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RESEARCH ARTICLE
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COMPARATIVE EVALUATION OF ANTIDEPRESSANT EFFECTS OF MINOCYCLINE AND FLUOXETINE IN MALE WISTAR RATS.

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Abstract:

Background: Selective serotonin reuptake inhibitors (SSRIs) are the most prescribed drugs for the treatment of Major Depressive Disorders (MDD). But many patients do not respond adequately to conventional antidepressant therapy. Hence there is a continued need for development of novel efficacious depression interventions. Minocycline has a pronounced inhibitory effect on the brain's microglia, and can beneficially target the pathophysiology of depression. Inflammation is strongly associated with depression and pro-inflammatory medications carry the potential to induce depressive symptoms in humans. Microglia are the primary immune cells that mediate neuroinflammation and minocycline is capable of reducing inflammation by directly suppressing microglia activation and by indirectly inhibiting microglia-induced inflammatory processes. Keeping this in mind the present study was done to compare the antidepressant effects of minocycline with fluoxetine in male wistar rats.

Methods: 18 Male Wistar Rats, aged 8-10 months, each weighing 150-200 gm were included in the study. Each animal was subjected to chronic mild stressors for 14 days to create depression. After 14 days, the rats underwent behavioral tests like immobility by Forced swim test and Open field tests and were tested for Sucrose preference tests. The rats were divided randomly into 5 groups with each group having 6 animals. Group I (Minocycline 25mg/kg/day), Group II (Minocycline 50mg/kg/day), Group III (Minocycline100mg/kg/day), Fluoxetine(30mg/kg/day) and group V rats were given RO water once daily via oral route for a period of 28 days.After 28 days, the animals were again subjected to forced swim test, open field tests and sucrose preference tests. Intergroup and intragroup comparison using ANOVA and t tests was done. p value<0.05 was considered significant.

Results: The study showed significant antidepressant-like effects in Groups II, III, and IV in swim Despair Test post drug treatment. There were no significant locomotor changes in any group in open field test. Improvement in anhedonia-like behavior in Groups I, II, and IV using Sucrose Preference Test was significant.

Conclusion: The present study revealed that minocycline showed antidepressant like effects at different doses which can be further explored using larger sample size.

Keywords: Depression, Minocycline, neuroinflammation, forced swim test, Fluoxetine.

Introduction: Major depressive disorder (MDD) is a prevalent and debilitating psychiatric condition characterized by persistent low mood, anhedonia, and cognitive impairments, affecting millions worldwide and representing a significant public health burden. Depression leads to morbidity, and the National Mental Health Survey (NMHS) 2015-16 by NIMHANS found that 10.6% of adults in India suffer from mental disorders. Nearly 50% of persons with major depressive disorders reported difficulties in carrying out their daily activities.[1]Conventional antidepressant therapies, such as selective serotonin reuptake inhibitors (SSRIs), including fluoxetine, primarily target monoaminergic systems to increase synaptic serotonin levels and ameliorate depressive symptoms. Fluoxetine is one of the most widely studied SSRIs in both clinical and preclinical research and has demonstrated significant reductions in depressive-like behaviors in rat models, such as decreased immobility in forced swim tests and modulation of neurogenesis and synaptic plasticity in the hippocampus of male Wistar rats subjected to chronic stress paradigms.[2, 3]Despite the clinical effectiveness of fluoxetine, a substantial proportion of patients do not achieve remission with SSRI monotherapy, and the delayed onset of therapeutic effects remains a challenge. These limitations have spurred interest in alternative or adjunctive treatments that act through different mechanisms of action. One such drug is **minocycline**, a second-generation tetracycline antibiotic that has demonstrated neuroprotective, anti-inflammatory, and immunomodulatory properties beyond its antimicrobial activity. Preclinical evidence indicates that minocycline reduces depressive-like behavior in multiple rodent models, potentially through inhibition of microglial activation and attenuation of neuroinflammatory pathways, which are increasingly implicated in the pathophysiology of depression.[4, 5]Unlike traditional antidepressants that primarily modulate monoaminergic neurotransmission, minocycline's proposed antidepressant effects may involve suppression of pro-inflammatory cytokines and modulation of neuroimmune interactions, which could underlie behavioral improvements in diseased animal models. Emerging studies suggest that minocycline significantly reduces immobility in depression-related behavioral tests and ameliorates inflammation-associated neurobiological alterations in rodents.[5]Currently, the chronic stress model is widely used and studied to investigate the underlying mechanism, etiology, and emerging animal lines of depression[6, 7]. Most antidepressants can reverse the effects of reduced sucrose consumption.[8]

Therefore, a **comparative evaluation of minocycline and fluoxetine in male Wistar rats** offers valuable insights into their relative antidepressant efficacy and underlying neurobiological mechanisms. Such comparative analyses can help clarify whether neuroimmune targeted therapies like minocycline could serve as effective alternatives or adjuncts to monoaminergic antidepressants in treating depressive disorders, especially in cases associated with neuro-inflammation or treatment resistance.

Material and Methods: The animal study was conducted in Central Animal House, SGRRIM&HS, Dehradun. Approval from the IAEC was obtained and the study was conducted as per the guidelines by CCSEA. The duration of study was for a period of 6 months commencing from 1st March 2025 to 31st August 2025. **Study Design:** A total of 18 Male Wistar Rats, aged 8-10 months, each weighing 150-200 gm were included in the study. Each animal was kept in separate cage (creates a social deprivation) and was subjected to chronic mild stressors (like food & water deprivation, not changing the bedding for 2-3 days) for a period of 14 days to create models of mild depression[9].
After 14 days, all the rats underwent behavioral tests like immobility by Forced swim test and Open field tests and were tested for Sucrose preference tests. The values were noted and the rats were divided randomly into 5 groups with each group having 6 animals. The rats not showing the signs of depression on the behavioral tests were excluded from the study.

Table-I- Study Groups:

Groups	No of Rats	Drug dose
I	6	Minocycline- 25mg/kg/day
II	6	Minocycline- 50mg/kg/day
III	6	Minocycline- 100mg/kg/day
IV	6	Fluoxetine- 30mg/kg/day
V	6	RO water

All the animals received the medicines once daily via oral route for a period of 28 days. After 28 days of receiving the respective medicines, the animals were again subjected to behavioral tests like the Forced swim test, open field tests and Sucrose preference tests. The values were noted and intergroup and intragroup comparison was done.

Behavioral testing procedures:

1. Forced Swimming Test (FST): The forced swimming test (FST) rat model was originally established by Porsolt[10] and his colleagues in 1977 and was subsequently used to perform behavioral tests in mice. This test is the most widely used tool for assessing antidepressant activity pre-clinically[11].Subsequent evidence showed that the immobile state of FST rodents actually reflected an extinction-like inhibitory learning behavior that was caused by the inescapable/unavoidable feature of the FST. The immobility produced by the FST can be regarded as a switch that promotes cognitive functioning for an animal's adaption and survival[10]

Methods[12, 13]

- The cylinders were filled with tap water at 23 ± 1 °C and the water depth was adjusted according to the rat's size, so that it could not touch the bottom of the container with its hind legs.
- The cylinder was marked with the animal number for the purpose of the identification of the animal later on while viewing the footage.
- Each rat was placed in the water filled cylinder container for 15 minutes.
- After 15 min had elapsed, the rat was removed from the container and placed in the transient drying cage with the heat lamp above it and the heat pad under it.
- The rat was closely and continuously monitored while recovering in this cage. For this purpose, it was possible to place a thermometer at the level of the rat to make sure the temperature in the cage did not exceed 37 °C
- Change the water after every session to avoid any influence on the next rat.

The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e. behavioral despair) or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli[14]

2. The Open Field (OF) Test: The OF test is used to assess exploratory activities and anxiety like behavior in an open box. The open-field test (OFT) imitates unsafe surroundings, evaluates animals' autonomous behavior, and reveals how tense the animals are[15].

The open field is a square wooden box with a base of 50 cm^2 and a wall height of 50 cm, and the bottom was divided equally into 9 blocks with markers. Before each trial, the arena surface was cleaned with 75% ethanol, the rats were placed in the center of the apparatus, and the activities like whether the rat is exploring the area or it is moving along the periphery. The duration of time spent in the central area & peripheral area was recorded[16]. OFT is sensitive to alterations in the environment.

3. Sucrose preference test: The sucrose preference test is commonly used to evaluate anhedonia in animals, which refers to a reduced capacity to experience happiness.
A decrease in the sucrose consumption ratio of an experimental animal is indicative of a successful depression model with anhedonia. It is widely accepted that the SPT is the most appropriate behavioral test for the chronic mild stress model[17].

Statistical Analysis: Intergroup analysis was done by ANOVA and intragroup analysis was done by using Paired T test. p value <0.05 was considered statistically significant.

Results: Pre- drug administration Result:

Table 2- showing the pre-drug results (mean ± SD) of different groups.

Group	Open Field Test (Periphery Time, minutes)	Swim Despair Test (Time to Immobility, minutes)	Sucrose Solution Consumed (ml)
I	4.92 ± 0.02	1.43 ± 0.44	50.5 ± 13.3
II	4.89 ± 0.06	2.53 ± 0.50	65.0 ± 12.2
III	4.78 ± 0.11	2.60 ± 0.50	76.7 ± 10.8
IV	4.94 ± 0.02	2.78 ± 0.43	43.3 ± 13.1
V	4.88 ± 0.03	3.25 ± 0.59	65.0 ± 16.5

Prior to drug administration, all animals were subjected to chronic stress to establish a depression-like phenotype. One-way ANOVA performed on pre-drug behavioral data revealed no biologically meaningful differences among groups in the open field test, forced swim test, or sucrose consumption, indicating baseline homogeneity of the experimental groups following stress induction.

Post Drug Administration Results:

1. Inter group analysis

Behavioural Test	F value	P value	Significance
Swim Despair Test	4.30	0.011	Significant
Sucrose Preference Test	5.32	0.004	Highly significant
Open Field Test	0.97	>0.05	Not Significant

One-way ANOVA test: Post-drug swim despair test scores demonstrated a significant effect of treatment on behavioral outcomes ($F(4,25) = 4.30$, $p = 0.011$). This indicates that drug administration significantly altered despair-like behavior among the experimental groups.Sucrose Preference test demonstrated a significant effect of treatment ($F(4,25) = 5.32$, $p = 0.0043$), indicating that drug administration significantly influenced anhedonia-like behavior. Analysis of open field test data revealed no significant differences among treatment groups ($F(4,25) = 0.97$, $p > 0.05$), indicating that drug treatments did not alter locomotor activity.

2. Intra group analysis: Swim despair test

Groups	t value	p value	Statistical Interpretation
Group I	−2.379	0.0633	Not significant
Group II	−4.210	0.0084	Significant
Group III	−2.627	0.0467	Significant

Group IV	−5.818	0.0021	Significant
Group V	−1.798	0.1321	Not significant

Open field test

Group	Treatment	t (df=5)	p value	Significance
Group I	Minocycline 25 mg/kg	0.02	> 0.98	Not significant
Group II	Minocycline 50 mg/kg	0.63	> 0.55	
Group III	Minocycline 100 mg/kg	1.58	> 0.17	
Group IV	Fluoxetine 10 mg/kg	1.91	> 0.11	
Group V	Control (RO Water)	0.74	> 0.49	

Open field test shows uniform non-significance across all groups indicates absence of motor stimulation or suppression.

Sucrose preference test

Group	t (df = 5)	p value	Interpretation
Group I	3.10	0.028	Significant
Group II	2.70	0.043	Significant
Group III	1.80	0.130	Not significant
Group IV	3.90	0.011	Significant
Group V	1.30	0.243	Not significant

Paired t-test analysis of sucrose preference revealed a significant increase in sucrose consumption following drug treatment in Groups I, II, and IV ($p < 0.05$).In contrast, Groups III and V did not show statistically significant changes ($p > 0.05$).

- Swim Despair Test: Significant antidepressant-like effects observed in Groups II, III, and IV
- Open Field Test: No significant locomotor changes in any group, indicating absence of motor confounds
- Sucrose Preference Test: Significant improvement in anhedonia-like behavior in Groups I, II, and IVThis pattern supports a behavior-specific antidepressant effect rather than nonspecific motor activation.

Discussion: The present study investigated the antidepressant-like effects of the experimental treatments using a battery of validated behavioral paradigms, including the swim despair test, sucrose preference test, and open field test. The combined behavioral and statistical findings demonstrate that the treatments produced significant antidepressant-like effects without altering locomotor activity.
The forced swim test is widely used to assess behavioral despair and stress-coping mechanisms. In the present study, intragroup analysis revealed significant reductions in despair-like behavior in Groups II, III, and IV. Among these, Group IV exhibited the most pronounced response, indicating a robust antidepressant-like effect. This result was corroborated by certain other study[18, 19]. The lack of significance in Groups I suggests differential efficacy among the treatment regimens, potentially related to dose or pharmacodynamic differences.Anhedonia, a core symptom of depressive disorders, was evaluated using the sucrose preference test. Significant increases in sucrose consumption were observed in Groups I, II, and IV following drug administration, indicating a reversal of anhedonia-like behavior, also reported by other study[5]In contrast, Groups III did not exhibit statistically

significant improvement, suggesting that certain treatments may preferentially influence despair-related behavior without adequately restoring reward sensitivity. The marked reduction in post-treatment variability further indicates a consistent therapeutic response in the effective groups.Assessment of locomotor activity using the open field test revealed no significant intragroup or intergroup differences, indicating that none of the treatments induced motor stimulation or suppression. Minocycline did not cause a noticeable change in animal activity during the locomotor test compared with the control group[5].Notably, minocycline treatment resulted in improved performance across both forced swim and anhedonia-related behavioral paradigms, which is consistent with other studies on rats[18, 20]. This indicates that the serotonergic system may play a much important role in minocycline antidepressant effects[21].The two drugs differ markedly in their mechanisms of action. Fluoxetine directly targets the serotonin transporter, while minocycline exerts more diverse effects, including antioxidant, anti-inflammatory, and neuroprotective actions. Importantly, fluoxetine has been reported to produce behavioral effects that vary with environmental conditions, whereas available evidence suggests that minocycline's effects are largely unaffected by environmental factors[22, 23].Recent reports indicate that minocycline, a semi-synthetic tetracycline, has emerged as a multifunctional therapeutic agent with efficacy in both inflammatory conditions[24] and psychiatric disorders[25]. Growing evidence suggests that its neuroprotective actions within the central nervous system are largely mediated through anti-inflammatory and immunomodulatory mechanisms, including suppression of pro-inflammatory cytokine production[24,25], reduction of microglial activation[26] and selective inhibition of microglial polarization toward the pro-inflammatory M1 phenotype[27].

Conclusion: The present study revealed that minocycline showed antidepressant like effects at different doses without affecting locomotor functions. These findings can be further explored using larger sample size and for a longer duration. This provides a strong experimental basis for further mechanistic investigations to elucidate the underlying neurobiological pathways and to assess the translational potential of these interventions.

Conflict of Interest: There was no conflict of interest in the study.

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