every 30 and 60 seconds. The units per milligram of protein were used to express the superoxide dismutase activity [20].

5) Assessment of Catalase activity

The activity of catalase was assessed by Claiborne's method (1985). The assay system contained 3.0 mL of reaction volume, consisting of 0.05 mL of postmitochondrial supernatant (10%), 0.019 M (H_2O_2, 1 mL), and 1.95 mL of pH 7.0 phosphate buffer (0.05 M). The rate of hydrogen peroxide breakdown was measured by tracking at 240 nm absorbance variations.

After that, the 0.07 mM H_2O_2 extinction coefficient was used to calculate catalase activity and expressed as μmol of H_2O_2 broken down per minute per milligram of protein [20].

6)Nitrite estimation

Nitric oxide production was assessed indirectly by measuring nitrite levels by the Griess reaction. 500 microliters of Griess reagent, consisting of 1% sulfanilamide in 5% phosphoric acid and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride in water, was reacted with 100 μL of postmitochondrial supernatant for the assay. A spectrophotometer was used to measure the absorbance at 546 nm (Green et al. 1982). A sodium nitrite standard curve was used to calculate the nitrite concentration. The units of measurement for nitrite were micrograms per milliliter [20].

2.8 Analysis of Data

Mean ± standard error of the mean (SEM) was used to express the data. One-way analysis of variance (ANOVA) and the Bonferroni multiple comparison post hoc test were used to compare the groups statistically. When the p-value was less than 0.05 ($p < 0.05$), differences were deemed statistically significant.

3. RESULTS

3.1 Total yield of Essential oil

The essential oil obtained is typically a pale yellow to golden brown or dark amber color, depending on purity, processing, and filtration, appearing as a viscous liquid with hints of green or brown. The yield of essential oil was calculated by the following equation:

y=V*100
W
y=350*100
1000
Volume= 350 ml
Weight=1000g
Yield= 35% (v/w)
Yield of Nigella sativa leaves essential oil= 35%
where y is the yield of essential oil (%), V is the volume of collected essential oil (ml)
and W is the weight of the plant material (g).

3.2 GC-MS analysis

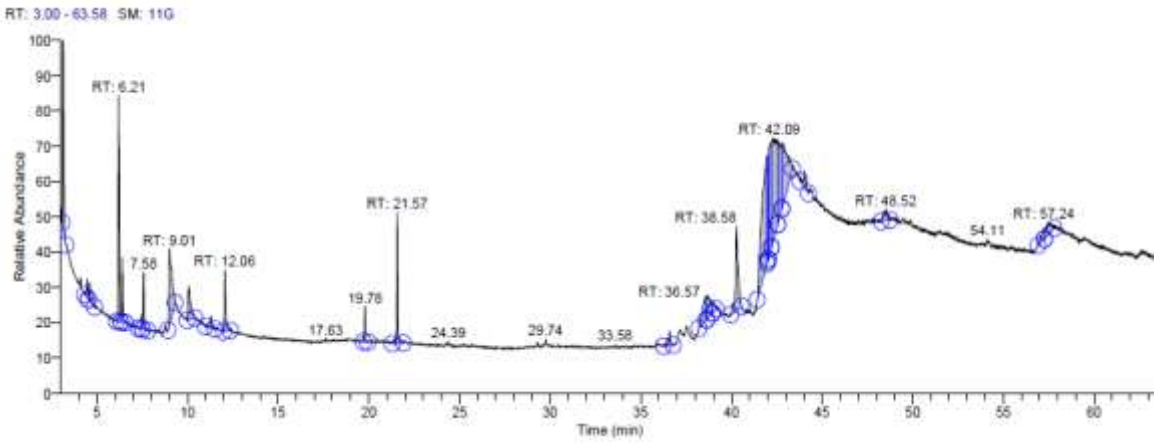


Figure 1. Peaks in the GC-MS chromatogram of Nigella sativa essential oil suggest that the plant oil contains 84 different chemicals.

GC–MS analysis of the essential oil identified 84 compounds using the NIST Mass Spectral Database by comparing fragmentation patterns with reference spectra. Some additional low-quality compounds were also detected. The compounds were catalogued with details such as peak area, peak height, peak area percentage, retention time (RT), and CAS number.

The major constituents of the oil were Thymoquinone (15.19%), 1,15-Pentadecanediol (12.98%), 2(1H)Pyridinone, 1,4,6-trimethyl (7.93%), o-Cymene (5.52%) and α-Pinene (1.65%).

Table 2. List of some important chemical compounds identified in Nigella sativa leaves essential oil by GC-MS results.

Compound Name	CAS	RT	Peak Area
5,10Pentadecadien1ol,(Z,Z)	64275-51-0	41.95	128622804.1
Thymoquinone	NA	41.95	128622804.1
cis,cis7,10,Hexadecadienal	56829-23-3	41.95	128622804.1
5,10Pentadecadien1ol,(Z,Z)	64275-51-0	42.26	109916548.2

2Methyl3(3methylbut2enyl)2(4methylpent3enyl)oxetane	NA	42.26	109916548.2
1,15Pentadecanediol	14722-40-8	42.26	109916548.2
2(1H)Pyridinone,1,4,6trimethyl	15031-89-7	42.8	67167540.34
(1S,15S)Bicyclo[13.1.0]hexadecan2one	102572-89-4	42.8	67167540.34
Pyrrolo[1,2b][1,2,4]oxadiazole2(1H)thione,1(4fluorophenyl)tetrahydro5,5dimethyl	50455-86-2	42.8	67167540.34
oCymene	527-84-4	9.01	46711640.87
2Methylbenzoic acid, 2,5dichlorophenyl ester	NA	9.01	46711640.87
2Methylbenzoic acid, 2fluorophenyl ester	NA	9.01	46711640.87
tButyl cyclopentaneperoxycarboxylate	25023-14-7	38.58	21785666.81
1,3Dioxolane, 2ethyl2isobutyl	935-45-5	38.58	21785666.81
2,6,10,14Tetramethylpentadecan6ol	104000-14-8	38.58	21785666.81
àPinene	80-56-8	6.4	13988666.47
Bicyclo[3.1.1]hept2ene, 3,6,6trimethyl	4889-83-2	6.4	13988666.47
Tricyclo[3.2.1.0(2,4)]octane, 8methylene, (1à,2à,4à,5à)	38310-48-4	6.4	13988666.47

3.3 In vitro antidepressant effect

3.3.1 Monoamine oxidase analysis

In our study, Nigella sativa essential oil inhibited MAO, which strongly advocates the contribution of the essential oil in exerting anti-depressant potential. The plant essential oil inhibited total MAO activity and the MAO-A isoform with comparable efficacy over a concentration range of 25–800 µg/mL (Figure 2), suggesting selective MAO-A inhibition at lower concentrations. However, at 800 µg/mL, inhibition of total MAO activity was markedly greater than that of MAO-A, indicating a loss of selectivity and additional inhibition of MAO-B. Nigella sativa essential oil showed IC_{50} values of 405.26 µg/mL for MAO-T and 700 µg/mL for MAO-A, as determined by linear regression. At concentrations below 400 µg/mL, MAO-B inhibition was negligible, and at the maximum dose tested, 800 µg/mL, it was estimated to be 24%. The effect of inhibition of MAO-A was more prominent than that of MAO-B.

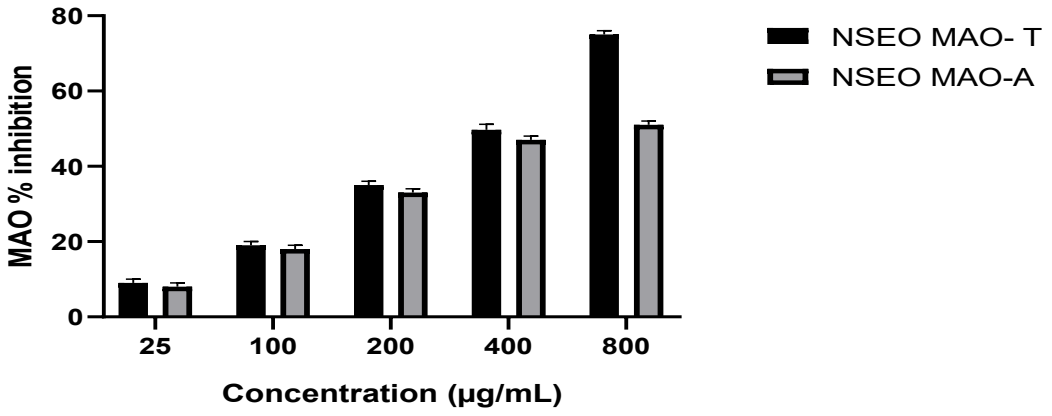


Figure 2. The inhibitory effect on monoamine oxidase (MAO) activity was expressed as percentage inhibition in rat brain homogenates. IC_{50} values were calculated through linear regression analysis of the N.sativa essential oil data.

3.4 In-vivo Antidepressant activity Unpredictable chronic mild stress (UCMS) induced Depression

3.4.1 Behavioural depression models Sucrose preference consumption test

Figure 3 represents the percentage of sucrose preference in rats before the UCMS and the effect of Nigella sativa essential oil on anhedonia. Before the UCMS, the sucrose preference was relatively very high (about 95%) in every animal group. After the UCMS, we noted a significant decrease in this percentage in all animals of the negative control group compared to normal animals. The treatment with Nigella sativa essential oil at a dose of 100 mg/kg has significantly increased ($p < 0.001$) the preference for sucrose in animals of the NS 100 group. Nevertheless, those treated with NS at a dose of 400 mg/kg and treated with fluoxetine presented a nonsignificant increase in preference for sucrose compared to stressed but non-treated animals.

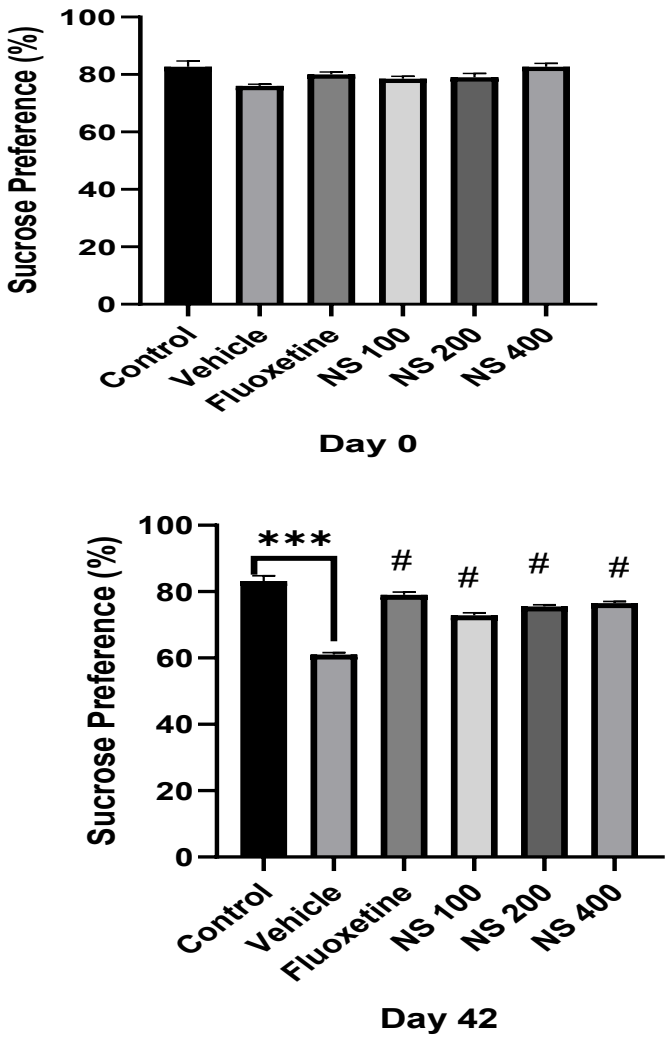


Figure 3: Nigella sativa essential oil's effects on the sucrose consumption test in the UCMS rat model. The mean ± SEM (n = 6) is used to express the results. One-way ANOVA and Bonferroni's multiple comparisons test were used to assess statistical significance. NC stands for normal control; (UCMS) Unpredictable long-term mild stress; Sesamum indicum (SI); Nigella sativa (NS).

Behavioral effects of essential oil in the tail suspension test

In the Tail Suspension Test (TST), rats under stress demonstrated a substantial increase in immobility time ($p < 0.001$) as compared to the normal control group. In comparison to the stress group, the period of immobility was considerably decreased by essential oil treatment where $p < 0.01$. Similarly, relative to agitated rats, fluoxetine administration significantly decreased immobility time ($p < 0.001$), suggesting an antidepressant-like effect (Figure 4).

Table 2: Immobility duration in experimental rats treated with Nigella sativa essential oil against the tail suspension test.

Experimental group	Duration of immobility (sec)
Normal Control	106.1666667 ±1.301708
UCMS	187.6666667 ±1.626175
Fluoxetine (10mg/kg)	120.6666667 ± 1.229273
NSEO 100 mg/kg	165.8333333 ± 1.166667
NSEO 200 mg/kg	144.8333333 ±1.166667
NSEO 400 mg/kg	121.5 ± 1.176152

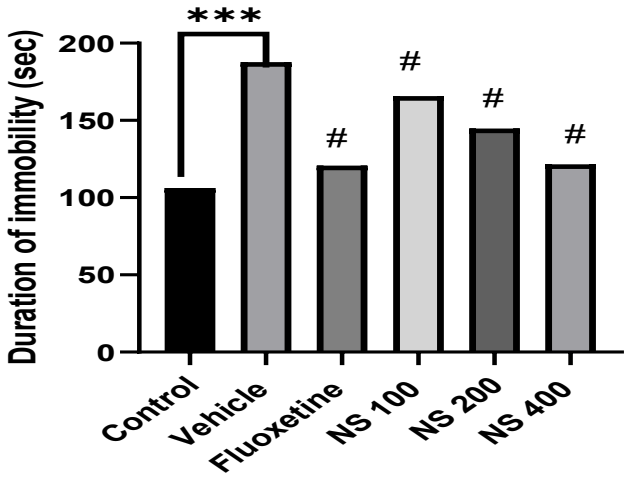
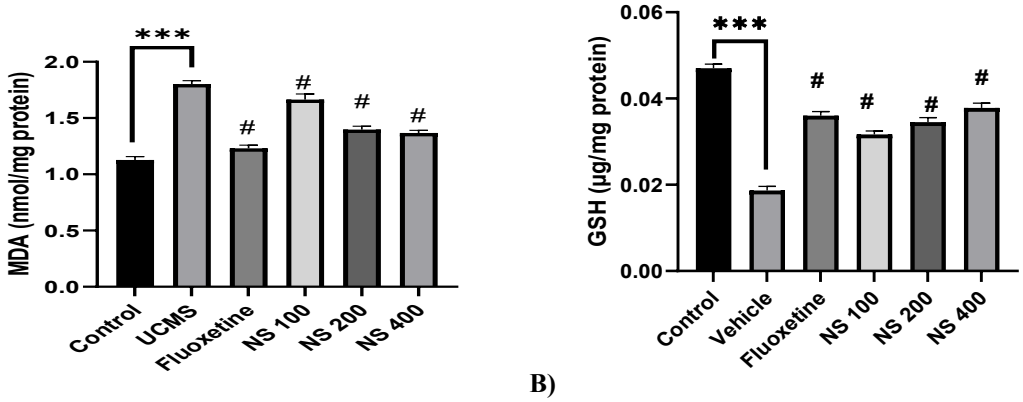


Figure 4. Evaluation of essential oil impact on tail suspension test (TST) outcomes in rats exposed to UCMS.

3.5 Biochemical Parameters



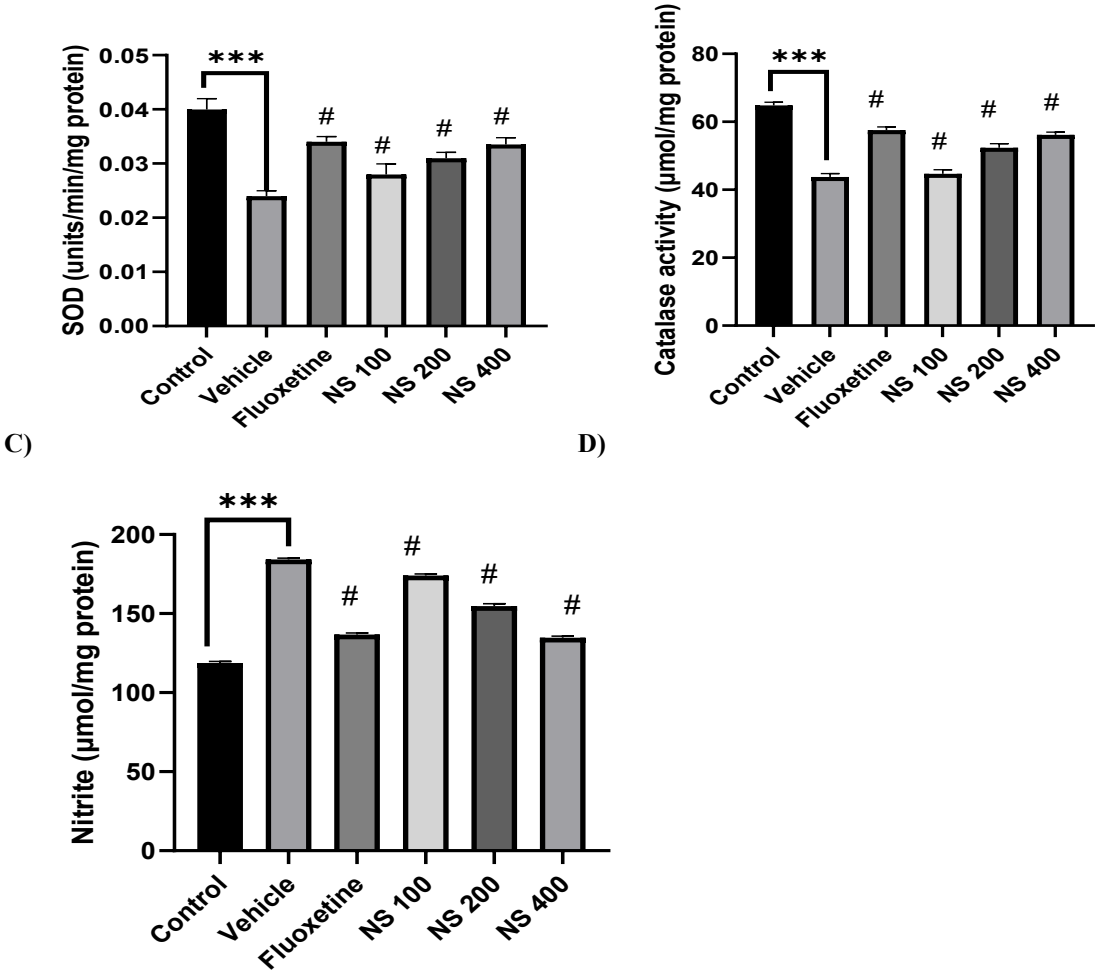


Figure 5: Effect of essential oil on various biochemical parameters in UCMS-induced rats, where; A) Lipid Peroxidation, B) Reduced glutathione, C) Superoxide Dismutase, D) Catalase activity, E) Nitrite estimation

Discussion

The present study demonstrated that Nigella sativa leaf essential oil possesses significant antidepressant-like activity in both in vitro and in vivo experimental models. GC–MS analysis identified thymoquinone (15.19%) as the major constituent, followed by 1,15-pentadecanediol, 2(1H)-pyridinone, o-cymene, and α-pinene. These phytochemicals possess antioxidant, anti-inflammatory, and neuroprotective properties that may contribute to the observed antidepressant effects. Similar chemical profiles have been reported previously, although constituent proportions vary with extraction methods and plant origin. Kokoska et al. identified thymoquinone and p-cymene as the principal volatile components of Nigella sativa essential oil, while Samadi et al. demonstrated that hydrodistillation preserves oxygenated monoterpenes with important biological activity [10,11]. Dalli et al. further confirmed the antioxidant and enzyme inhibitory potential of Nigella sativa phytochemicals relevant to neurological disorders [12].

A major finding of the present study was the concentration-dependent inhibition of monoamine oxidase, particularly MAO-A. Since MAO-A metabolizes serotonin, norepinephrine, and dopamine, its inhibition may enhance monoaminergic neurotransmission and produce antidepressant effects. Similar MAO inhibitory activity of plant-derived phytochemicals has been reported by Borba et al., supporting monoamine oxidase inhibition as an important mechanism underlying antidepressant activity [13].

The UCMS model successfully induced depressive-like behavior, as demonstrated by reduced sucrose preference and increased immobility time. These findings are consistent with previous reports showing that chronic unpredictable stress reproduces behavioral and neurochemical changes resembling human depression. Ping et al. demonstrated that chronic stress decreases hippocampal BDNF expression and neurogenesis, whereas Singh et al. validated UCMS as a reliable model for evaluating antidepressant agents [14,15].

Treatment with Nigella sativa essential oil significantly improved sucrose preference, indicating reversal of stress-induced anhedonia. Likewise, immobility time in the tail suspension test was significantly reduced, particularly at 400 mg/kg, approaching the effect of fluoxetine. Similar behavioral improvements following treatment with

plant-derived essential oils have been reported by Ekeanyanwu et al [16]. Gholamnezhad et al. also attributed the antidepressant potential of Nigella sativa and thymoquinone to antioxidant, anti-inflammatory, neuroprotective, and monoaminergic mechanisms, while Alqahtani et al. emphasized the synergistic pharmacological actions of medicinal plant phytochemicals [17,18].

Oxidative stress plays a major role in depression pathogenesis. In the present study, UCMS increased lipid peroxidation and nitrite levels while reducing glutathione, superoxide dismutase, and catalase activities. Administration of Nigella sativa essential oil significantly restored these biochemical parameters, indicating strong antioxidant activity. Similar restoration of oxidative stress markers following antidepressant treatment has been reported by Foyet et al. and Kumar et al., who demonstrated that reduction of oxidative stress contributes substantially to antidepressant efficacy [19,20].

Overall, the findings indicate that Nigella sativa leaf essential oil exerts antidepressant effects through multiple mechanisms, including MAO-A inhibition, enhancement of antioxidant defenses, reduction of oxidative stress, and neuroprotection. These results support its potential as a promising natural therapeutic candidate for depression.

Conclusion

The present study demonstrated that Nigella sativa leaf essential oil possesses significant antidepressant activity in both in vitro and in vivo models. The oil, rich in bioactive compounds including thymoquinone, exhibited MAO-A inhibitory activity, improved depressive-like behaviors by increasing sucrose preference and reducing immobility time, and attenuated oxidative stress by restoring antioxidant defenses. These findings suggest that its antidepressant effects are mediated through monoamine oxidase inhibition, antioxidant, and neuroprotective mechanisms. Nigella sativa leaf essential oil may therefore represent a promising natural candidate for depression management, warranting further mechanistic and clinical investigations.

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Declaration:

Ethical Approval:

This study used healthy Albino Wistar male and female rats weighing 150–200 g, with ethical approval no. (caf/mm(DU)/26/IAEC-77) from M.M. College of Pharmacy, Ambala approved in January, 2026.

Competing Interest:

The authors declare no competing interests.

Authors contribution:

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and to be accountable for all aspects of the work. All the authors are eligible to be author as per the requirements/ guidelines.

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Data Availability:

All the data is available with the authors and shall be provided upon request.

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