https://doi.org/10.33472/AFJBS.6.5.2024. 4671-4686

AfricanJournalofBiological Sciences

Evaluation of Minocycline in post-traumatic stress disorder induced in Wistar rats with alcohol use disorder

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Article History
Volume 6, Issue 5, 2024
Received: 11 May 2024
Accepted: 17 May 2024
doi:*10.33472/AFJBS.6.5.2024. 4671-4686*

Abstract:

Objective: Post-traumatic stress disorder (PTSD) often co-occurs with alcohol use disorder (AUD), leading to exacerbated symptoms and poorer treatment outcomes. Despite the significant comorbidity, therapeutic options remain limited. This study aimed to evaluate the efficacy of Minocycline, a second-generation tetracycline with anti-inflammatory and neuroprotective properties, in a comorbid model of PTSD and AUD in Wistar rats.

Methods: A study involving 48 male Wistar rats aged 6-8 weeks was conducted to investigate the effects of Minocycline and Fluoxetine on predator scent-induced anxiety. The rats were divided into control, disease control, Fluoxetine, and Minocycline groups, with two-bottle choice paradigms for alcohol and sucrose consumption. Predator scent stress was induced using cat urine, and anxiety was assessed using behavioral tests including Elevated Plus Maze (EPM) and Open Field Test (OFT). Serum cortisol levels and brain IL-1α were measured to evaluate stress and inflammation.

Results: Study showed a significant reduction in ethanol consumption in rats treated with Minocycline compared to the disease control group, indicating an ameliorating effect on anxiety and alcohol use disorder. Behavioral tests revealed decreased anxiety-like behavior and freezing responses in Minocycline-treated animals compared to controls. Additionally, Minocycline administration led to reduced serum cortisol levels and brain IL-1α levels, suggesting modulation of stress response and neuroinflammation.

Conclusion: These findings support the potential anxiolytic and anti-inflammatory effects of Minocycline in the context of comorbid PTSD and AUD. Further research is warranted to explore the clinical implications and underlying mechanisms of Minocycline in this complex psychiatric comorbidity.

Keywords: Alcohol, Alcohol use disorder, Post traumatic stress, Minocycline

Introduction

Post-traumatic stress disorder (PTSD) is a chronic and disabling psychiatric disorder with an estimated lifetime prevalence of 6.8%.(1) Prevalence rates vary, but extensive epidemiological studies show a lifetime prevalence of PTSD in the United States of about 8%, but the rate increases drastically after catastrophic events.(2) The prevalence of PTSD in India during covid 19 pandemic was reportedly as high as 40%.(3) Increasing awareness is reflected in the revisions of the diagnostic criteria for PTSD, and its reclassification from a form of anxiety disorder to its current place in traumaand stressor-related disorders is observed in the DSM-V criteria. Adverse physical health co-morbidities are also common in individuals with PTSD, including obesity, diabetes, and cardiovascular disease. (4) Individuals with PTSD have high rates of comorbid alcohol and substance use disorders; rates range from 28% to as high as 75%. (5)

People with post-traumatic stress disorder are approximately three times more likely to develop alcohol use disorder than people without PTSD.(6) PTSD appears to play an essential role in perpetuating the vicious cycle leading to AUD and is associated with relapse to alcohol use among those who achieved abstinence.(7) The pathophysiology of PTSD is not completely clear, and many hypotheses also overlap the pathophysiology of other psychiatric disorders like anxiety and depression. One biological process that has been increasingly interrogated over the last decade is the inflammatory system, as it has a clear role in the pathophysiology of chronic mental and physical illness. Immune signalingregulates the hypothalamic-pituitary-adrenal (HPA) axis and other neurobiological processes that modulate affective behaviorduring stressor exposure. Exposure to traumatic and stressful events (including exposure to fear- and anxiety-provoking stimuli) results in HPA axis reactivity, activation of the immune system, and the release of pro-inflammatory cytokines. (8)These studies point towards immune alterations in PTSD that indicate the immunological balance skewed toward a pro-inflammatory state. (9)

The SSRI, Fluoxetine, is recommended as a first-line drug treatment for PTSD. Despite the frequency of co-occurring PTSD and addiction, there are limited therapeutic agents. Anticonvulsants, atypical antipsychotics, and naltrexone have been tried with modest effects.(10) Minocycline is a second-generation tetracycline with anti-microbial, anti-inflammatory, anti-apoptotic, and neuroprotective effects. It promotes neurogenesis and neuroplasticity, reduces oxidative stress and glutamate excitotoxicity, and attenuates the decrease of serotonin, dopamine, norepinephrine, and their derivatives. (11)Minocycline can reduce inflammation by acting on various immune cells and mediators. (11) Minocycline, a

tetracycline derivative,may be effective in alcohol use disorders as it crosses the blood-brainbarrier. (12) Minocycline has shown antidepressant and anti-anxiety effects in preclinical models.(13,14) There have been studies to demonstrate the anxiolytic effects of this drug. There are studies to show the effect of drugs on AUD. However, there are no studies to explore its effect on the comorbid PTSD and AUD models. With this background onMinocycline and the need for therapy for PTSD and AUD co-morbidity, we decided to evaluate the role of Minocycline in comorbid PTSD and AUD models in rats.

Materials and Methods

Institutional Animal Ethics Committee permission was taken before the commencement of the study under reference number AEC/15/2018. Animals randomly bred were used. The study was conducted following the CPCSEA guidelines.

The study was conducted with 48 male Wistar rats aged 6-8 weeks weighing 150-250 gm. There were three groups of Wistar rats, with 12 rats in each group used during the study.

Animals were randomly bred as per CPCSEA guidelines. Animals were quarantined in appropriate temperature conditions (18-29° C with 30-70%humidity) without handling for one week with a daily chow diet. They were housed 4 per cage and with a dark-light cycle of 14:9 hours. The animals were housed in air-conditioned rooms with 12 – 15 filtered fresh air changes, with a temperature of 22 ± 3° C and relative humidity of 30 – 70 %. The cages with a stainless-steel top grill containing food and drinking water facilities were used in polypropylene bottles with stainless steel sipper tubes. The animals were fed in the form of pellets and were provided water ad libitum. Pure drinking water through aqua guards was supplied.

Minocycline hydrochloride was procured from Sigma Aldrich in pure powder form.Fluoxetine hydrochloride was procured from Sigma Aldrich in pure powder form.Serum cortisol kits for rats were purchased from KrishgenLimited. Brain IL-1α kits for rats were purchased from KrishgenLimited. There were 4 study groups with 12 animals in each group: control, disease control, fluoxetine group, and test group. Two bottle choices were given to the disease control, Fluoxetine, and test groups. Disease control was given normal saline, the fluoxetine group was given Fluoxetine (10 mg/kg) i.p, and the test group was given Minocycline (35 mg/kg) i.p.

During days 1 to 7, animals were allowed to consume alcohol in a two-bottle choice paradigm voluntarily, sucrose and alcohol, respectively, in two bottles. Alcohol concentration was started at 4% and increased by 2% every alternate day till a maximum of 10% was

achieved. Similarly, sucrose concentration was decreased by 4% on alternate days and was brought to 0% from 12%.This was followed by alcohol and water administration for the remaining 21 days using a two-bottle drinking procedure.

From days 8 to 28, animals were housed singly (one per cage). Animals were weighed, and the fluid levels in the bottles were monitored to the nearest 0.5 ml at 24-hour intervals to determine daily alcohol intake (ml and g/kg). The position(left–right) of the alcohol and water bottle was changed each day to control side preferences.

The study drugs were administered 30 minutes before the predator-scent stress protocol fromthe 11^{th}daytill 17 days upto day 27,as shown in Table I. The predator-scent stress was given to all groups in which 20 ml of cat urine was mixed with 100 mL of bedding (standard corn husk) and shaken in a flask. It was then spread evenly in an identical open field chamber. Rats were placed in the chamber for 15 minutes, and activity was recorded during exposure. The stress test was carried out in aseparate room. Rat cages were taken in the room only when carrying out the test.

On day 19, the rats underwent behavior tests on Elevated Plus Maze (EPM) and Open Field Test (OFT) to testwhether predator scent stress-induced anxiety.The behavioral variables assessed on EPMwere time spent in open arms, closed arms, and on the central platform,number of entries to the open and closed arms, and total exploration of the maze.On OFT,horizontal locomotor activity (grid lines crossed), vertical locomotor activity (rearing),and grooming (rubbing the nose with its forepaws). Duration of time spent in Central Square (anxiety)

On day 20, retro-orbital blood collection was done in phase 2. 0.5 ml of blood was collected from each animal for cortisol estimation. The blood was immediately centrifuged to separate the serum. The serum was stored at -80° C in a deep freezer until the ELISA test was performed. The ELISA test was performed after thawing the serum at room temperature.

On day 24, a situational reminder was given to the rats by placing them in unused cat litter, to which the rat was exposed for 10 min; the freezing time indicateda response to fear associated with the predator. The variable recorded wasthe total seconds spent freezing during each assessment period.Behavior was recorded with an overhead video camera, and immobility (freezing) was scored using the recorded video.

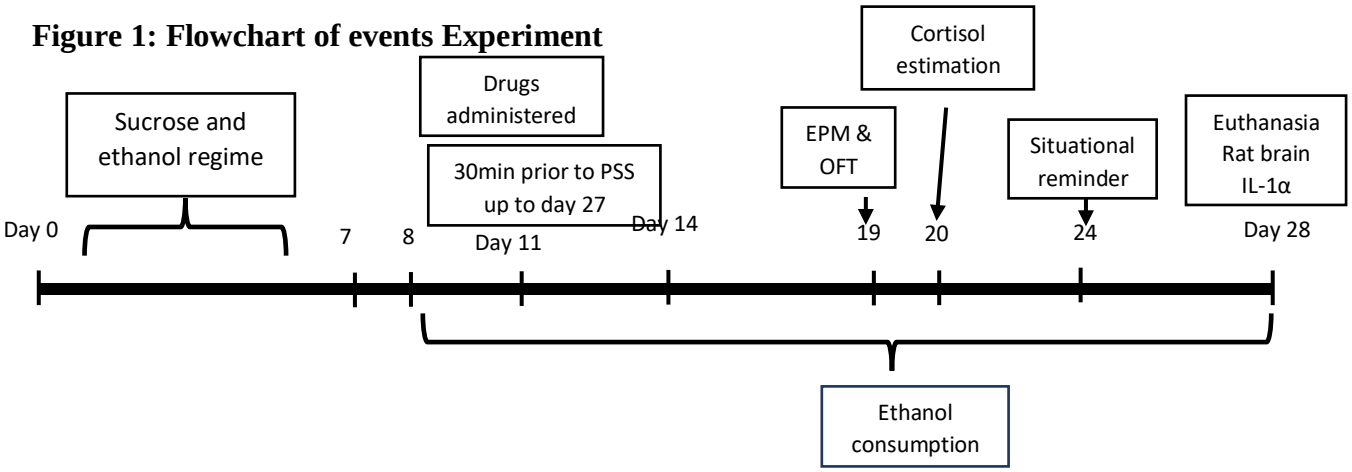
On day 28, animals were sacrificed, and levels of inflammatory marker IL-1α in rat brainswere measured usingan ELISA kit.

Variables recorded wereBehavioral tests (EPM and OFT), Freezing time, Serum cortisol levels at baseline and 20th day, and Rat brain IL-1α after the 28th day.

Anxiety was assessed using EPM as described by Serovoet al. (15).The apparatus used in these experiments consists of two open arms and two closed arms, each of diameter of 50× 10× 40 cm with an open roof, arranged so that the open arms are opposite to each other. The arms are configured in a plus orientation and elevated 50 cm above the floor. Two closed arms havea 17× 13× 1cm wall enclosing the arm, while the other two arms open with a lip around the platform of the arm. The procedure was conducted in a dark room. Rats were allowed to acclimatize in the experimental room for 30 minutes before the test. The tracking software was used to record and analyze the behavior. Each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was taken as the time the animal took to move into any closed arms with all its four legs inside the closed arm. The animals were allowed to explore the maze for 10 seconds and returned to their home cage. The maze was cleaned thoroughly with alcohol-water solution after the removal of each rat to remove any confounding olfactory clues.

OFT Apparatus dimensions were floor 60*60 cm, wall height 25 cm. The floor and wall were black, and the ceiling and the floor were divided into nine equal squares. The central square was considered as a central area. The apparatus was coupled with a video device positioned above the apparatus so that each trial could be recorded for later analysis using a video camcorder. The apparatus was tested in a sound-attenuated, dark room with minimal background lighting. The mice were placed either in the center or against one of the retaining walls. Mice were allowed to explore the apparatus for 5 minutes. The field was cleaned thoroughly with an alcohol-water solution after the removal of each rat.

Figure 1: Flowchart of events Experiment



The results are expressed as mean± standard deviation. Parametric data was analyzed using one-way ANOVA or two-way repeated measures ANOVA followed by post-hoc Tukey's test. Nonparametric data wasanalyzed using the Kruskal-Wallis test. P-value < 0.05 was considered as statistically significant.

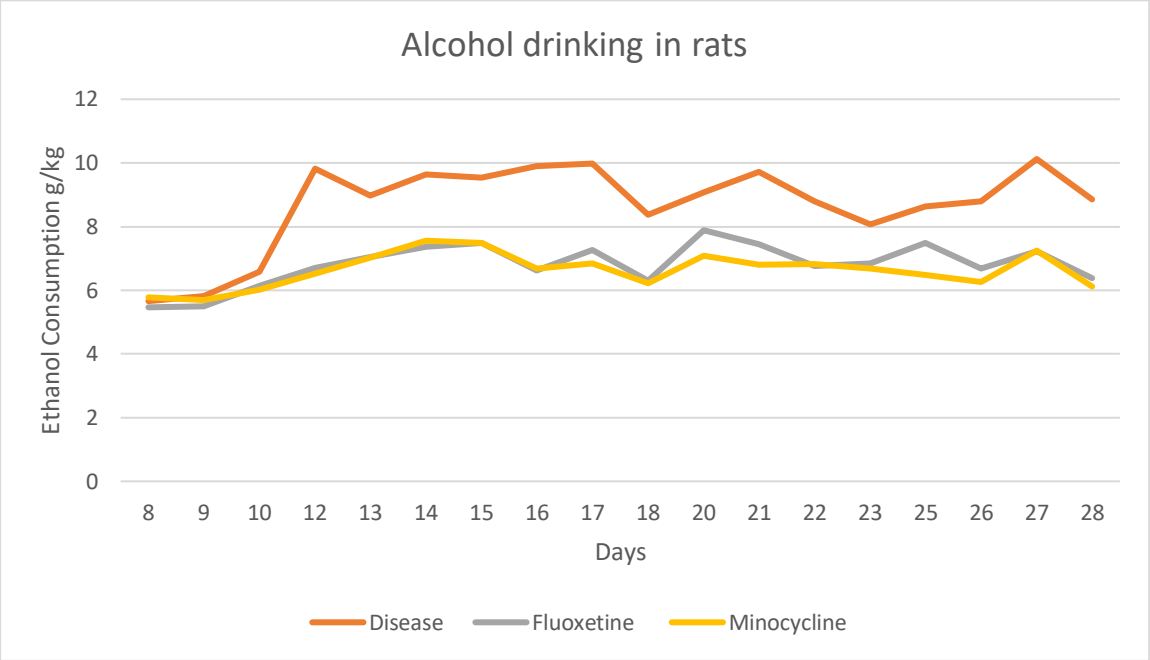
Results

A two-way repeated measures-ANOVA revealed a significant effect for group [$F(2,22) = 20.375$, $p<0.0001$], a significant effect for days [$F(17,187) =7.780,15$ $p<0.001$], and a significant group*days interaction ($F(34,374)=3.090$, $p=0.013$; Fig. 1). Post-hoc comparisons revealed that the pretreatment with Fluoxetine and Minocycline significantly decreased the consumption of EtOH compared to saline-treated rats. There was a significant difference between saline-treated rats and Fluoxetine on days 13 ($p=0.009$), 14 ($p=0.002$), 16 ($p=0.006$), 17 ($p=0.027$), 18 ($p=0.019$), 21 ($p=0.028$), 22 ($p=0.004$), 26 ($p=0.002$), 27 ($p=0.009$) and 28 ($p=0.007$). There was a significant difference between saline-treated rats and Fluoxetine on days 13 ($p=0.004$), 14 ($p=0.003$), 16 ($p=0.012$), 17 ($p=0.020$), 18 ($p=0.014$), 20 ($p=0.012$), 21 ($p=0.007$), 22 ($p=0.012$), 23 ($p=0.045$), 25 ($p=0.014$), 26 ($p=0.002$), 27 ($p=0.008$) and 28 ($p=0.003$).

The disease control group showed significant changes in EPM as compared to the normal control group, indicating predator stress-induced anxiety. There is a statistically significant increase in time spent in open arms and a decrease in time spent in closed arms in Fluoxetine and minocycline-treated groups in elevated plus maze (table 1). Also, there is an increase in entries in the open arm and a decrease in entries in the closed arm in animals treated with Fluoxetine and Minocycline as compared to saline treated group (table 2). There is no difference between horizontal and vertical locomotor activity groups on the open field test. The duration of time spent in Central Square significantly increased in the Fluoxetine and minocycline-treated group compared to the saline-treated group. (table 4)

The freezing time decreased significantly in fluoxetine-treated and minocycline-treated animals (table 4). The corticosterone levels on day 20 were significantly reduced in fluoxetine-treated and minocycline-treated animals compared to the control group (figure 3). The animals were sacrificed on day 29, and the rat brain IL-1alpha was determined. These levels were significantly reduced in drug-treated groups as compared to the control PSS group (table 5).

Figure 2: Effects of administration of Fluoxetine (10 mg/kg i.p) and Minocycline (35 mg/kg) on the ethanol consumption from day 8 to day 28



Data represent the mean ± SD (n = 12 per group)
one-way ANOVA followed by post hoc Tukey's test

Table 1: Effects of administration of Fluoxetine (10 mg/kg i.p) and Minocycline (35 mg/kg) on time spent in the open arms and time spent in the closed arms in 5 min in the EPM test

Groups	**Time spent in open arm**	**P value**	**Time spent in closed arm**	**P value**
Control	82.2 ± 11.26	p<0.01*	189 ± 17.8	p=0.025*
PSS	51.08 ± 10.54	_	211.5 ± 16.88	_
Fluoxetine	77 ± 11.69	p=0.023*	182.5 ± 21.19	p=0.013*
Minocycline	73.91 ± 9.14	p=0.021*	189.25 ± 17.78	p=0.022*

Data represent the mean ± SD (n = 12 per group)
*p<0.05 vs PSS group using one-way ANOVA followed by post-hoc Tukey's test

Table 2: Effects of administration of Fluoxetine (10 mg/kg i.p) and Minocycline (35 mg/kg) on the entries in the open arms and closed arms in 5 min in the elevated plus-maze test

Groups	**Entries in open-arm**	**p-value**	**Entries in closed-arm**	**p-value**
Control	7.42 ± 2.31	p=0.163	17 ± 3.16	p=0.090
PSS	5.66 ± 1.96	_	20.5 ± 4.75	

Fluoxetine	8 ± 2.25	p=0.013*	16.41 ± 3.42	p=0.036*
Minocycline	7.66 ± 1.43	p=0.088	15.91 ± 2.50	p=0.015*

Data represent the mean ± SD (n = 12 per group)
*p<0.05 vs PSS group using one-way ANOVA followed by post hoc Tukey's test

Table 3: Effects of administration of Fluoxetine (10 mg/kg i.p) and Minocycline (35 mg/kg) on the horizontal and vertical locomotor activity and duration of time spent in the central square in the open field test

Groups	**Horizontal locomotor activity**	**p-value**	**Vertical locomotor activity (rearing)**	**p-value**	**Duration of time spent in Central Square**	**p-value**
Control	3968 ± 662	p=0.262	14.8 ± 3.52	p=0.881	19.8 ± 1.28	p=0.001*
Disease	3578 ± 419	_	13.9 ± 1.83	_	13.91 ± 3.65	
Fluoxetine	3898 ± 510	p=0.431	15.3 ± 2.77	p=0.596	20 ± 3.13	p=0.001*
Minocycline	3646 ± 430	p=0.988	14.7 ± 2.67	p=0.910	18.83 ± 2.55	p=0.007*

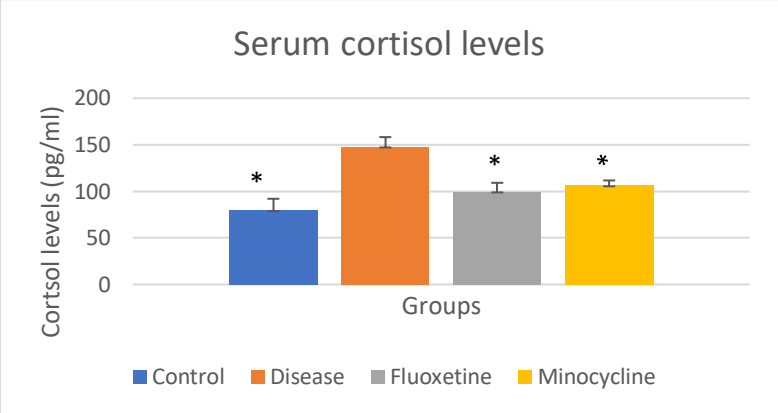
Data represent the mean ± SD (n = 12 per group)
*p<0.05 vs PSS group using one-way ANOVA followed by post hoc Tukey's test

Table 4: Effects of administration of Fluoxetine (10 mg/kg i.p) and Minocycline (35 mg/kg) on the freezing time (total seconds spent freezing during each assessment period) after situational reminder on day 24 and on the brain IL-1 alpha levels

Groups	**Freezing time**	**p-value**	**Interleukin 1 alpha levels**	**p-value**
Control	8.67± 6.93	p=0.002*	104.38 ± 1.27*	p<0.001
Disease	24.75± 8.44	_	142.85 ± 2	_
Positive	13.83 ± 2.59	p=0.015*	119.36 ± 1.39*	p<0.001
Test	14.38 ± 2.26	p=0.047*	131.92 ± 1.58*	p<0.001

Data represent the mean ± SD (n = 12 per group)
*p<0.05 vs PSS group using Kruskal Wallis test followed by post hoc Dunn's test for freezing time and *p<0.05 vs PSS group using one-way ANOVA followed by post hoc Tukey's test for IL-1 alpha levels.

Figure 3: Effects of administration of Fluoxetine (10 mg/kg i.p) and Minocycline (35 mg/kg) on the baseline and day 20 cortisol levels



Data represent the mean ± SD (n =6 per group)
*p<0.05 vs PSS group using one-way ANOVA followed by post hoc Tukey's test

Discussion

We carried out the study in the predator scent stress model, which is a validated model of PTSD in rats with alcohol use disorder. We observed a statistically significant decrease in ethanol consumption in the minocycline group compared to disease control, indicating an amelioratingeffect on anxiety and, thus,alcohol use disorder in the animals. The predator scent stress-induced stress disorder was associated with a higher risk of alcohol-drinking behavior in rats. The protective effect observed with Minocycline could be due to its anxiolytic as well as its per se effect on alcohol reward potential.(16-17) The effect of Minocycline may be due to its effect on its inhibition of microglial cell activation and its inhibition of pro-inflammatory cytokines. (18)

Our results showed that the PSS rats with alcohol use demonstrate increased anxiety-like behavior as demonstrated on EPM and open field tests. An increase in freezing time is also considered anxiety-like behavior, which was seen in PSS rats with alcohol use. The study drugs Fluoxetine and Minocycline were able to reduce anxiety-like behavior in animals. Our study confirms the findings of the study done by Patankar et al. in a model of PTSD in hamsters; it showed similar results to our studies with the significant anxiolytic effect of Minocycline. (19) Additionally, the above study showed decreased pro-inflammatory cytokines in the brain, including IL-1, IL-6, and TNF-α, consolidating the Minocycline anti-inflammatory hypothesis. A study using chronic administration of Minocycline in a Wistar rat model of Intermittent Foot Shock (IFS) was done by Wang et al. (9). The results for behavioral tests were similar to our study, as evidenced by an increase in time spent in the central square in OFT and increase in time spent in open arms in EPM. The study mentioned above also showed a decrease in microglial cell activation in the group receiving Minocycline. Various pro-inflammatory activations activate microglial cells in the brain.

Thus, a decrease in the same can be attributed to the anti-inflammatory effect of Minocycline on PTSD-like states. Similar results are seen in the study by Liu et al. (20), which was done using chronic restraint on C57BL/6 mice and chronic administration of Minocycline. In this study, the efficacy of Minocycline was comparable to buspirone.

SSRIs are considered first-line therapy for PTSD by treatment guideline recommendations and the results of numerous clinical trials. Although the overall response rate with the use of SSRIs is approximately 60% in patients with PTSD, only 36% of patients achieve complete remission five months after trauma. (21,22)We decided to use Fluoxetine as our positive control as the model used in the study induces acute PTSD associated with distress and anxiety componentsin PTSD. The prevalence of PTSD is comparatively higher in individuals with alcohol use disorder than in the general population. (23-24) Also, it is observed that the comorbid presentation of PTSD and substance use disorder is associated with a more severe clinical presentation and poorer treatment prognosis.(25) Despite this fact, we have not been able to successfully study the comorbid condition and delineate the best possible treatment. The reasons for the same could be the need for a better animal model of the comorbid condition, better designed clinical research, the difference in subpopulations,etc.(26) Amongst the numerous models for PTSD, we chose the predator scent stress model as its key advantages are etiological correlation with exposure of single stressor, robust behavioral phenotypes after trauma. (27)Similar to the study conducted by Manjoch et al., we induced the alcohol use disorder and subsequently exposed animals to predator odor. We found similar results to those of Manjoch et al., where the freezing time increased and the time spent in the open arm in EPM decreased in the exposed group. (28)

The effect of models of PTSD on plasma corticosterone has been studied in different models. Few studies have shown an increase in plasma corticosterone levels following predator stress. (29-30)A study conducted by Wilson et al. has shown an increase in serum corticosterone and adrenal gland hypertrophy at day 31, considered a means to cope with stress.(30) Other studies have reported reduced plasma concentration of corticosterone in rats following predator stress, especially with repeated exposures.(31) It is also theorized that stress can modulate the circadian rhythm of corticosterone secretion and disrupt the feedback; this facilitates the HPA axis to over-release corticosterone and form a vicious cycle.(32)Our study showed an increase in corticosterone response to stress as PTSD was induced by a predator scent exposure followed by a reminder. Our study is in agreement with Patankar et al., who showed a reduced corticosteroid level with minocycline therapy in PTSD in hamsters.(19)

The neuroinflammatory hypothesis is a known pathophysiological theory of PTSD. Studies have shown that individuals with PTSD exhibit increased blood levels of inflammatory markers, such as IL-1β, IL-6, TNF-α, and CRP, relative to healthy control subjects. (33)The animal model of predator scent stress model has shown activated B cells (NF-κB) mediated activation of the nuclear factor k light-chain enhancer pathway,which promotes anxiety.(34) It was shown that the inhibition of this pathway reduced IL-1β concentrations andanxiety levels. (34)Similar to the findings in our study, where we saw inflammatory markers raised at four weeks, Muhie S et al. found upregulated inflammatory mediators immediately and upto four weeks after withdrawal of stress in an animal model of PTSD.(35)

One of the limitations of this study is that we assessed the prophylactic role of Minocycline in PTSD as the drugs were administered before exposure to predator scent stress. However, future studies to evaluate its effect after PTSD sets in must be conducted. Also, the study can be supported with other stress markers, like serum ACTH, serum cortisol-releasing hormone (CRH), and serum testosterone levels.

Our study elucidates the anxiolytic effect of Minocycline in the model of PTSD in rats with alcohol use disorder as confirmed with behavioral parameters assessed using EPM and OFT along with a reduction in the serum cortisol levels (biochemical parameter) and inflammatory marker (reduced IL-1) indicating that the effects may be mediated through anti-inflammatory mechanisms of Minocycline. Our study demonstrates the effect of Minocycline. Further studies are required to consolidate our findings and further take the test drug in drug development for the benefit of the affected population.

References

1. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime Prevalence and Age-of-Onset Distributions of. Arch Gen Psychiatry [Internet]. 2005;62(June):593–602. Available from: http://archpsyc.jamanetwork.com/article.aspx?doi=10.1001/archpsyc.62.6.593

2. Gradus JL. Prevalence and prognosis of stress disorders: A review of the epidemiologic literature. Clin Epidemiol. 2017;9:251–60.

3. Georgieva I, Lepping P, Bozev V, Lickiewicz J, Pekara J, Wikman S, et al. Prevalence, new incidence, course, and risk factors of PTSD, depression, anxiety, and panic disorder during the covid-19 pandemic in 11 countries. Healthc. 2021;9(6).

4. Edmondson D, von Känel R. Post-traumatic stress disorder and cardiovascular disease. The Lancet Psychiatry. 2017;4(4):320–9.

5. Petrakis IL, Simpson TL. Post-traumatic Stress Disorder and Alcohol Use Disorder: A Critical Review of Pharmacologic Treatments. Alcohol Clin Exp Res. 2017;41(2):226–37.

6. Quiñones-Laracuente K, Hernández-Rodríguez MY, Bravo-Rivera C, Melendez RI, Quirk GJ. The effect of repeated exposure to ethanol on pre-existing fear memories in rats. Psychopharmacology (Berl). 2015;232(19):3615–22.

7. Neupane SP, Bramness JG, Lien L. Comorbid post-traumatic stress disorder in alcohol use disorder: Relationships to demography, drinking and neuroimmune profile. BMC Psychiatry. 2017;17(1):1–10.

8. Michopoulos V, Powers A, Gillespie CF, Ressler KJ, Jovanovic T. Inflammation in Fear-and Anxiety-Based Disorders: PTSD, GAD, and beyond. Neuropsychopharmacology. 2017;42(1):254–70.

9. Wang Z, Caughron B, Young MRI. Post-traumatic stress disorder: An immunological disorder? Front Psychiatry. 2017;8(NOV):1–7.

10. Shorter D, Hsieh J, Kosten TR. Pharmacologic Management of Comorbid Post-Traumatic Stress Disorder and Addictions. Focus (Madison). 2017;15(4):454–61.

11. Naderi Y, Panahi Y, Barreto GE, Sahebkar A. Neuroprotective effects of Minocycline on focal cerebral ischemia injury: A systematic review. Neural Regen Res. 2020;15(5):773–82.

12. Shultz RB, Zhong Y. Minocycline targets multiple secondary injury mechanisms in traumatic spinal cord injury. Neural Regen Res. 2017;12(5):702–13.

13. Wong ML, Inserra A, Lewis MD, Mastronardi CA, Leong L, Choo J, et al. Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition. Mol Psychiatry [Internet]. 2016;21(6):797–805. Available from: http://dx.doi.org/10.1038/mp.2016.46

14. Zheng LS, Kaneko N, Sawamoto K. Minocycline treatment ameliorates interferon-alpha-induced neurogenic defects and depression-like behaviors in mice. Front Cell Neurosci. 2015;9(JAN):1–10.

15. Serova LI, Laukova M, Alaluf LG, Sabban EL. Intranasal infusion of melanocortin receptor four (MC4R) antagonist to rats ameliorates development of depression and anxiety related symptoms induced by single prolonged stress. Behav Brain Res

[Internet]. 2013;250:139–47. Available from: http://dx.doi.org/10.1016/j.bbr.2013.05.006

16. Camargos QM, Silva BC, Gonc D, Cristina E, Toscano DB, Oliveira S, et al. Minocycline treatment prevents depression and anxiety-like behaviors and promotes neuroprotection after experimental ischemic stroke. Brain Res Bull. 2020;155(1).

17. Gajbhiye S V, Tripathi RK, Salve B, Petare A, Potey A V. Neuroscience Letters Evaluation of effect of Minocycline on rewarding potential and alcohol relapse in place preference model in mice. Neurosci Lett [Internet]. 2017;649:28–33. Available from: http://dx.doi.org/10.1016/j.neulet.2017.04.007

18. Karelina K, Nicholson S, Weil ZM. Minocycline blocks traumatic brain injury-induced alcohol consumption and nucleus accumbens inflammation in adolescent male mice. Brain BehavImmun [Internet]. 2018;69:532–9. Available from: https://doi.org/10.1016/j.bbi.2018.01.012

19. Patankar P, Joshi S, Mane A, Manjesh P, Kokate D. Anxiolytic effect of Minocycline in post-traumatic stress disorder model of Syrian hamsters. Tzu Chi Med J [Internet]. 2021;33(3):263. Available from: www.buffalobirdnerd.com

20. Liu HY, Yue J, Hu LN, Cheng LF, Wang XS, Wang XJ, et al. Chronic minocycline treatment reduces the anxiety-like behaviors induced by repeated restraint stress through modulating neuroinflammation. Brain Res Bull [Internet]. 2018;143:19–26. Available from: https://doi.org/10.1016/j.brainresbull.2018.08.015

21. Morimoto N, Shimazawa M, Yamashima T. Minocycline inhibits oxidative stress and decreases in vitro and in vivo ischemic neuronal damage. 2005;1044:8–15.

22. Morina N, Wicherts JM, Lobbrecht J, Priebe S. Remission from post-traumatic stress disorder in adults: A systematic review and meta-analysis of long term outcome studies. Clin Psychol Rev [Internet]. 2014;34(3):249–55. Available from: http://dx.doi.org/10.1016/j.cpr.2014.03.002

23. Blanco C, Xu Y, Brady KT, Perez-Fuentes G, Okuda M, Wang S. Co-morbidity of post-traumatic stress disorder with alcohol dependence among US adults: Results from NESARC. Drug Alcohol Depend. 2013;132(3):630–8.

24. Debell F, Fear NT, Head M, Batt-Rawden S, Greenberg N, Wessely S, et al. A systematic review of the co-morbidity between PTSD and alcohol misuse. Soc Psychiatry Psychiatr Epidemiol. 2014;49(9):1401–25.

25. Jenna L McCauley, Ph.D.1, Therese Killeen, Ph.D., A.P.R.N.1, Daniel F. Gros, Ph.D.1, 2, Kathleen T. Brady, MD, Ph.D.1, 2, and Sudie E. Back PD. Post-traumatic Stress Disorder and Co-Occurring Substance Use Disorders: Advances in Assessment and Treatment. Clin Psychol (New York). 2012;19(3):10.1111/cpsp.12006.

26. Gilpin NW, Weiner JL. Neurobiology of comorbid post-traumatic stress disorder and alcohol-use disorder. Genes, Brain Behav. 2017;16(1):15–43.

27. Albrechet-Souza L, Gilpin NW. The predator odor avoidance model of post-traumatic stress disorder in rats. BehavPharmacol [Internet]. 2019 Apr;30(2 and 3):105–14. Available from: https://journals.lww.com/00008877-201904000-00003

28. Manjoch H, Vainer E, Matar M, Ifergane G, Zohar J, Kaplan Z, et al. Predator-scent stress, ethanol consumption and the opioid system in an animal model of PTSD. Behav Brain Res [Internet]. 2016;306:91–105. Available from: http://dx.doi.org/10.1016/j.bbr.2016.03.009

29. Zoladz PR, Conrad CD, Fleshner M, Diamond DM, Zoladz PR, Conrad CD, et al. Acute episodes of predator exposure in conjunction with chronic social instability as an animal model of post-traumatic stress disorder. 2009;3890.

30. Wilson CB, Mclaughlin LD, Nair A, Ebenezer PJ, Dange R, Francis J. Inflammation and Oxidative Stress Are Elevated in the Brain , Blood , and Adrenal Glands during the Progression of Post-Traumatic Stress Disorder in a Predator Exposure Animal Model. 2013;8(10):1–10.

31. Cohen H, Zohar J, Gidron Y, Matar MA, Belkind D, Loewenthal U. Blunted HPA Axis Response to Stress Influences Susceptibility to Post-traumatic Stress Response in Rats. 2005;

32. : Cai Zhang, Yong-Ping Zhang, Yu-Yu Li B-P, Liu, Hao-Yin Wang, Kang-Wei Li CS. Minocycline ameliorates depressive behaviors and neuro-immune dysfunction induced by chronic unpredictable mild stress in the rat. Behav Brain Res. 2018;

33. Lee D, Lee J, Hong D, Lee E, Park S, Lee M, et al. Neuroinflammation in Post-Traumatic Stress Disorder. Biomedicines [Internet]. 2022;10:1–17. Available from: https://doi.org/10.3390/%0Abiomedicines10050953%0D

34. Zimmerman G, Shaltiel G, Barbash S, Cohen J, Gasho CJ, Shalev H, et al. Post-traumatic anxiety associates with failure of the innate immune receptor TLR9 to evade the pro-inflammatory NF j B pathway. 2012;(October 2011).

35. Muhie S, Gautam A, Chakraborty N, Hoke A, Meyerhoff J, Hammamieh R, et al. Molecular indicators of stress-induced neuroinflammation in a mouse model simulating features of post-traumatic stress disorder. 2017;7(5):e1135-9. Available from: http://dx.doi.org/10.1038/tp.2017.91