Vol. 4 Issue No. 2, April-June 2022 e-ISSN 2456-7701
Journal of Science and Technological Researches
A Peer Reviewed Journal
Origin of Innovation
Domain: www.jstr.org.in, Email: editor@jstr.org.in

EFFECT OF COMBINATION OF DOXYCYCLINE AND MINO CYCLINE ANTIBIOTIC ON KETAMINE INDUCED EXPERIMENTAL SCHIZOPHRENIA IN MICE

Sher Singh*, Deepa Khanna, Rampal Singh Negi and Sanjeev Kalra
Rajendra Institute of Technology and Sciences, Sirsa, Haryana, India
Email: sschopra.ssc@gmail.com

Date of Received **23 April, 2022**

Date of Revised **18 May, 2022**

Date of Acceptance **23 June, 2022**

Date of Publication **30 June, 2022**

DOI : https://doi.org/10.51514/JSTR.4.2.2022.14-43

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

EFFECT OF COMBINATION OF DOXYCYCLINE AND MINO CYCLINE ANTIBIOTIC ON KETAMINE INDUCED EXPERIMENTAL SCHIZOPHRENIA IN MICE

Sher Singh*, Deepa Khanna, Rampal Singh Negi and Sanjeev Kalra

Rajendra Institute of Technology and Sciences, Sirsa, Haryana, India

Email: sschopra.ssc@gmail.com

ABSTRACT

Schizophrenia is a complex, chronic and widespread psychiatric problem that affects about 1% of the world's total population. The risk of developing Schizophrenia is about the same for men and women, but there are gender differences in the development of the disease. Doxycycline and Minocycline are tetracyclines with potent antioxidant, anti-inflammatory, immunomodulatory, neuroprotective, antidepressant, anti-anxiety, antipsychotic, memory enhancing, neuroprotective properties and better pharmacokinetic profile. This tetracycline is used to treat psychosis and to evaluate its protective effects against Ketamine-induced behavioral, biochemical, neurochemical and histological changes in mice. Olanzapine is a drug that acts on the brain to treat Schizophrenia. Olanzapine restores the balance of Dopamine and Serotonin, which improves thinking, mood and behavior.

Results: In the present study, pretreatment with a combination of Doxycycline and Minocycline was studied for Ketamine-induced psychosis in mice. Pretreatment with the combination of Doxycycline and Minocycline showed valuable antipsychotic activity that would be exploited due to its antibiotic activity. Combination of the two antibiotics ameliorated behavioural changes as represented by hyper locomotion and stereotype behavior, increased immobility, increased step down latency on 7th, 14th and 21st day respectively. Further the combination showed biochemical, neurochemical and histological changes as assessed on 22nd day.

Conclusion: The results indicate that the combination of Doxycycline and Minocycline may be a new option for the treatment of Ketamine-induced psychotic symptoms in animal models. The combination of Doxycycline and Minocycline has been shown to be more effective than monotherapy in the treatment of the positive, negative and cognitive symptoms of Ketamine-induced Schizophrenia. GABAnergic, antioxidant, anti-inflammatory, acetyl cholinesterase inhibition exhibited by these antibiotics probably be mechanisms involved in the treatment of psychosis.

Keywords: *Schizophrenia, Doxycycline, Minocycline, Olanzapine, Ketamine*

INTRODUCTION

Swiss psychiatrist Bleuler, describes Schizophrenia as a syndrome of distorted perceptions and behaviors. The original expression defines it as a chronic mental disorder or disease described as "disorder or disruption of mental function" [1]. Positive and negative for a variety of symptoms including delusions, hallucinations, thinking disorders, movement disorders, anhedonia, regression, apathy, apathy, unconsciousness, emotional control disorders, and disorganized behavior with slow decline in mental functioning and social relationships and cognitive Schizophrenia [2-4]. The onset occurs before the age of 40 years in 98% of population from total of 5 million people suffering and male gender dominates this figure [5]. Altered Dopamine, Serotonin, Glutamate, N-methyl-D-aspartate (NMDA), Acetylcholine, Adrenaline, Aspartate, Glycine, and Gamma-aminobutyric acid (GABA) are formed, leading to Schizophrenia [6-7], Many of the other hypotheses are based on the involvement of enzymes, neuropeptides on oxidative stress, neuroinflammation, and mitochondrial dysfunction [6-8]. Ketamine, a Phencyclidine derivative, developed in 1963 produces a state of "dissociative anaesthesia", amnesia, and, at the same time, maintained the respiratory drive effect and supports the systemic arterial blood pressure [9]. S-Ketamine has better analgesic and anesthetic effects than R-Ketamine and is less likely to cause Schizophrenia and other side effects. [10]. Ketamine induced positive, negative and cognitive symptoms of Schizophrenia [11]. Olanzapine is an atypical antipsychotic drug with broad efficacy. It elicits responses to both positive and negative symptoms of Schizophrenia, acute mania, and relapse inhibition of bipolar disorder. The anti-manic and antipsychotic effects are probably mediated primarily by blockade

*Author for correspondence

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

of Dopamine D_2 and Serotonin 5-HT_{2A} receptors in the mesolimbic pathway [12].

Although Minocycline and Doxycycline are second-generation tetracycline antibiotics with similar chemical structures, Minocycline has been shown to be a good substitute for Doxycycline for several diseases in present scenario [13]. Minocycline showed promising results in experimental neurology, which was due to its high lipophilic nature. It can accumulate in cells of the cerebrospinal fluid and central nervous system**,** thus enabling its use in the treatment of many central nervous system diseases [14]. Minocycline is clinically nontoxic and effective adjunct to antipsychotic medications. It is helpful in increasing antipsychotic efficacy used for the treatment of Schizophrenia [15]. Mizoguchi *et al.,* We proposed the therapeutic potential of Minocycline in the treatment of cognitive impairment in patients with Schizophrenia and Methamphetamine-induced psychosis [16]. Similarly, Doxycycline alone and combination with Risperidone prevented and reversed the negative and cognitive symptoms associated with Schizophrenia [17].

As a follow-up, the present study assessed the locomotor, stereotype behaviors, immobility duration, SDL period, biochemical, neurochemical and histological changes of Ketamine.

METHODS OF PREPARATION

Drugs and Chemicals

Doxycycline Hydrochloride from Bionomics Life Sciences Private Limited, Ambala, Minocycline Hydrochloride from Sun pharmaceutical India Ltd, Ketamine from Pil Company manufactured by Psychotropics India Ltd, Olanzapine from Intas Pharmaceutical Ltd, Dopamine from Neon Laboratories Ltd, India & Trichloroacetic acid from RANKEM, New Delhi, India, Tris HCL was purchased from SD Fine-Chem Ltd, Mumbai, India. Acetylcholine Iodide from Sigma Aldrich, St Louis, MO, USA & 5, 5-dithiobis-(2-nitrobenzoic acid was purchased from Sigma Aldrich, St Louis, MO, USA. Volume of oral administration and *i.p*. (intraperitoneally) injection was 1ml/100g of mouse.

Experimental Animals

In present investigation, the mice were randomly divided into 6 groups. Each group comprised of 6 mice. *Swiss albino* mice (males) weighing approximately 20-25 g were used. Animals were obtained from disease free small animal house, Lala Lajpat Rai University of Veterinary and animal science, Hisar, Haryana, India. Animals were housed in polyacrylic cages with wire mesh and soft linen. Animals were maintained in environmental conditions with a 12-hour light-dark cycle and fed with cooked dahlias and water ad libitum. Experiments were carried out between 0900 and 1800 h. Animals were acclimatized to laboratory conditions for at least 5 days. The experimental protocol used in this study was approved by the "Institutional Animal Ethics Committee" in accordance with the recommendations of the "Committee for Control and Supervision of Animal Experiments (CPCSEA)" in New Delhi, India (Registration Number 888/ac/05/CPCSEA 29-04-2005).

Preparation of Drugs

Doxycycline hydrochloride (50 mg/kg) and Minocycline hydrochloride (50 mg/kg) were dissolved in 0.9% saline and orally administered to mice. Olanzapine was dissolved in 0.9% physiological saline and administered intraperitoneally at a dose of 5 mg/kg. Ketamine (50 mg/kg) was dissolved in 0.9% saline and administered intraperitoneally.

EXPERIMENTAL DESIGN

In this present study, mice were randomly divided into six groups. Each group consisted of 6 mice. Behavioral and biochemical studies were performed to evaluate the benign (hallucinations, delusions), negative and cognitive symptoms of Schizophrenia. Mice were administered Ketamine (50 mg/kg, *i.p*.) for 21 successive days for inducing psychosis and Olanzapine was administered as standard antipsychotic agent at the dose of 5 mg/kg, *i.p*. Doxycycline Hydrochloride and Minocycline Hydrochloride dissolved in normal saline 0.9% solution was given orally at the dose of 50 Hydrochloride mg/kg for 21 succeeding days. After 60 min of management of the dose given on 7th day, 14th day and 21st day behavioral parameters were accessed on 7th day, mice were exposed to Actophotometer for measurement of locomotor activity and also for stereotype behaviors (rearing score, falling behavior). Forced swimming test (FST) for measurement of immobility duration was carried after 60 min of management of the dose on 14th day and Step down latency (SDL) was recorded on passive avoidance paradigm after 60 min of

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

administration of the dose on 21st day. On 22nd day biochemical estimations were carried on blood and brain homogenate. The choice of doses and behavioral parameters were made on the basis of research references [18-19]. Isolated brain sample from each group was preserved in formalin (10% v/v) for histopathology.

Group 1 (Normal group): Mice were well-maintained on standard food and water and animal were administered 0.9% normal saline orally as vehicle for 21 successive days.

Group 2 (Ketamine group): Mice were administered Ketamine (50 mg/kg, *i.p.*) for 21 succeeding days.

Group 3 (Pre-treated Doxycycline group): Mice were administered Doxycycline Hydrochloride (50 mg/kg, *p.o.*), followed by Ketamine (50 mg/kg, *i.p.*) later 30 min of Doxycycline Hydrochloride administration for 21 successive days.

Group 4 (Pre-treated Minocycline group): Mice were administered Minocycline Hydrochloride (50 mg/kg, *p.o.*), followed by Ketamine (50 mg/kg, *i.p.*) after 30 min of Minocycline Hydrochloride administration for 21 successive days.

Group 5 (Pre-treated Doxycycline + Minocycline combination group): Mice were administered Doxycycline Hydrochloride (50 mg/kg, *p.o.*) and Minocycline Hydrochloride (50 mg/kg, *p.o.*), followed by Ketamine (50 mg/kg, *i.p.*) later 30 min of combination administration for 21 successive days [18-19].

Group 6 (Pre-treated Olanzapine group): Mice were administered Olanzapine (5 mg/kg, *i.p.*) [18], followed by Ketamine (50 mg/kg, *i.p.*) after 30 min of Olanzapine administration for 21 successive days.

BEHAVIORAL MODELS FOR ASSESSMENT OF SCHIZOPHRENIA IN MICE

Behavioral assessments were carried out on 7th, 14th and 21st day of experiment in accordance to the earlier study. After behavioral assessments, mice were sacrificed on 22nd day for collection of blood and brain samples for biochemical and neurochemical evolutions.

Measurement of Locomotor activity

Actophotometer was used to measure the outcome of combination of Doxycycline and Minocycline on locomotor activity according to procedure given by Da Silva *et al.,* 2010. Each animal was placed in the device's activity chamber and the total number of exercises was expressed as a 10-minute light beam block. [20]

Rearing is a typical vertical movement in which an animal stands on its hind legs, raises its forearms into the air, or leans against the wall of a cage, and exhibits increased exploratory behavior, which is a measure of central nervous system arousal. Induced falling describe as number of falls on the floor. These stereotype behaviors were checked in animals for a total period of 10 minutes [21].

Measurement of Immobility duration

Forced swimming test was used to measure the outcome of combination of Doxycycline and Minocycline on immobility period according to the procedure given by Chillar and Dhingra, 2013. In this experiment, animals were forced to swim in a glass chamber (25 x 15 x 25 cm) containing water up to a height of 15 cm (23 ± 2°C). Reaching immobility with minimal floating is measured as behavioral distress similar to the depressive symptoms of psychosis. After the mice were placed in the chamber for 2 minutes and acclimatized, the next 4 minutes of immobility was recorded [22].

Measurement of Step down latency

Passive avoidance tests are commonly used to measure SDL, a parameter used to test small animal memory. SDL is defined as the time it takes an animal to move from a wooden platform to a grid floor with all its paws. A Passive avoidance paradigm was used to measure the effect of combination of Doxycycline and Minocycline on cognitive perspective of psychosis according to procedure given by Soni and Parle, 2017 [23]. The model consists of a chamber (27 x 27 x 27 cm) with one wall made of plexiglass and three walls made of wood, excluding slate floors with wooden platforms. A 15W light bulb was used to light the chamber and the floor was exposed to a current of 20 VAC during the experiment. The experiment consisted of two training sessions and one memory session. During the first training session, animals were placed on a wooden platform and subjected to electrical shocks for 15 seconds when all paws were stepped on a slate floor for up to 60 seconds. The following training sessions were performed after a 90-minute interval in which the animals were not in shock. The next day, a memory session was performed and the animals were observed for 300

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

seconds to assess their cognitive function. Animals were not subjected to electric shocks during the memory session [23].

BIOCHEMICAL PARAMETERS FOR ASSESSMENT OF SCHIZOPHRENIA IN MICE

Collection of blood samples for C-reactive protein (CRP) levels

After evaluating the behavioral parameters of animals in groups 1-6, they were used to determine CRP in serum. On day 22 of the protocol, 24 hours after the last treatment, animals were weighed and sacrificed; 3 ml of blood was collected from each animal. The collected blood was allowed to clot for 15 minutes. Samples were centrifuged at 3000 rpm for 15 min and serum was used for biochemical analysis of CRP levels.

Dissection and homogenization

Animals in groups 1-6 were sacrificed by decapitation and brains immediately removed, washed, weighed, and divided into 4 equal parts. The homogenate formation procedure is described in Yadav *et al.,* 2017. Single portion homogenates were prepared in 0.1 M phosphate buffer (pH 7.4) and used to evaluate Reduced glutathione (GSH) and Malondialdehyde (MDA). The remaining brains were homogenized in phosphate buffer (pH 8) and centrifuged at 3000 rpm for 10 minutes. The resulting supernatant was used to evaluate the activity of Acetylcholinesterase (AChE). One third of the brain was homogenized in 3 ml HCL-butanol (0.1MHCL in butanol) to assess dopamine levels, and a quarter of the brain was homogenized in 5 ml 0.01M HCL to assess GABA levels.

Assessment of Total protein in brain homogenate

Total protein was assessed by the burette method using a commercial kit (Agappe Diagnostics Ltd). Protein content was assessed by the burette method using a diagnostic kit for protein determination. A standard material was prepared and tested by adding a working reagent (1000 µl) containing potassium iodide, potassium tartrate, copper sulfate, and sodium hydroxide to 20 µl of the standard protein and 20 µl of the sample (brain homogenate). The blank contains only 1000 µl of reagent. All tubes were incubated at 37°C for 10 min, and optical densities for tests and standards were measured spectrophotometrically on blanks at 546 nm. Proteins are present as blue complexes in the sample when treated with divalent copper ions in alkaline solution. The strength of blue color was relative to proteins existing in sample.

$$\text{Total protein concentration (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X } \text{Concentration of standard (g/dl)}$$

Where, calibrator concentration of standard = 6 g/dL

Assessment of Oxidative parameters in brain homogenate

Estimation of MDA level

MDA (lipid peroxidation product) levels were evaluated with TBAR (thiobarbituric acid reaction substance). About 0.5 ml of brain homogenate and 0.5 ml of Tris-HCl buffer (pH 7.4) were mixed and incubated at 37°C, for 2 hours. After incubation, 1 ml of trichloroacetic acid (TCA) was added and centrifuged at 1000 rpm for 10 minutes. About 1 ml of the supernatant thus obtained was added to 1 ml of 0.67% thiobarbituric acid (TBA) and maintained in a boiling water bath for 10 minutes. After cooling, 1 ml of secondary distilled water was added and the optical density was measured at 532 nm. The level of TBARS was calculated using an extinction coefficient of 1.56 X 10^5 M^{-1} cm^{-1} and the result was expressed as nmol MDA/mg brain protein (Wills, 1964) [24].

$$\text{MDA level (nmol/mg protein)} = \frac{\text{Absorbance}}{1.56 \,/\, \text{Protein concentration}}$$

Estimation of Nitrite/Nitrate level

The accumulation of nitrite in the supernatant, an indicator of nitric oxide formation, is determined by colorimetric analysis using Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green *et al.,* (1982) [25]. Mix equal volumes of supernatant and Greiss reagent, incubate the mixture in the dark at room temperature for 10 min and measure the absorbance spectrophotometer at 540 nm. The nitrite concentration of the supernatant on the sodium nitrite standard curve, expressed in µM/mg protein.

$$\text{Nitrite/ Nitrate level} = \frac{\text{Absorbance X 3.38}}{\text{Protein concentration}}$$

Estimation of Reduced GSH level

The Ellman method was used to assess GSH levels in the brain. To 1 ml of the prepared homogenate, 1 ml of 4% TCA was added to precipitate and

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

centrifuged at 5000 rpm for 10 minutes. Then, 2 ml of disodium hydrogen phosphate buffer (0.3M, pH 8.4) and secondary distilled water (0.4 ml) were added to 0.5 ml of the resulting supernatant. To the mixture was added 0.25 ml of 0.001M DTNB [5,5-dithiobis-(2-nitrobenzoic acid) dissolved in 1% w/v. sodium citrate] and incubate for 10 min at room temperature. The developed yellow was read at 412 nm using a UV-visible spectrophotometer. GSH levels were calculated using a molar extinction coefficient of 1.36 x 10^4 M^{-1} cm^{-1} and the results are expressed in µmol/mg brain protein (Ellman, 1959) [26].

$$\text{GSH level (nmol/mg protein)} = \frac{\text{Absorbance X 1000}}{\text{136 X Protein concentration}}$$

ASSESSMENT OF COGNITIVE FUNCTIONS IN BRAIN HOMOGENATE

Estimation of AChE level

Taken following solutions like 0.5 ml separated supernatant, 0.1 ml DTNB, 3 ml of 0.01M sodium phosphate buffer (pH 8) and made a reaction mixture, its absorbance was taken on UV-Visible Spectrophotometer at 412 nm. Additional, 0.10 ml Acetylcholine iodide solution (7% aqueous solution) was added and then kept stand for 15 min. Finally, absorbance was once more taken at 412 nm. The alteration in absorbance was calculated and effects expressed as mmol/mg protein (Ellman, 1961) [27].

$$\text{AChE level (mol/min/mg)} = \frac{5.74 \text{ X } 10^{-4} \text{ X } \Omega A}{\text{Brain weight (mg/ml) X 15}}$$

Where, ΩA = Absorbance of sample

ASSESSMENT OF INFLAMMATION IN SERUM

Estimation of CRP level

his was done by determining serum CRP. CRP was performed using the CRP-turbiletex method. A quantitative measurement of CRP in serum or plasma. Latex particles coated with specific anti-mouse CRP aggregates when mixed with a sample comprising CRP. Aggregation causes a change in absorbance depending on the CRP content of the sample, which can be quantified compared to a calibrator with a known CRP concentration. Reagents and photometers (cuvette holder) were maintained at 37°C. The wavelength was adjusted to 540 nm, the temperature to 37°C, and the length of the optical path to 1 cm, and zeroed with distilled water. 5.0 µl brain homogenate/calibrator was mixed with 900 µl diluent (trisbuffer 20 mmol/l, pH 8.2) and 100 µl latex (particles coated with anti-mouse CRP IgG, pH 7.3). Absorbance was measured immediately after adding the sample (A1) and after 2 minutes (A2).

$$\text{CRP level (mg/dl)} = \frac{(A_2\text{-}A_1)\ \text{Sample}}{(A_2\text{-}A_1)\ \text{Calibrator}} \text{ X Calibrator concentration}$$

ASSESSMENT OF NEUROCHEMICALS IN BRAIN HOMOGENATE

Estimation of Dopamine level

The method of Schlumpf *et al.,* 1974 was used to quantify dopamine levels in the brain. About 0.8 ml of supernatant was removed by centrifugation of the brain homogenate (2000 rpm for 10 min) and added to 0.25 ml of 0.1M HCl and 2 ml of heptane. The resulting mixture was shaken for 10 minutes and centrifuged to separate the aqueous phase. To 0.02 ml of the separated aqueous phase, 0.01 ml of sodium acetate buffer (pH 6.9) and 0.05 ml of 0.4M ethylenediaminetetraacetic acid (EDTA) were added. Then 0.01 ml of iodine solution (0.1M in ethanol) was added to oxidize and 0.5 ml sodium thiosulfate (5M in NaOH) was used to stop oxidation after 2 min. After a rest of 90 seconds, acetic acid solution (0.5 ml of 10 M) was added and heated at 100°C for 6 minutes. After cooling, the mixture was read at 330/375 nm with a spectrofluorometer. Finally, brain values (fluorescence of tissue extract - fluorescence of tissue blank) were obtained with reference to the internal reagent standard (fluorescence of internal reagent standard - fluorescence of internal reagent blank). To prepare the tissue blank, the reagents for the oxidation step were mixed in reverse order (sodium thiosulfate before iodine) and all other parameters were left unchanged. Internal reagent standard was prepared by adding 2.5 ml HCl-butanol (0.1M HCl in butanol), 0.125 ml distilled water in 500 ng dopamine standard, and internal reagent blank was prepared by adding 0.125 ml distilled water in 2.5 ml HCl-butanol . . (0.1M HCl in butanol) [28].

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

DA (Test) – DA (Blank) = DA

DA / Brain weight / 31.333

Where, DA = Dopamine

HISTOLOGICAL STUDIES

Formalin (10% v/v) was used to preserve the isolated brain. Brains were excised and soaked in 10% buffered formalin. They were then dehydrated in various concentrations of ethanol, soaked in xylene, and embedded in paraffin. Thin sections of 4 mm thickness were cut from paraffin blocks and stained with hematoxylin (0.6% w/v) for 15 min and then counterstained with eosin (1% w/v) for 2 min. They were then examined under a light microscope for motive integrity analysis.

STATISTICAL ANALYSIS

All the standards are expressed as mean ± variance. Data gotten from numerous groups were evaluated at graph pad prism 5 and statistical using one way ANOVA (analysis of variance), followed by Tukey test for evaluation of all the groups. A 'P' value of but 0.05 was measured statistically significant

RESULTS

Effect of combination of Doxycycline and Minocycline on Behavioral parameters:

Effect of combination of Doxycycline and Minocycline on Locomotor activity using Actophotometer

Ketamine (50 mg/kg, *i.p*.) administration for 7 consecutive days significantly increased the locomotor activity of mice (703.00 ± 43.27, $p < 0.001$), when matched with normal group (479.50 ± 40.34). Olanzapine, as standard anti-psychotic drug treatment (5 mg/kg, *i.p.)* on 7th day significantly reversed hyper locomotor activity (124.66 ± 20.81; $p < 0.001$) induced by Ketamine. Pre-treated Doxycycline and Minocycline (50 mg/kg, *i. p.*) groups showed significant decrease in the hyper locomotor movement (373.33 ± 43.04, $p < 0.001$; 388.16 ± 13.36, $p < 0.001$), when compared to Ketamine group. Combination of Doxycycline and Minocycline (50 mg/kg *i.p*.) was also found highly significant in reversing (332.00 ± 15.00, $p < 0.001$), Ketamine induced hyper locomotor activity but the combination showed non-significant results in comparison to Doxycycline and Minocycline pretreated groups (Fig. 1, Table 1)

Effect of combination of Doxycycline and Minocycline on stereotype behaviors

Ketamine administered for 7 successive days, significantly elicit stereotype behavior like, rearing (24.00 ± 1.39, $p < 0.001$), and falling (7.83 ± 0.93, $p < 0.001$) in mice, when observed for a period of ten minutes each in comparison with normal group (17.66 ± 1.30; 0.66 ± 0.21). Pre-treated Doxycycline and Minocycline showed reduction (16.16 ± 0.94, $p < 0.001$; 15.16 ± 0.98, $p < 0.001$) in rearing behavior as well as reduction (3.50 ± 0.56, $p < 0.001$; 4.16 ± 0.47, $p < 0.001$) in falling behavior in mice. Combination of Doxycycline and Minocycline (50 mg/kg, *i.p.*) was also found effective in reversing Ketamine occurred rearing and falling behaviors (10.50 ± 0.84, $p < 0.001$; 2.00 ± 0.36, $p < 0.001$). Combination of Doxycycline and Minocycline was significant in reducing rearing ($p < 0.01$), when linked to Doxycycline and ($p < 0.05$), when matched with Minocycline pretreated groups, while combination was significant in reducing no of falls of animals ($p < 0.05$), when compared to Minocycline pretreated. Non-significant results were seen with respect to Doxycycline pretreated group. Olanzapine, (5 mg/kg, *i.p.)* on 7th day significantly reduced stereotype rearing (0.33 ± 0.21, $p < 0.001$) and falling (0.00 ± 0.00, $p < 0.001$) in mice, when related to Ketamine group (Fig. 2-3, Table 1).

Effect of combination of Doxycycline and Minocycline on Immobility duration using FST

Immobility period in FST was significantly increased (197.66 ± 6.66, $p < 0.001$) through the administration of Ketamine (50 mg/kg, *i.p*.) for 14 successive days, when compared to normal group (88.00 ± 6.25). Pre-treated Doxycycline and Minocycline groups showed effective reduction in the immobility duration (110.16 ± 3.16, $p < 0.001$; 110.16 ± 4.20, $p < 0.001$), when compared to Ketamine group. Combination of Doxycycline and Minocycline (50 mg/kg*, i.p*.) was also found highly significant in reversing (80.33 ± 4.95, $p < 0.001$) Ketamine induced increase immobility duration. Combination was initiate statistically significant in falling immobility ($p < 0.01$) in comparison to Doxycycline and Minocycline pretreated groups. Olanzapine, standard anti-psychotic drug treatment (5 mg/kg, *i.p.)* on 14th day significantly reversed (98.33 ± 2.62, $p < 0.001$) Ketamine induced increase in immobility duration (Fig. 4, Table 1).

Effect of combination of Doxycycline and Minocycline on SDL using Passive avoidance test

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

Significant reduction in step down latency of mice (102.16 ± 2.28; $p < 0.001$), in Passive avoidance test was observed in Ketamine (50 mg/kg, *i.p*.) group administered for 21 successive days in comparison to normal group (265.00 ± 3.58), which indicates loss of memory. Pre-treated Doxycycline and Minocycline showed significant reversal (130.33 ± 2.45; $p > 0.05$: 134.83 ± 5.51; $p < 0.05$), whereas Combination of Doxycycline and Minocycline (50 mg/kg, *i.p.*) was found highly significant in reversing Ketamine induced decrease step down latency (188.83 ± 12.28; $p < 0.001$). Combination was found significant ($p < 0.001$) in comparison to Doxycycline and Minocycline pretreated groups. Olanzapine, (5 mg/kg, *i.p.)* on 21st day significantly increased (226.66 ± 11.72; $p < 0.001$) the step down latency in comparison to Ketamine treated group (Fig. 5, Table 1).

Effect of combination of Doxycycline and Minocycline on Oxidative parameters in brain homogenate:

Effect of combination of Doxycycline and Minocycline on MDA level

MDA levels were initiate significantly increased in mice brain (2.07 ± 0.27; $p < 0.001$) with Ketamine (50 mg/kg, *i.p*.) administration for 21 successive days, when compared to normal group (0.35 ± 0.034). However, Pre-treated Doxycycline and Minocycline groups significantly reversed (0.94 ± 0.09; $p < 0.01$, 1.03 ± 0.11; $p < 0.01$), Ketamine induced changes of MDA level in mice brain. Combination of Doxycycline and Minocycline (50 mg/kg, *i.p.*) showed notable decrease (0.59 ± 0.04; $p < 0.001$) in MDA level, when compared to Ketamine group. However, the combination did not showed significant results with respect to Pre-treated Doxycycline and Minocycline pretreated groups. Olanzapine used as positive control (5 mg/kg; *i.p*.) showed significant reduction in MDA level (0.25 ± 0.44; $p < 0.001$), when matched with Ketamine group (Fig. 6, Table 2).

Effect of Combination of Doxycycline and Minocycline on Nitrite/Nitrate level

Ketamine (50 mg/kg, *i.p*.) administration for 21 successive days showed significant changes in mice brain as estimated in term of elevated level of brain nitrate (0.050 ± 0.003; $p < 0.001$), when compared normal group (0.019 ± 0.003). Pre-treatment with Doxycycline pretreated and Minocycline pretreated (50 mg/kg, *i. p.*) showed significant fall in brain nitrate (0.021 ± 0.002; 0.021 ± 0.004; $p < 0.001$), when compared to Ketamine group. Combination of Doxycycline and Minocycline (50 mg/kg, *i.p.*) (0.015 ± 0.001; $p < 0.001$) was significantly successful in reversing Ketamine induced increase in brain nitrate. Olanzapine treatment (5 mg/kg, *i.p.)* on 21st day significantly reversed (0.010 ± 0.003; $p < 0.001$) rise in brain nitrate, when compared to Ketamine treated group (Fig. 7, Table 2).

Effect of Combination of Doxycycline and Minocycline on GSH level

Ketamine (50 mg/kg, *i.p*.) administration for 21 successive days significantly declined brain GSH level of mice (1.57 ± 0.11; $p < 0.001$), when compared to normal group (5.44 ± 0.19). Pre-treated Doxycycline and Minocycline groups significantly reversed (4.10 ± 0.44; $p < 0.01$, 4.04 ± 0.49; $p < 0.01$) Ketamine induced changes in enzyme level. Combination of Doxycycline and Minocycline (50 mg/kg, *i.p.*) was found highly significant (5.22 ± 0.57; $p < 0.001$) in reversing Ketamine induced decrease in GSH level. Combination was statistically successful in bringing GSH values back to normal. Olanzapine treatment (5 mg/kg, *i.p.)* on 21st day significantly increased (7.34 ± 0.42; $p < 0.001$) the brain GSH level declined by Ketamine (Fig. 8, Table 2).

Effect of Combination of Doxycycline and Minocycline on Cognitive functions in brain homogenate:

Effect of Combination of Doxycycline and Minocycline on AchE level

Increased AchE level reflects cognitive impairment. Ketamine (50 mg/kg, *i.p*.) administration for 21 successive days significantly rise brain AchE levels (4.46 ± 0.11; $p < 0.001$), when compared to normal control (2.64 ± 0.44). Pre-treated Doxycycline and Minocycline (50 mg/kg, *i. p.*) pretreated groups showed high reduction (1.60 ± 0.12; 2.89 ± 0.11; $p < 0.001$) in brain AchE level, when compared to Ketamine group. Combination of Doxycycline and

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

Minocycline (50 mg/kg, *i. p.*) was also significant in reversing (1.88 ± 0.15; $p < 0.001$) Ketamine induced increase in brain AchE level. Combination also showed significant result with Minocycline pretreated group. Olanzapine showed significantly reduction in brain AchE levels (1.56 ± 0.12; $p < 0.001$) in mice, when compared to Ketamine group (Fig. 9, Table 2).

Effect of Combination of Doxycycline and Minocycline on Inflammation in serum

Effect of Combination of Doxycycline and Minocycline on CRP level

Ketamine (50 mg/kg, *i.p.*) administration for 21 successive days significantly increased serum CRP levels (2.07 ± 0.22; $p < 0.001$), when compared to normal group (0.68 ± 0.05). Pre-treatment with Doxycycline and Minocycline (50 mg/kg, *i. p.*) showed significant reduction (1.25 ± 0.11; $p < 0.01$; 1.38 ± 0.08; $p < 0.01$) of serum CRP levels, when compared to Ketamine group. Combination of Doxycycline and Minocycline (50 mg/kg, *i. p.*) was also found highly significant result (0.99 ± 0.09; $p < 0.001$) in reversing Ketamine induced increase serum CRP levels. Olanzapine treatment (5 mg/kg, *i.p.)* on 21st day significantly reversed increase in serum CRP levels (0.84 ± 0.12; $p < 0.001$), when compared to Ketamine treated group (Fig. 10, Table 3).

Effect of Combination of Doxycycline and Minocycline on Neurochemicals in brain homogenate:

Effect of Combination of Doxycycline and Minocycline on Dopamine level

Ketamine (50 mg/kg, *i.p.*) administration for 21 successive days significantly increased Dopamine level (16.54 ± 0.57; $p < 0.001$), when compared normal group (4.55 ± 0.48). Pre-treatment with Doxycycline and Minocycline (50 mg/kg, *i. p.*) showed significant reduction (10.30 ± 0.17; 9.61 ± 1.14; $p < 0.01$) in Dopamine levels, when compared to Ketamine group. Combination of Doxycycline and Minocycline (50 mg/kg, *i. p.*) was also found highly significant result (6.61 ± 0.65; $p < 0.001$) in reversing Ketamine induced increase Dopamine levels, combination also gave significant results in comparison to Doxycycline ($p < 0.01$) and Minocycline ($p < 0.05$) pretreated groups. Olanzapine treatment (5 mg/kg, *i.p.)* on 21st day significantly reversed increase in Dopamine level, when compared to Ketamine treated group (4.78 ± 0.34; $p < 0.001$) (Fig. 11, Table 2).

Effect of combination of Doxycycline and Minocycline on mice Body weight

Ketamine (50 mg/kg, *i.p.*) administration for 21 successive days decreased the body weight of mice (29.50 ± 0.95), when compared to normal group (37.16 ± 2.00). Pre-treated Doxycycline and Minocycline (50 mg/kg, *i. p.*) groups showed significant recovery in body weight (33.50 ± 1.38; 35.66 ± 1.28). Combination of Doxycycline and Minocycline (50 mg/kg, *i.p.*) was also found significant in reversing (32.50 ± 0.95), Ketamine induced decreased in body weight. Olanzapine, standard anti-psychotic drug treatment (5 m/kg, *i.p.)* on 21st day significantly reversed decrease in body weight (31.83 ± 0.70), when compared to Ketamine treated group (Table 4).

Effect of combination of Doxycycline and Minocycline on brain Histology

Ketamine (50 mg/kg, *i.p.*) administration for 21 successive days produced histopathology changes like perinuclear vacuolization, dilated vascular channels, red neurons and hyperchromatic nuclei in mice brain cortex. Pretreatment with Doxycycline and Minocycline showed histopathology changes as represented by less perinuclear vacuolization, hyperchromatic nuclei and red neurons. Combination of Doxycycline and Minocycline (50 mg/kg, *p.*o.) was also found significant in reversing histopathology changes, when compared to Doxycycline, Minocycline and Ketamine groups. Olanzapine, standard anti-psychotic drug treatment (5 m/kg, *i.p.)* on 21st day significantly reversed histopathology changes, when compared to Ketamine treated group (Fig. 12).

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

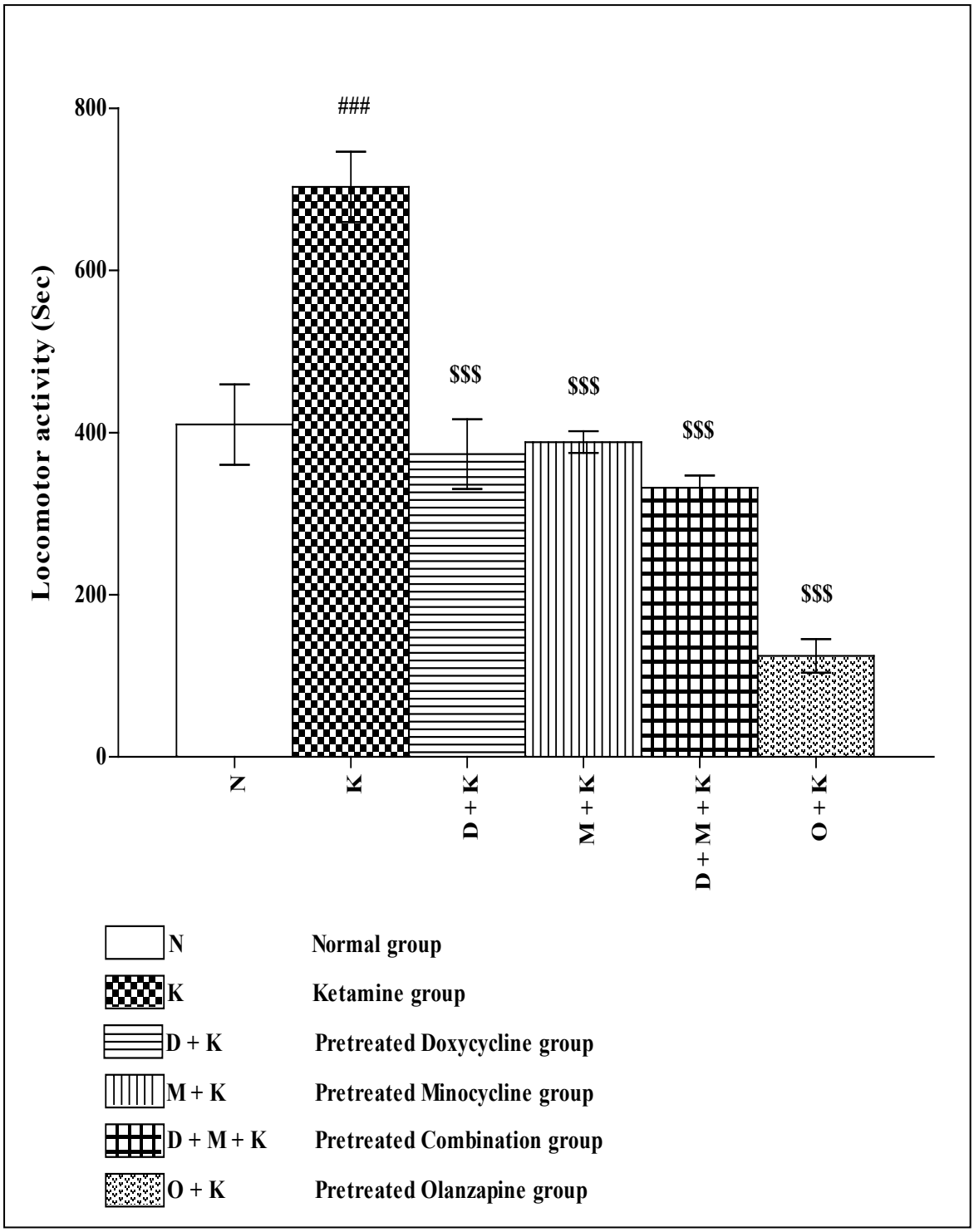

Fig. 1 Effect of combination of Doxycycline and Minocycline on Locomotor activity using Actophotometer

Value were expressed as mean ± *SEM*, ###; $p < 0.001$ versus Normal group, $$$; $p < 0.001$ versus Ketamine group.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

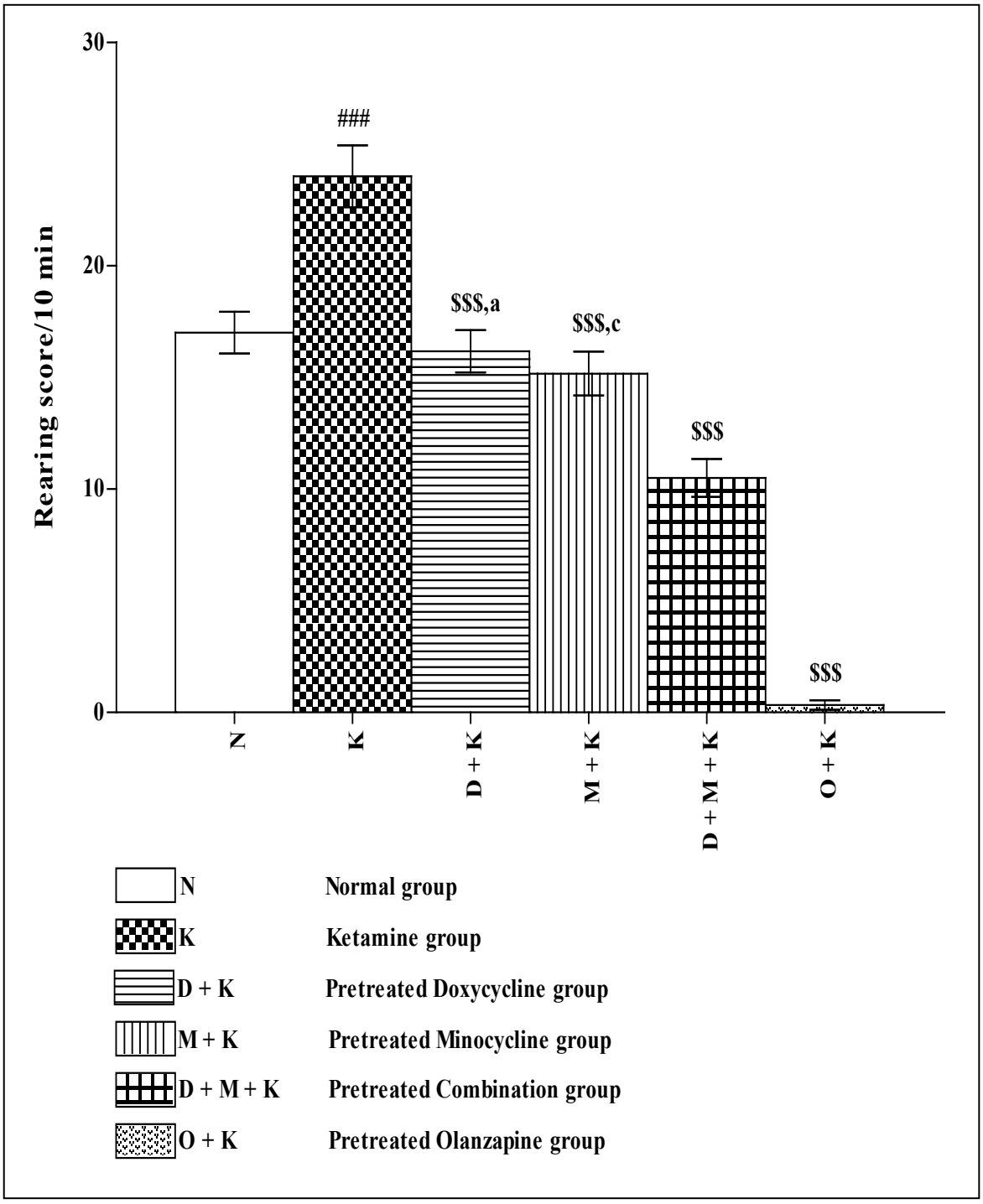

Fig. 2 Effect of combination of Doxycycline and Minocycline on Rearing behavior

Value were expressed as mean ± *SEM*, ###; $p < 0.001$ versus Normal group, $$$; $p < 0.001$ versus Ketamine group, a; $p < 0.001$ versus Doxycycline group, c; $p < 0.05$ versus Minocycline group.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

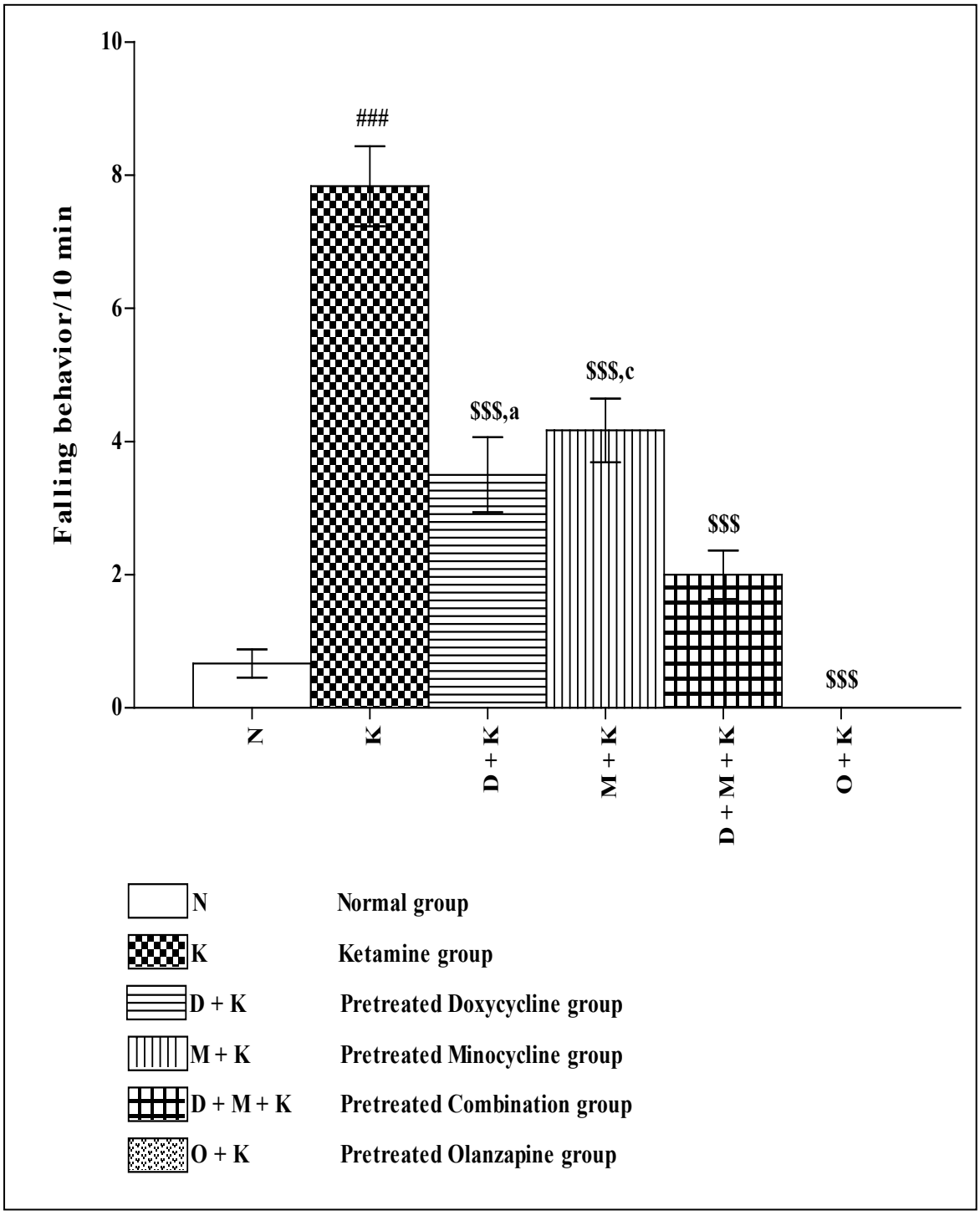

Fig. 3 Effect of combination of Doxycycline and Minocycline on falling behavior

Value were expressed as mean ± *SEM*, ###; $p < 0.001$ versus Normal group, $$$; $p < 0.001$ versus Ketamine group, a; $p < 0.001$ versus Doxycycline group, c; $p < 0.05$ versus Minocycline group.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

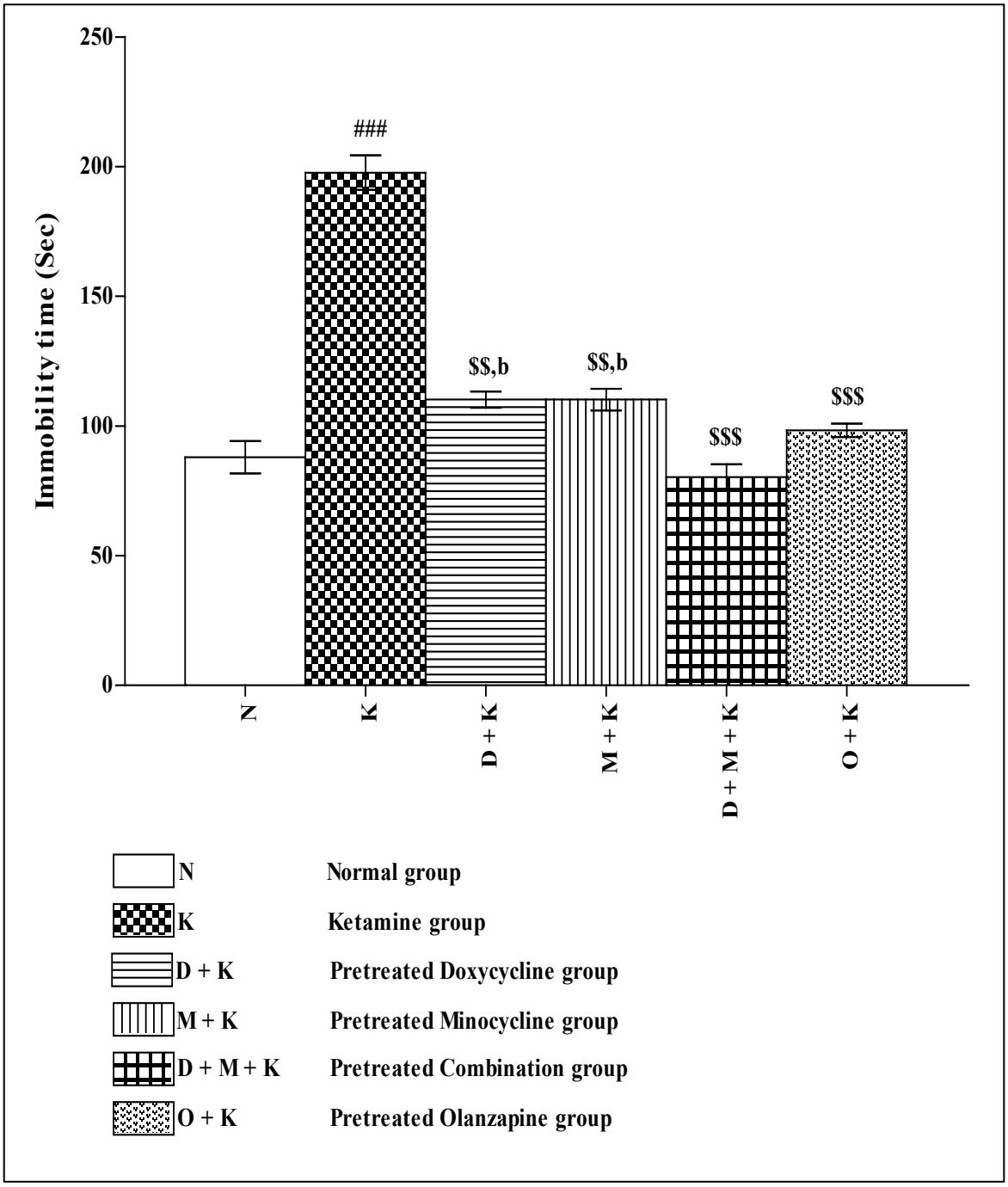

Fig. 4 Effect of combination of Doxycycline and Minocycline on Immobility duration using FST

Value were expressed as mean ± *SEM*, ###; $p < 0.001$ versus Normal group, $$$; $p < 0.001$ versus Ketamine group; $$; $p < 0.01$ versus Ketamine group, b; $p < 0.01$ versus Doxycycline and Minocycline group.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

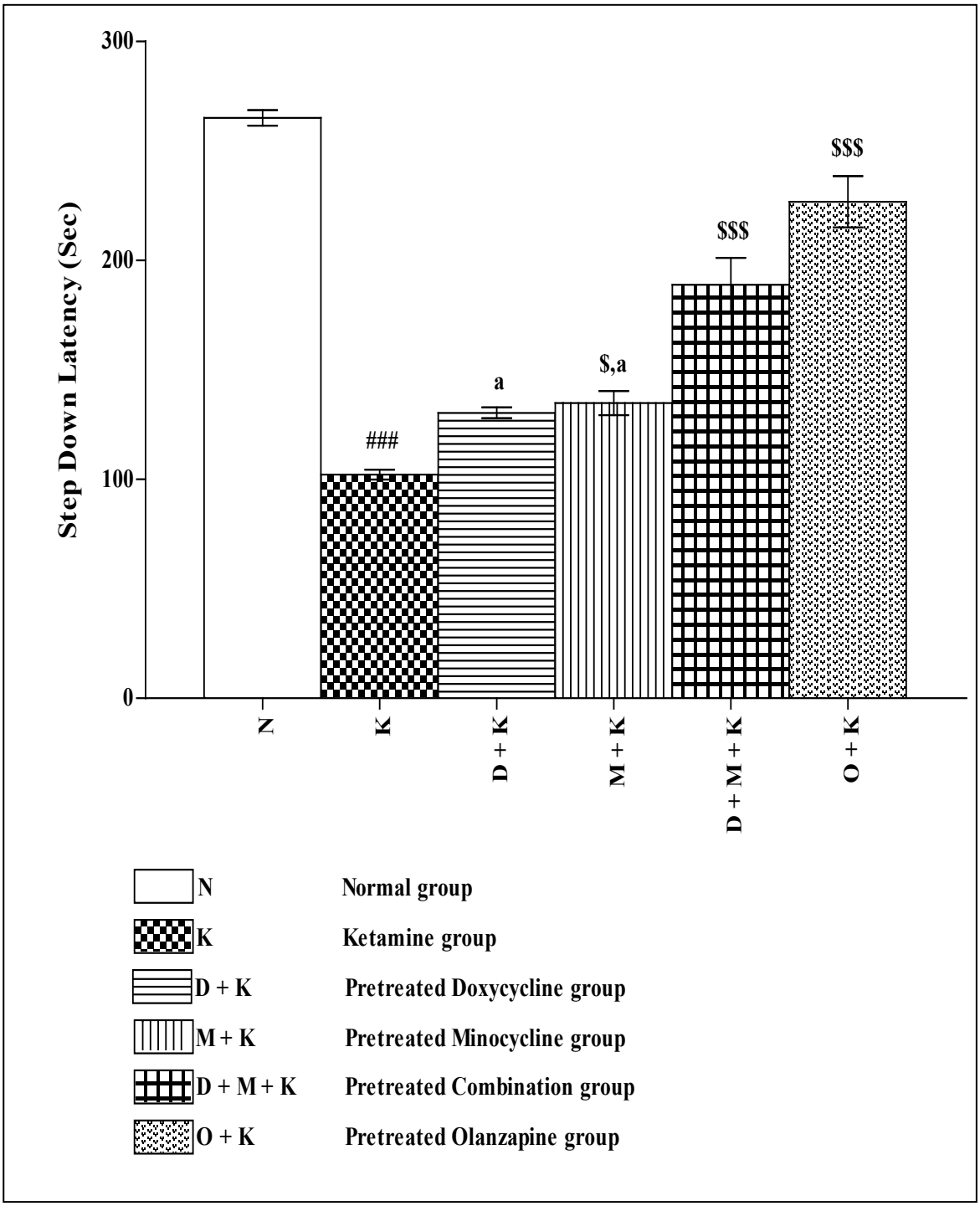

Fig. 5 Effect of combination of Doxycycline and Minocycline on SDL using Passive avoidance test
Value were expressed as mean ± *SEM*, ###; $p < 0.001$ versus Normal group, $$$; $p < 0.001$ versus Ketamine group, $; $p < 0.05$ versus Ketamine group; a $p < 0.001$ versus Doxycycline and Minocycline group.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

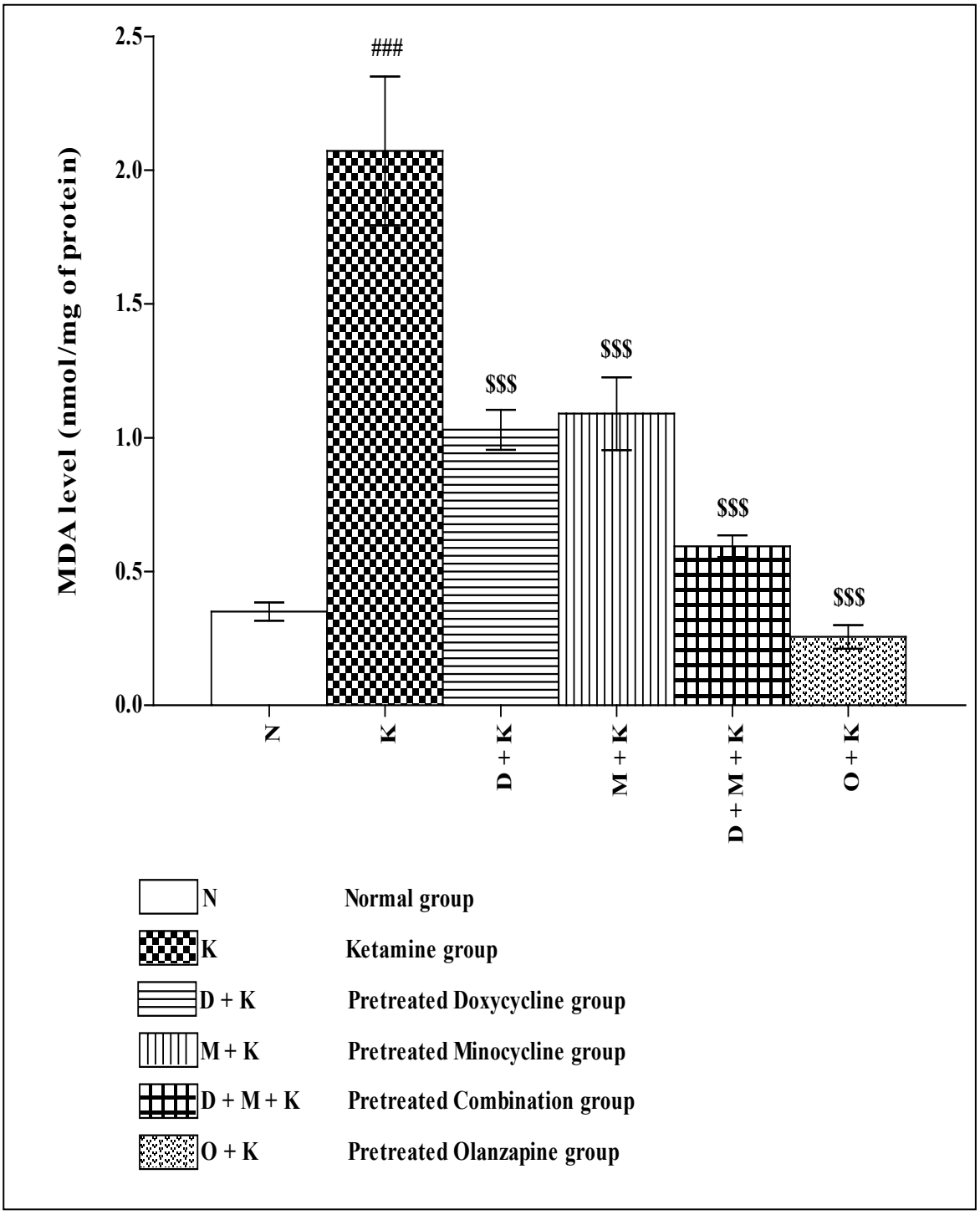

Fig. 6 Effect of combination of Doxycycline and Minocycline on MDA level

Value were expressed as mean ± *SEM*, ###; $p < 0.001$ versus Normal group, $$$; $p < 0.001$ versus Ketamine group.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

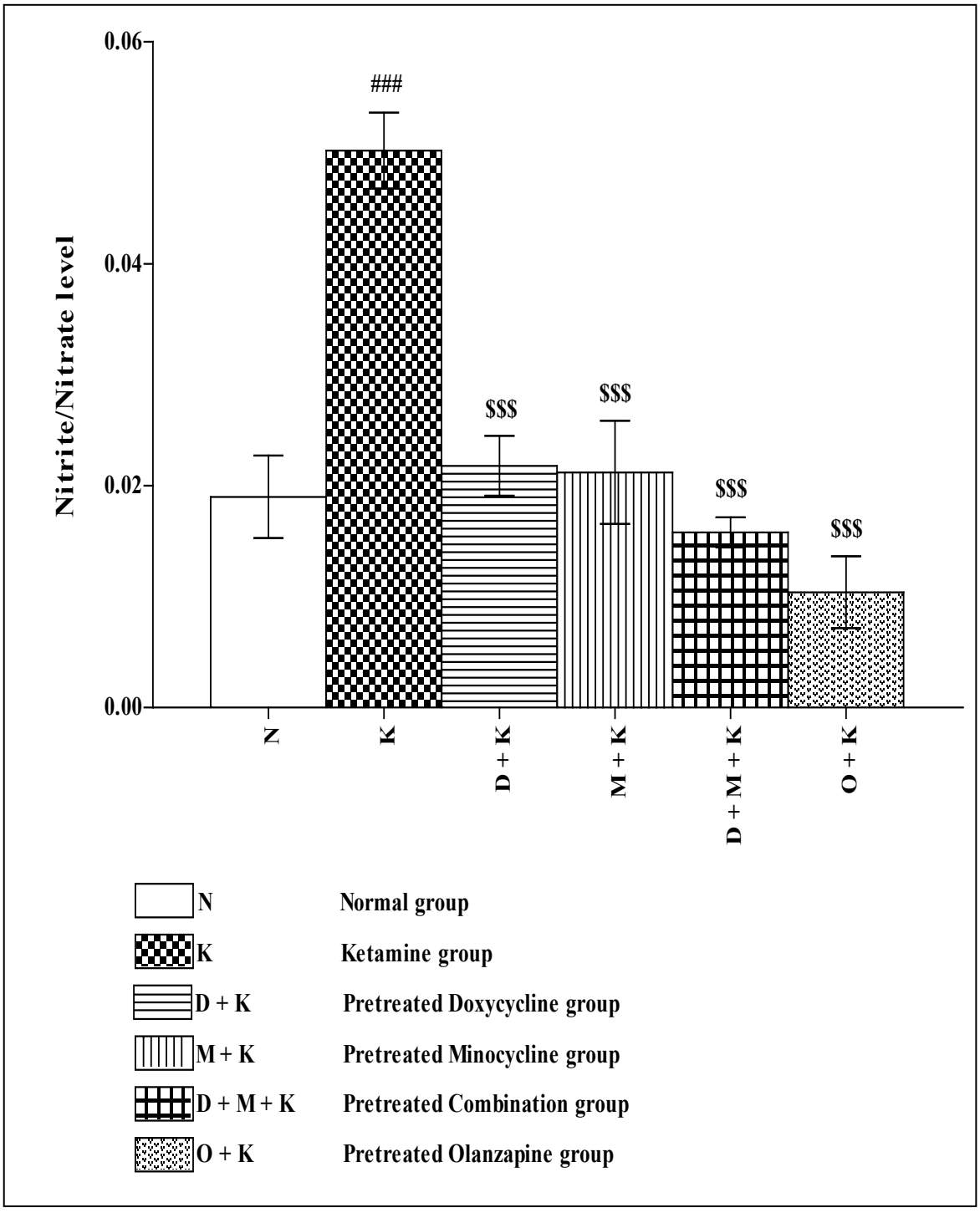

Fig. 7 Effect of combination of Doxycycline and Minocycline on brain Nitrite/Nitrate level

Value were expressed as mean ± *SEM*, ###; $p < 0.001$ versus Normal group, $$$; $p < 0.001$ versus Ketamine group.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

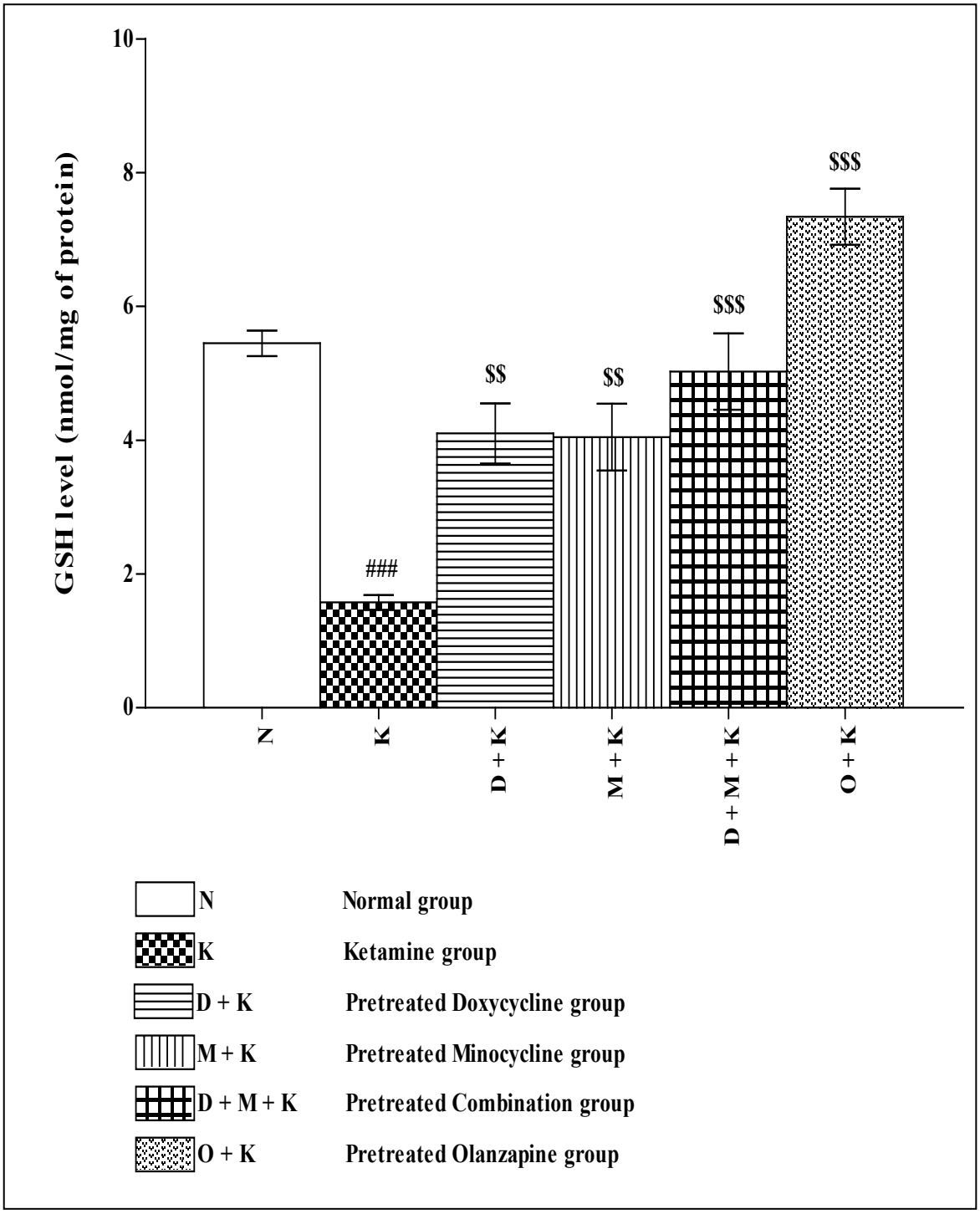

Fig. 8 Effect of combination of Doxycycline and Minocycline on GSH level

Value were expressed as mean ± *SEM*, ###; $p < 0.001$ versus Normal group, $$$; $p < 0.001$ versus Ketamine group, $$; $p < 0.01$ versus Ketamine group.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

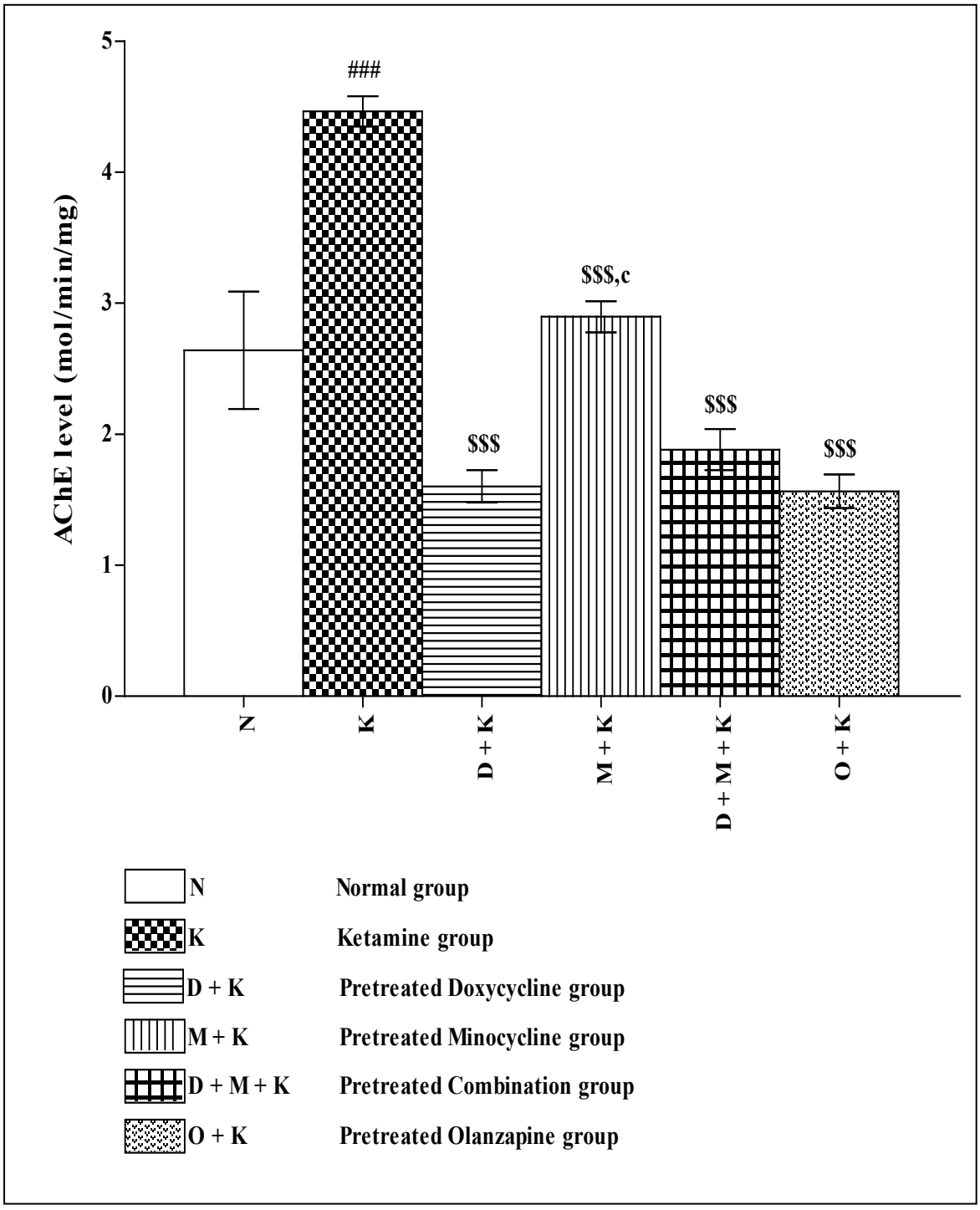

Fig. 9 Effect of combination of Doxycycline and Minocycline on AchE level

Value were expressed as mean ± *SEM*, ###; $p < 0.001$ versus Normal group, $$$; $p < 0.001$ versus Ketamine group, c; $p < 0.05$ versus Minocycline group.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

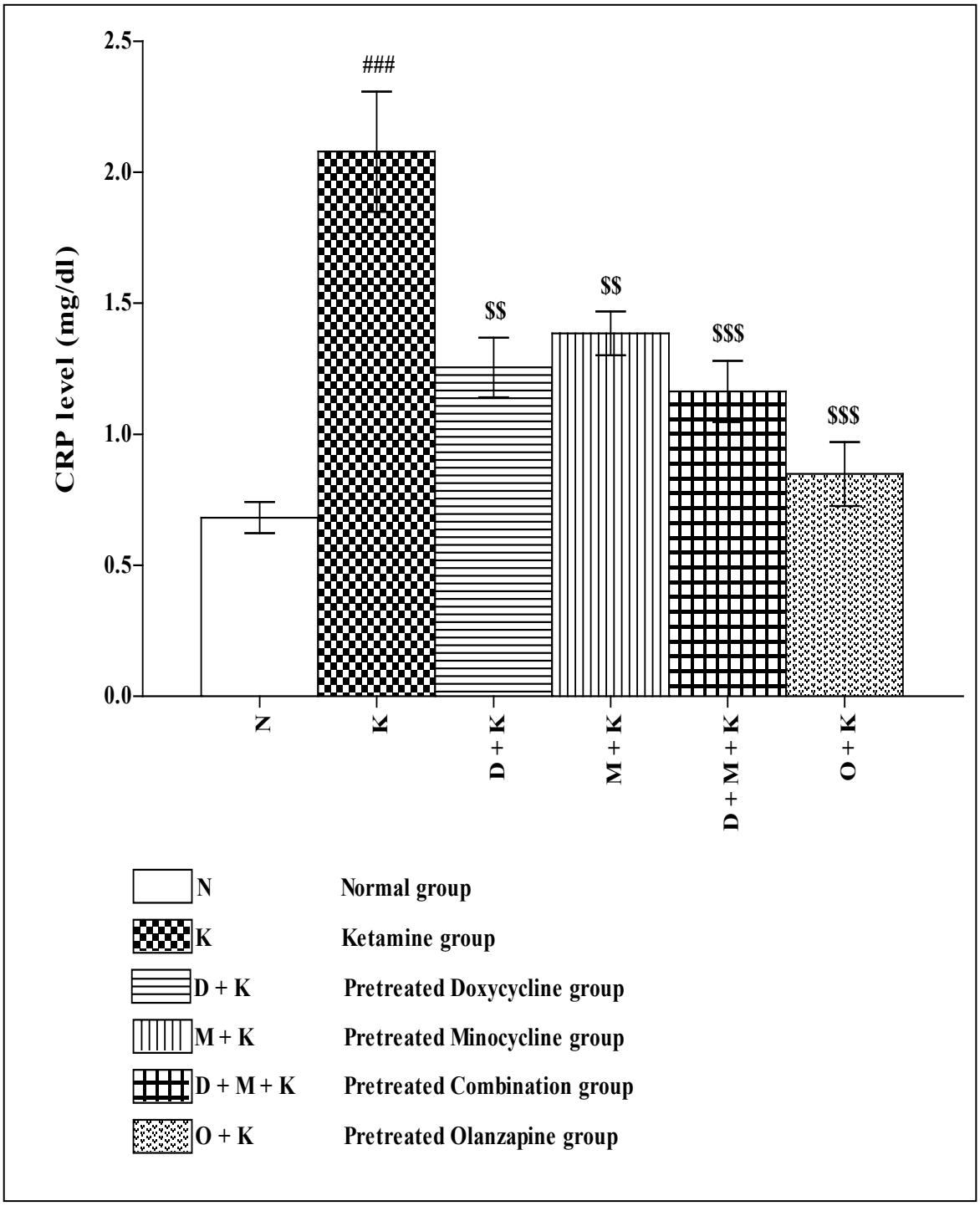

Fig. 10 Effect of combination of Doxycycline and Minocycline on CRP level

Value were expressed as mean ± *SEM*, ###; p< 0.001 versus Normal group,$$$; p< 0.001 versus Ketamine group, $$; p< 0.01 versus Ketamine group.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

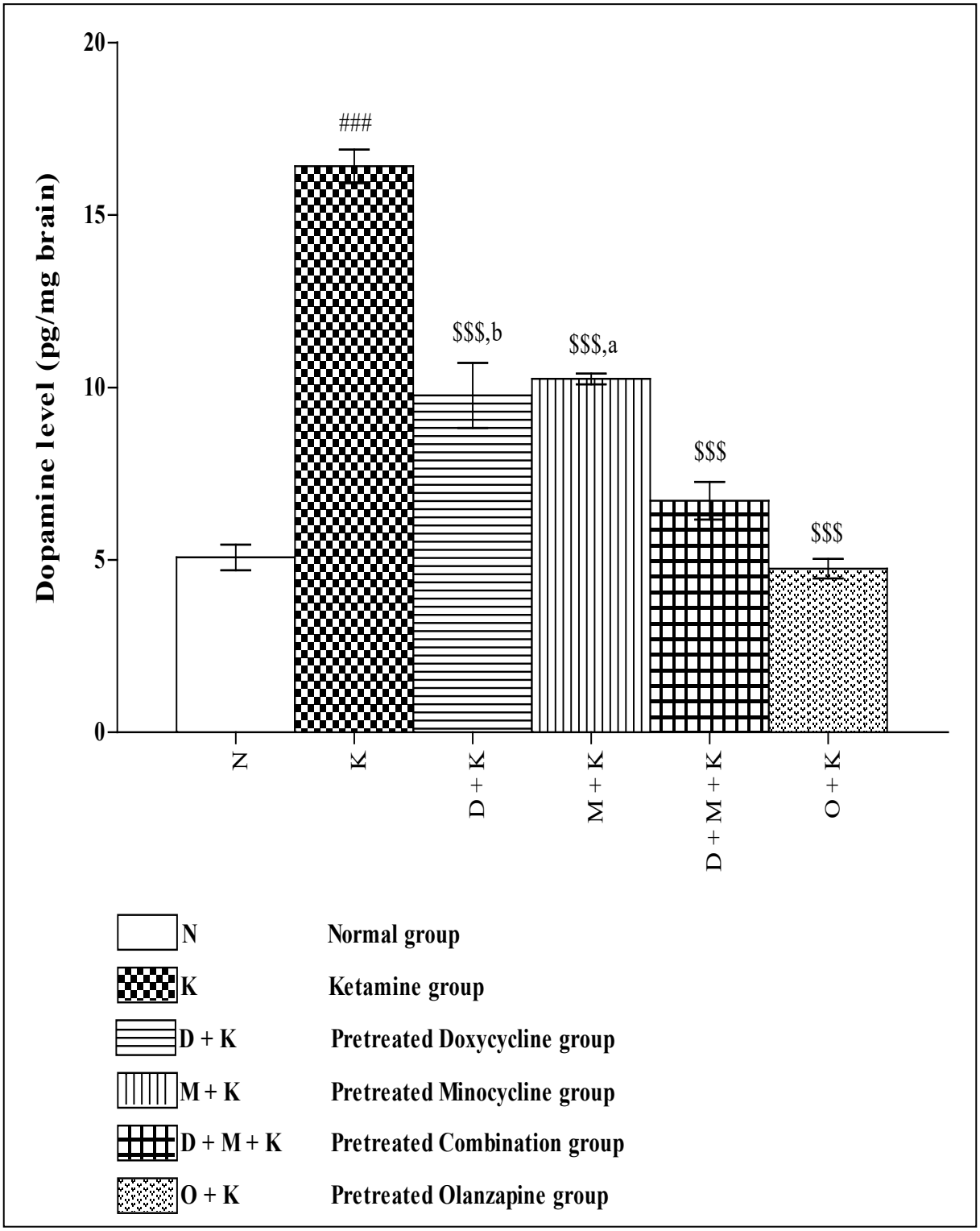

Fig. 11 Effect of combination of Doxycycline and Minocycline on Dopamine level

Value were expressed as mean ± *SEM*, ###; $p < 0.001$ versus Normal group, $$$; $p < 0.001$ versus Ketamine group, b; $p < 0.01$ versus Doxycycline group, a; $p < 0.001$ versus Minocycline group.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

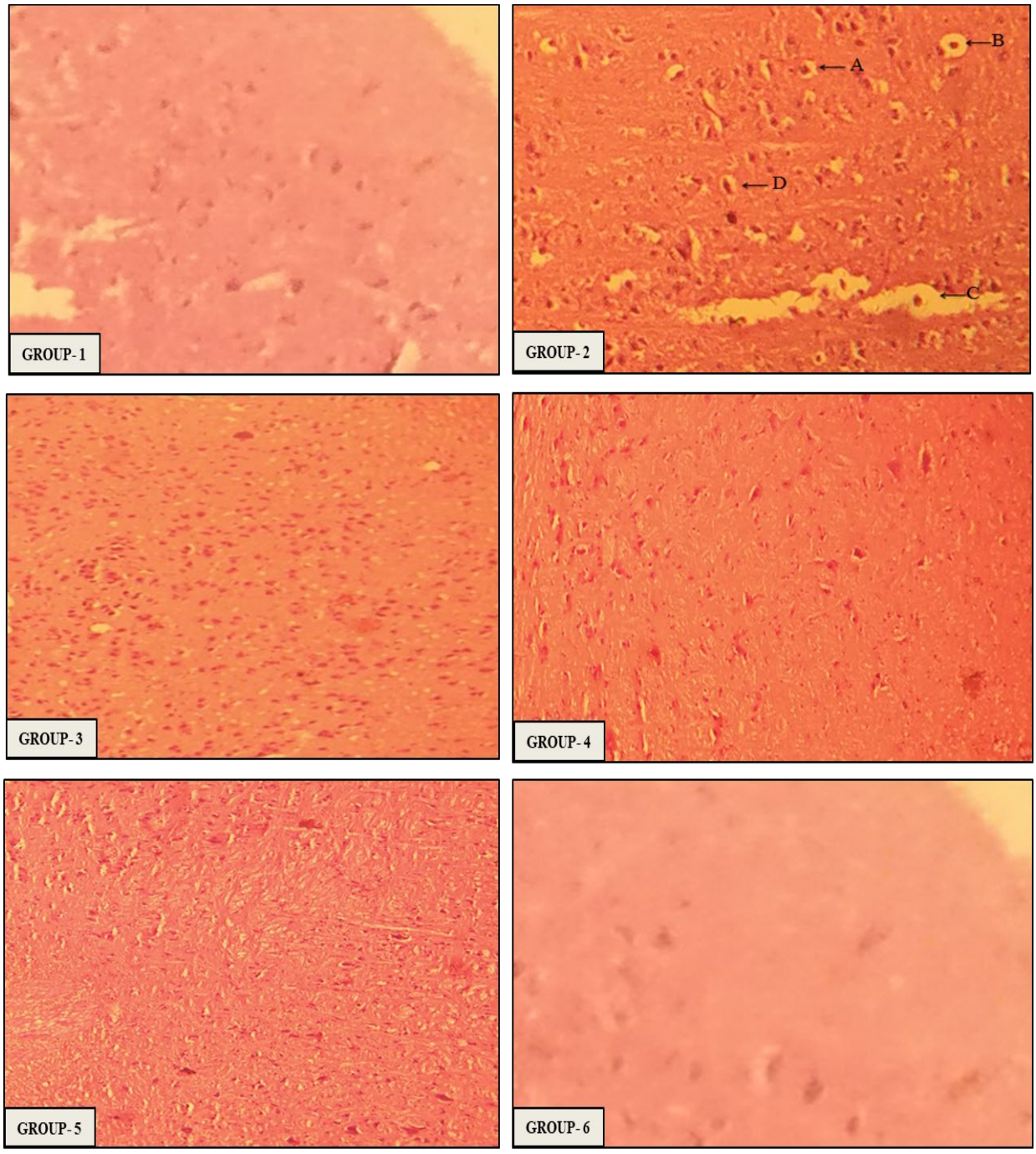

Fig. 12 Effect of combination of Doxycycline and Minocycline on brain histology of mice

Representative photomicrographs of sections of the mice cortex. 1; Normal group, 2; Ketamine group, 3; Pretreated Doxycycline group, 4; Pretreated Minocycline group, 5; Pretreated combination of Doxycycline + Minocycline group, 6; Pretreated Olanzapine group.

Abbreviations: A, Hyperchromatic nuclei; B, Perinuclear vacuolization; C, Dilated vascular channels; D, Red neurons etc.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

Table 1: Effect of combination of Doxycycline and Minocycline treatment against Ketamine induced Behavioral parameter changes in mice

Treatment	Locomotor activity (sec)	Rearing Score/10 min	Falling behavior/ 10 min	Immobility duration (sec)	Step down latency (sec)
	7th day	7th day	7th day	14th day	21st day
Group 1 (Normal group)	479.50 ± 40.34	17.00 ± 0.93	0.66 ± 0.21	88.00 ± 6.25	265.00 ± 3.58
Group 2 (Ketamine group)	703.00 ± 43.27	24.00 ± 1.39	7.83 ± 0.60	197.66 ± 6.66	102.16 ± 2.28
Group 3 (Pre-treated Doxycycline group)	373.33 ± 43.04	16.16 ± 0.94	3.50 ± 0.56	110.16 ± 3.16	130.33 ± 2.45
Group 4 (Pre-treated Minocycline group)	388.16 ± 13.36	15.16 ± 0.98	4.16 ± 0.47	110.16 ± 4.20	134.83 ± 5.51
Group 5 (Pre-treated combination group)	332.00 ± 15.00	10.5 ± 0.84	2 .00 ± 0.36	80.33 ± 4.95	188.83 ± 12.28
Group 6 (Pre-treated Olanzapine group)	124.66 ± 20.81	0.33 ± 0.21	0.00 ± 0.00	98.33 ± 2.62	226.66 ± 11.72

Table 2: Effect of combination of Doxycycline and Minocycline treatment against Ketamine induced Biochemical changes in mice brain

Treatment	MDA level (nmol/mg protein)	Nitrite/ Nitrate Level	GSH level (nmol/mg protein)	AchE level (mol/min/mg)	Dopamine level (pg/mg brain)
Group 1 (Normal group)	0.35 ± 0.03	0.019 ± 0.003	5.44 ± 0.19	2.64 ± 0.44	4.55 ± 0.48
Group 2 (Ketamine group)	2.07 ± 0.27	0.050 ± 0.003	1.57 ± 0.11	4.46 ± 0.11	16.54 ± 0.57
Group 3 (Pre-treated Doxycycline group)	0.94 ± 0.09	0.021 ± 0.002	4.10 ± 0.44	1.60 ± 0.12	10.30 ± 0.17
Group 4 (Pre-treated Minocycline group)	1.03 ± 0.11	0.021 ± 0.004	4.04 ± 0.49	2.89 ± 0.11	9.61 ± 1.14
Group 5 (Pre-treated combination group)	0.59 ± 0.04	0.015 ± 0.001	5.02 ± 0.57	1.88 ± 0.15	6.61 ± 0.65
Group 6 (Pre-treated Olanzapine group)	0.25 ± 0.44	0.010 ± 0.003	7.34 ± 0.42	1.56 ± 0.12	4.78 ± 0.34

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

Table 3: Effect of combination of Doxycycline and Minocycline treatment against Ketamine induced Biochemical changes in mice serum

Treatment	CRP level (mg/dl)
Group 1 (Normal group)	0.68 ± 0.05
Group 2 (Ketamine group)	2.07 ± 0.22
Group 3 (Pre-treated Doxycycline group)	1.25 ± 0.11
Group 4 (Pre-treated Minocycline group)	1.38 ± 0.08
Group 5 (Pre-treated combination of Doxycycline + Minocycline group)	0.99 ± 0.09
Group 6 (Pre-treated Olanzapine group)	0.84 ± 0.12

Table 4: Effect of combination of Doxycycline and Minocycline treatment against Ketamine induced Body weight changes in mice

Treatment	*Swiss Albino* mice – Body weight (gms)			
	1st day	7th day	14th day	21st day
Group 1 (Normal group)	33.66 ± 1.68	35.31 ± 1.51	35.66 ± 1.45	37.16 ± 2.00
Group 2 (Ketamine group)	33.00 ± 1.29	29.00 ± 1.50	27.16 ± 1.19	24.50 ± 0.95
Group 3 (Pre-treated Doxycycline group)	33.00 ± 0.57	33.16 ± 1.16	33.66 ± 1.02	33.50 ± 1.38
Group 4 (Pre-treated Minocycline group	33.16 ± 1.40	34.83 ± 1.44	34.00 ± 1.41	35.66 ± 1.28
Group 5 (Pre-treated combination group)	33.83 ± 0.74	32.00 ± 1.23	32.16 ± 0.94	32.50 ± 0.95
Group 6 (Pre-treated Olanzapine group)	33.00 ± 0.77	31.60 ± 0.80	31.50 ± 0.71	31.83 ± 0.70

DISCUSSION

Schizophrenia is a heterogeneous psychiatric disorder of distorted perceptions and behaviors which is considered via positive, negative and cognitive symptoms. Multifaceted pathophysiological mechanisms are changed in the development of psychosis. Formation of positive, negative and cognitive symptoms includes Dopaminergic, GABAergic, Cholinergic, NMDA receptors dysfunctioning along with neuro-inflammation & oxidative stress [1, 18, 30]. In the current experimental study, the combination result of Doxycycline and Minocycline as a drug treatment was evaluated in Ketamine induced Schizophrenia in mice and combination of Doxycycline and Minocycline was found effective in treating positive, negative and cognitive changes in animals, when matched with Ketamine preserved group and the results were also comparable to Doxycycline and Minocycline pretreated groups in certain parameters. Olanzapine, an established antipsychotic agent also showed its proven properties.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

Ketamine at the dose of 50 mg/kg was used animal model of psychosis [18]. In the present study, Ketamine (50 mg/kg, *i.p.*) was taken as a model for investigational psychosis and as an implement for screening behavioral, biochemical and neurochemical changes and there alterations by combination of Doxycycline and Minocycline. Behavioral Biochemical and neurochemical changes were assessed. Behavioral changes were observed on 7th, 14th and 21st days of Ketamine administration. Ketamine showed hyperlocomotor activity, enhancement of immobility in FST and stereotyped behaviors and reduction in step down latency period indicating positive, negative and cognitive appearance of Schizophrenia and changes in biochemical and neurochemical parameters also confirmed Ketamine made Schizophrenia in mice.

Ketamine produces a state of "dissociative anaesthesia", amnesia, and, at the same time, maintained the respiratory drive effective and supports the systemic arterial blood pressure [9]. Ketamine has psychotomimetic and other adverse effects. Sub-anaesthetic doses of Ketamine induced behavioral and biochemical changes in animals, which makes the basis for its use as one of the reliable rodent model in psychosis analysis [31]. Ketamine causes positive, negative & cognitive symptoms of Schizophrenia [11]. Ketamine-induced positive and negative symptoms and cognitive impairment are explained in part by NMDAR blockade, hyperactivity of Dopamine communication in the mesolimbic system, and hypoactivity of Dopamine in the brain mesocotical system. [32]. In a review given by Geyer *et al*., Phencyclidine and Ketamine induce behavioral abnormalities in rats and monkeys that are important for symptoms of Schizophrenia [33]. Ketamine raise Dopamine levels in the prefrontal cortex by antagonizing NMDA (N-methyl-D-aspartate) receptors establish on GABAergic neurons, thereby stimulating motor activity and stereotyped behavior, resulting in positive symptoms of psychosis [34-36]. In the present study, Ketamine (50 mg/kg*, i.p.*) on 7th day induces hyperlocomotion in mice. Doxycycline and Minocycline pretreated groups showed significant reduction ($p < 0.001$) in hyperlocomotion induced by Ketamine as indicated by the decrease in the number of photocell counts and increase in duration of ambulation of the animals. These effects are in accordance with the results investigated by Benneth Ben-Azu *et al*., 2016 and Zhang *et al.,* 2014, where both Doxycycline and Minocycline possesses mild to moderate ability to ameliorate positive symptoms of Schizophrenia respectively by attenuating behavioral changes like hyperlocomotion via stimulate the NMDA receptors located on GABAergic neurons causing in decreased Dopamine level in prefrontal cortex region of the brain [37-38]. Combination was more significant ($p < 0.001$) in attenuating locomotor counts as compared to Ketamine indicating that the combinatorial effects of the two antibiotic drugs are better for controlling positive behavioural changes in psychosis.

According to Ridley 1994, psychiatric disorders like Schizophrenia and Obsessive-compulsive disorder, showed stereotyped behavioral features and in experimental animals models of Schizophrenia, stereotyped behavior seems to be related mainly to excess dopaminergic activity in the basal ganglia [39]. Reduction of GABA levels modulates dopaminergic neurotransmission and increases ketamine-induced hyperactivity, including stereotyped behavior [40].

Ketamine (50 mg/kg, *ip*) induced falls. number of falls on the floor, flips; number of turns, head tremors; the number of vertical and horizontal rotations and weaving of the neck; The amount of grooming, rearing, ataxia, intermittent twirling and reciprocal forepaw behavior in mice [21, 41-42]. In present study, Ketamine (50 mg/kg*, i.p*.) induced stereotypic behaviors viz., rearing and falling. Doxycycline and Minocycline pretreated groups showed significant reduction ($p < 0.001$) in rearing and falling behaviors induced by Ketamine and results are similar to established changes shown by Dokuyucu *et al.,* 2014 and Yadav *et al*., 2017. These positive behavioral symptoms were possibly changed by blocking D_2 receptors as suggested by Dokuyucu *et al.,* 2014 and Yadav *et al*., 2017 [21, 43]. Combination was also found highly significant ($p < 0.001$) in changing Ketamine induced stereotype behavior of rearing and falling. Combination was significant in reducing rearing and falling behavior with respect to Doxycycline and Minocycline pretreated groups suggesting that the combination could be better option for preventing positive stereotype symptoms.

So far, the effects of drugs on negative symptoms have been positively correlated with their blocking

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

effects on 5-hydroxytryptaminergic ($5HT_{2A}$) receptors. This is because, in some studies, immobility time enhanced by phencyclidine and ketamine was inhibited by 5-HT_{2A} receptor antagonists, including: Ritanserine, Clozapine, Risperidone and Paroxetine, respectively [44-46]. Study conducted by Fan *et al.,* suggested the role of higher CRP levels in exacerbation of negative symptoms and general psychopathology of Schizophrenia [47]. Reactive oxidative species and hyperactivated microglia are also associated with negative symptoms of psychosis, along with other psychiatric disorders [47]. Ketamine induced enhancement of immobility in FST in animal model, which is an indicative of negative symptoms of Schizophrenia [45-46]. In the present study, Ketamine (50 mg/kg*, i.p.*) administration for 14 days enhanced immobility time in FST in mice. Doxycycline and Minocycline pretreated groups showed significant reduction ($p < 0.001$) in immobility time induced by Ketamine, which were in accordance with the results illustrated by Ben-Azu *et al*., 2018, Ben-Azu *et al*., 2016, Saeedi Saravi *et al.,* 2016 and Liu *et al*., 2014 [17, 31, 48-49]. Combination was found statistically more significant ($p < 0.001$) in reducing immobility in comparison to Ketamine. The immobility time was effectively ($p < 0.01$) reduced by combination, when compared to Doxycycline and Minocycline pretreated groups suggesting that combination could be better option for increasing the effectiveness of the two drugs and it does so by reducing the elevated CRP levels as revealed by CRP assessment in the present study.

Cognitive deficiencies played a crucial role in different subtypes of Schizophrenia and considered as one of the important parameter to distinguish Schizophrenia from other forms of psychosis [33, 50]. Passive avoidance behaviors based on negative reinforcement were used to study long-term memory [51-52]. The passive avoidance test has previously been used to access cognitive behavioral parameters in relation to Ketamine-induced psychosis. [18]. In the present study, cognitive impairment in Schizophrenia was well observed by administration of Ketamine (50 mg/kg, *i.p.*) for 21st days, which showed decrease in SDL period in passive avoidance test in Schizophrenia affected mice, which is in accordance with results of Yadav *et al*., 2017 and Chatterjee *et al.,* 2012 [18, 34]. Minocycline pretreated groups showed significant increase ($p < 0.05$) in SDL values, when compared to Ketamine group, whereas Doxycycline pretreated group was not found significant in attenuating Ketamine induced step down latency changes. Combination was found statistically more significant ($p < 0.001$) in increasing step down latency period in comparison to Ketamine, Doxycycline and Minocycline pretreated groups. The changes could be due to probable reduction in CRP and AchE values as accessed in the present study.

Increased levels of reactive oxygen species have detrimental effects on signal transduction, structural plasticity and cellular stability, primarily through membrane lipid peroxidation, protein and nucleic acid damage. [53]. Ketamine induces Schizophrenia by causing oxidative and nitrigenic alteration, and by increasing AchE activity. Ketamine significantly increased malonyladehyde, nitrite levels and decreased level of glutathione, superoxide dismutase and catalase in the brain of mice [17, 44]. In the present study, administration of Ketamine for 21 successive days induced alterations in oxidative stress biomarkers. Ketamine administration increased the levels of brain MDA, brain nitrate, and reduced the GSH level, which were in accordance to studies conducted by Ben-Azu *et al.,* 2018; Naderi *et al.,* 2017; and Chatterjee *et al*., 2012 [17, 44, 54]. Doxycycline and Minocycline pretreated groups showed significant reduction ($p < 0.001$) in MDA and nitrite/nitrate levels and increase in reduced glutathione levels induced by Ketamine. The results were in accordance with the results reported by Antonio *et al*., 2014 and Monte *et al.,* 2013 for antioxidant activity exhibited by Doxycycline and Minocycline [55-56]. Further, combination of Doxycycline and Minocycline significantly ($p < 0.001$) ameliorated Ketamine-induced lipid peroxidation as indicated by reduced MDA and nitrite/nitrate levels, and restored antioxidant GSH values, whereas the combination group was non-significant, when compared to Doxycycline and Minocycline pretreated groups.

Dopamine alters the transmission of Acetylcholine in cortex region of brain, which contributes to cognitive impairment in rodents [57]. Acetylcholinestrease enzyme decreases the concentration of available Acetylcholine at muscranic and nicotinic receptor sites leading to cognitive dys-

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

functioning [58]. Peripheral inflammation may also be connected with cognitive deficits in Schizophrenia and bipolar disorder. According to Maisik *et al*., there is a positive correlation between peripheral levels of cytokines and CRP, cytokine gene polymorphisms, and cognitive function in patients with Schizophrenia and bipolar disorder [59]. It has already been established that Ketamine reduces Acetylcholine concentrations in the hippocampal region of the brain by blocking nAChR, along with an increase in AChE activity associated with cognitive symptoms of psychosis [44, 60]. In present study Ketamine administration for 21 successive days alter AchE levels as observed with their increase. Doxycycline and Minocycline inhibits AchE activity ($p < 0.001$) in Ketamine induced schizophrenic mice, which was in accordance to results reported by Yadav *et al.,* 2017, Ben-Azu *et al*., 2018, and Sharma *et al*., 2010 [17-18, 61]. Combination of Doxycycline and Minocycline showed more significant results ($p < 0.001$) in altering AchE level, when compared to Ketamine group. Combination also showed significant ($p < 0.05$) result, when compared with Minocycline pretreated group, while non-significant results were found in comparison to Doxycycline pretreated group. These results were possibly correlated with increased Acetylcholine levels and reduced CRP and Dopamine levels mediated by Doxycycline and Minocycline.

Animal models show that chronically elevated levels of pro-inflammatory cytokines in the brain can trigger abnormal neural connections in the developing brain, which may contribute to the pathogenesis of psychosis [62]. CRP is a non-specific serum marker of inflammation and elevated blood levels of CRP are observed in Schizophrenia [61-64]. Negative Symptoms and cognitive impairment were also linked with elevated CRP level in Schizophrenia and bipolar disorders correlate increased CRP and cognition in Schizophrenia [28, 59, 63, 65]. Study conducted by Sun *et al.,* 2004 on rat liver suggested increase in the expression of inflammatory cytokines (TNF-α and NF-Kappa β) with high dose (50 mg/kg *i.p.*) of Ketamine [66]. In the present study, administration of Ketamine for 21 successive days induced changes in CRP level, which was in accordance with Sun *et al*, reported results. The anti-inflammatory action was suggested by Di Caprio *et al.,* for Doxycycline [67], and Chen *et al*., and Kloppenburg for Minocycline [68-69]. According to Di Caprio *et al,* low dose Doxycycline modulate inflammatory cytokines and can safely be used as anti-inflammatory in progressive and periodically recurring inflammation [67]. Chen *et al.,* reported that Minocycline can be used as adjunct psychotherapy to antipsychotics in patients with deficit Schizophrenia to control elevated serum levels of inflammatory cytokines [68]. See also Kloppenburg *et al.,* it has been suggested that Minocycline is useful in the treatment of several forms of chronic inflammation because of its different effects on the regulation of cytokine production by T cells and monocytes [69]. In the present study Doxycycline and Minocycline pretreated proved their anti-inflammatory action by attenuating the CRP levels. Combination of Doxycycline and Minocycline (50 mg/kg, *i.p.*) was also found highly significant ($p < 0.001$) in changing Ketamine induced increase serum CRP levels, but combination showed non- significant results in comparison with Doxycycline and Minocycline pretreated groups.

Ketamine produces reliable changes in parameter of dopaminergic, glutamatergic, 5-hydroxytryptaminergic and cholinergic neurotransmission in Schizophrenia. Ketamine increases Dopamine release by increasing glutamate release, which stimulates postsynaptic glutamate receptors other than NMDA associated with impaired dopaminergic neurotransmission in the prefrontal cortex as well as cognitive function, and induces schizophrenic behavior and major neurochemical changes in mice [17, 70-74]. In the present study, Ketamine administered (50 mg/kg, *i.p*.) for 21 successive days showed increase in Dopamine levels, which was in accordance to the above reported studies. Doxycycline showed effective result in revising Ketamine induced increase Dopamine level in schizophrenic mice [18]. Methamphetamine attenuated with Minocycline increased extracellular levels of Dopamine in the nucleus accumbens in mice. Present study results indicated that Doxycycline and Minocycline pretreated group significantly changed Ketamine caused increase of Dopamine level, which was in accordance to the reported studies. Combination Doxycycline and Minocycline (50 mg/kg, *p.o.*) was more effective ($p < 0.001$) in reducing the Dopamine levels than Doxycycline and Minocycline pretreated treatment. This is possibly by due to blocking of excessive release of Dopamine in brain region.

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

Ketamine administered (50 mg/kg, *i.p*.) for 21 successive days showed decrease in GABA levels. Doxycycline has significant effect against Ketamine induced decrease GABA level, which were in accordance to studies reported by Yadav *et al.,* 2017 [18].

In a study of Long-Evans and Sprague-Dawley rats, animals were observed to lose weight 24 hours after anesthesia pretreated with ketamine/xylazine/acepromazine [72-73]. In present study Ketamine (50 mg/kg, *i.p*.) administration for 21 successive days decreased the body weight of mice, when compared to normal group. Pre-treated Doxycycline and Minocycline groups showed significant recovery in body weight, when liked to Ketamine group. Combination of Doxycycline and Minocycline (50 mg/kg, *p.o*.) was also initiated significant in restoring the change of body weight in comparison to Ketamine group.

Histopathological studies have identified a significant reduction in ketamine-induced histopathological changes in the mouse brain. Ketamine (50 mg/kg, *i.p.*) induced histopathological changes such as perinuclear vacuolization, dilated vascular channels, red neurons and hyperchromic nuclei in mouse cerebral cortex similar to those described by Yadav *et al.,* 2017 [18]. Combination of Doxycycline and Minocycline (50 mg/kg, *p.o*.) for 21 succeding days was highly significant in mediating the histological changes induced by Ketamine in mice brain.

CONCLUSION

In conclusion, the combination of Doxycycline and Minocycline was more effective as compared to their pretreated groups in controlling positive, negative and cognitive changes in mice. In future, different combination doses of Doxycycline and Minocycline can be explored as an adjunctive drug for the treatment of Schizophrenia-like behaviors.

Consent for publication

None

Funding

None

Conflict of interest

None

Acknowledgements

Declared none

ABBREVIATIONS

AChE – Acetylcholinesterase
CPCSEA - Committee for the Purpose of Control and Supervision of Experiment on Animals
CRP - C-reactive protein
DTNB - 5,5-dithiobis-(2-nitrobenzoic acid
FST - Forced swimming test
GABA - Gamma-aminobutyric acid
GSH - Reduced glutathione
IP - Intraperitoneally
MDA - Malondialdehyde
NMDA - N-methyl-D-aspartate
PO - Per oral
SDL - Step down latency
TBA - Thiobarbituric acid
TCA - Trichloroacetic acid

REFERENCES

[1]. Picchioni MM and Murray RM. Schizophrenia. BMJ. 2007; 335(7610):91-5. doi: 10.1136/bmj.39227.616447

[2]. Patel KR, Cherian J, Gohil K, Atkinson D. Schizophrenia: Overview and treatment options. P T. 2014; 39(9):638-45.

[3]. Kaneko K. Negative Symptoms and Cognitive Impairments in Schizophrenia: Two Key Symptoms Negatively Influencing Social Functioning. Yonago Acta Med. 2018; 61(2):91-102.

[4]. Carlborg A, Winnerback K, Jonsson EG, Jokinen J, Nordstrom P. Suicide in schizophrenia. Expert Rev Neurother, 2010; 10(7):1153-64. doi: 10.1586/ern.10.82

[5]. Altamura C, Fagiolini A, Galderisi S, Rocca P, Rossi A. Schizophrenia today: Epidemiology, diagnosis, course and models of care. Italian Journal of Psychopathology. 2014; 20(3):223-243.

[6]. Gaur N, Gautam, S, Gaur M, Sharma P, Dadheech, G, Mishra S. The biochemical womb of schizophrenia: A review. Indian J Clin Biochem. 2008; 23(4):307-27. doi: 10.1007/s12291-008-0071-x

e-ISSN 2456-7701
Vol. 4 Issue No. 2, April-June 2022

[7]. Kumar A, Yadav M, Parle M, Dhingra S, Dhull DK. Potential drug targets and treatment of schizophrenia. Inflammopharmacology. 2017; 25(3):277-92. doi: 10.1007/s10787-017-0340-5

[8]. Howes O, McCutcheon R, Stone J. Glutamate and dopamine in schizophrenia: an update for the 21st century. J Psychopharmacol. 2015; 29(2):97-115. doi: 10.1177/0269881114563634

[9]. Ivani G, Vercellino C, Tonetti F. Ketamine: a new look to an old drug. Minerva Anestesiol. 2003; 69(5):468-471.

[10]. Andrade C. Ketamine for Depression, 3: Does Chirality Matter? J Clin Psychiatry. 2017; 78(6):e674-7. doi: 10.4088/JCP.17f11681

[11]. Monte AS, De Souza GC, McIntyre RS, Soczynska JK, Dos Santos JV, Cordeiro RC *et al*. Prevention and reversal of ketamine-induced schizophrenia related behavior by minocycline in mice: Possible involvement of antioxidant and nitrergic pathways. J Psychopharmacol. 2013; 27(11):1032-43. doi: 10.1177/0269881113503506

[12]. Narasimhan M, Bruce TO, Masand P. Review of olanzapine in the management of bipolar disorders. Neuropsychiatr Dis Treat. 2007; 3(5):579-87.

[13]. Carris NW, Pardo J, Montero J, Shaeer KM. Minocycline as A Substitute for Doxycycline in Targeted Scenarios: A Systematic Review. Open Forum Infect Dis. 2015; 2(4):ofv178. doi: 10.1093/ofid/ofv178

[14]. Yong VW, Wells J, Giuliani F, Casha S, Power C, Metz LM. The promise of minocycline in neurology. Lancet Neurol. 2004; 3(12):744-51. doi: 10.1016/S1474-4422(04)00937-8

[15]. Miyaoka T. Clinical potential of minocycline for schizophrenia. CNS Neurol Disord Drug Targets. 2004; 7(4):376-81.

[16]. Mizoguchi H, Takuma K, Fukakusa A, Ito Y, Nakatani A, Ibi D *et al*. Improvement by minocycline of methamphetamine-induced impairment of recognition memory in mice. Psychopharmacology (Berl). 2008; 196(2):233-41. doi: 10.1007/s00213-007-0955-0

[17]. Ben-Azu B, Omogbiya I A, Aderibigbe AO, Umukoro S, Ajayi AM, Iwalewa EO. Doxycycline prevents and reverses schizophrenic-like behaviors induced by ketamine in mice via modulation of oxidative, nitrergic and cholinergic pathways. Brain Res Bull. 2018; 139:114-24. doi: 10.1016/j.brainresbull.2018.02.007

[18]. Yadav M, Parle M, Sharma N, Dhingra S, Raina N, Jindal DK. Brain targeted oral delivery of doxycycline hydrochloride encapsulated Tween 80 coated chitosan nanoparticles against ketamine induced psychosis: behavioral, biochemical, neurochemical and histological alterations in mice. Drug Deliv. 2017; 24(1):1429-40. doi: 10.1080/10717544.2017.1377315

[19]. Levkovitz Y, Levi U, Braw Y, Cohen H. Minocycline, a second-generation tetracycline, as a neuroprotective agent in an animal model of schizophrenia. Brain Res. 2007; 1154:154-62. doi: 10.1016/j.brainres.2007.03.080

[20]. Da Silva FC, Do Carmo de Oliveira Cito M, da Silva MI, Moura BA, De Aquino Neto MR, Feitosa ML *et al*. Behavioral alterations and pro-oxidant effect of a single ketamine administration to mice. Brain Res Bull. 2010; 83(1-2):9-15. doi: 10.1016/j.brainresbull.2010.05.011

[21]. Yadav M, Parle M, Dhingra MS. Protective effect of Brassica oleracea juice against Ketamine-induced stereotypic behaviours in mice. Journal of Medicinal Plants Studies. 2017; 5(1):200-4.

[22]. Chillar R and Dhingra D. Antidepressant-like activity of gallic acid in mice subjected to unpredictable chronic mild stress. Fundam Clin Pharmacol. 2013; 27(4):409-18. doi: 10.1111/j.1472-8206.2012.01040.x

[23]. Soni K and Parle M. *Trachyspermum ammi* seeds supplementation helps reverse scopolamine, alprazolam and electroshock induced amnesia. Neurochem Res. 2017; 42(5):1333-44. doi: 10.1007/s11064-017-2177-0

[24]. Wills ED. The effect of inorganic iron on the thiobarbituric acid method for the determination of lipid peroxides. Biochim Biophys Acta. 1964; 84:475-7. doi: 10.1016/0926-6542(64)90016-2

[25]. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 1982; 126(1):131-8. doi.org/10.1016/0003-2697(82)90118-x

[26]. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82(1):70-7. org/10.1016/0003-9861(59)90090-6

[27]. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7(2):88-95. doi.org/10.1016/0006-2952(61)90145-9

[28]. Schlumpf M, Lichtensteiger W, Langemann H, Waser PG, Hefti F. A fluorimetric-micromethod for the simultaneous determination of serotonin, nor-adrenaline and dopamine in milligram amount of brain tissue. Biochem Pharmacol. 1974; 23(17):2437-46. doi.org/10.1016/0006-2952(74)90235-4

[29]. Lowe IP, RobinsE, Eyerman GS. The fluorimetric measurement of glutamic, decarboxylase measurement and its distributionin brain. J Neurochem. 1958; 3(1):8-18. doi.org/10.1111/j.1471-4159.1958.tb12604.x

[30]. Tomasik J, Rahmoune H, Guest PC, Bahn S. Neuroimmune biomarkers in schizophrenia. Schizophr Res. 2016; 176(1):3-13. doi: 10.1016/j.schres.2014.07.025

[31]. Becker A, Peters B, Schroeder H, Mann T, Huether G, Grecksch G. Ketamine-induced changes in rat behaviour: A possible animal model of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2003; 27(4):687-700. doi: 10.1016/S0278-5846(03)00080-0

[32]. Hou Y, Zhang H, Xie G, Cao X, Zhao Y, Liu Y *et al*. Neuronal injury, but not microglia activation, is associated with ketamine-induced experimental schizophrenic model in mice. Prog Neuropsychopharmacol Biol Psychiatry. 2013; 45:107-16. doi: 10.1016/j.pnpbp.2013.04.006

[33]. Geyer MA and Moghaddam B. Animal models relevant to schizophrenia disorders. Neuropsychopharmacology: The Fifth Generation of Progress. 2002; 689-702.

[34]. Chatterjee M, Singh S, Kumari R, Verma AK, Palit G. Evaluation of the antipsychotic potential of Panax quinquefolium in ketamine induced experimental psychosis model in mice. Neurochem Res. 2012; 37(4):759-70. doi: 10.1007/s11064-011-0670-4.

[35]. Canever L, Oliveira L, D'Altoe de Luca R, Correa PT, De B Fraga D, Matos MP *et al*. A rodent model of schizophrenia reveals increase in creatine kinase activity with associated behavior changes. Oxid Med Cell Longev. 2010; 3(6):421-7.

[36]. Hunt MJ, Olszewski M, Piasecka J, Whittington MA, Kasicki S. Effects of NMDA receptor antagonists and antipsychotics on high frequency oscillations recorded in the nucleus accumbens of freely moving mice Psychopharmacology (Berl). 2015; 232(24):4525-35. doi: 10.1007/s00213-015-4073-0

[37]. Ben-Azu B, Omogbiya IA, Aderibigbe AO, Umukoro S, Ajayi AM, Eneni AO *et al.* Doxycycline Ameliorates Schizophrenia-Like Behaviors in Experimental Models in Mice by Targeting Underlying Oxidative Stress. Journal of Behavioral and Brain Science. 2016; 6:539-62. doi: 10.4236/jbbs.2016.613048.

[38]. Zhang L and Zhao J. Profile of minocycline and its potential in the treatment of schizophrenia. Neuropsychiatr Dis Treat. 2014; 10:1103-1111. doi: 10.2147/NDT.S64236.

[39]. Ridley RM. The psychology of perserverative and stereotyped behaviour. Prog Neurobiol. 1994; 44(2):221-31.

[40]. Kadian R and Parle M. Evaluation of Terminalia bellerica for its antipsychotic potential. International Journal of Pharmaceutical Sciences Review and Research. 2015; 30:247-52.

[41]. Razoux F, Garcia R, Lena I. Ketamine, at a dose that disrupts motor behavior and latent inhibition, enhances prefrontal cortex synaptic efficacy and glutamate release in the nucleus accumbens. Neuropsychopharmacology. 2007; 32(3):719-27. doi: 10.1038/sj.npp.1301057

[42]. Sotoing Taiwe G, Ngo Bum E, Talla E, Dawe A, Okomolo Moto FC, Temkou Ngoupaye G *et al.* Antipsychotic and sedative effects of the leaf extract of Crassocephalum bauchiense (Hutch.) Milne-Redh (Asteraceae) in rodents. J Ethnopharmacol. 2012; 143(1):213-20. doi: 10.1016/j.jep.2012.06.026

[43]. Dokuyucu R, Kokacya H, Inanir S, Copoglu US, Erbas O. Antipsychotic-like effect of

minocycline in a rat model. Int J Clin Exp Med . 2014; 7(10):3354-61.

[44]. Chatterjee M, Singh S, Kumari R, Verma AKL, Palit G. Evaluation of the Antipsychotic Potential of Panax quinquefolium in Ketamine Induced Experimental Psychosis Model in Mice. Neurochemical Research. 2012; 37(4):759-70. doi: 10.1007/s11064-011-0670-4

[45]. Noda Y, Yamada K, Furukawa H, Nabeshima T. Enhancement of Immobility in a Forced Swimming Test by Subacute or Repeated Treatment with Phencyclidine: A New Model of Schizophrenia. Br J Pharmacol. 1995; 116(5):2531-7. doi: 10.1111/j.1476-5381.1995.tb15106.x

[46]. Chindo BA, Adzu B, Tijani AY, Karniyus SG. Ketamine-Enhanced Immobility in Forced Swim Test: A Possible Animal Model for the Negative Symptoms of Schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2012; 38(2):310-6. doi: 10.1016/j.pnpbp.2012.04.018

[47]. Fan X, Pristach C, Liu EY, Freudenreich O, Henderson DC, Goff DC. Elevated serum levels of C-reactive protein are associated with more severe psychopathology in a subgroup of patients with schizophrenia. Psychiatry Res. 2007; 149(1-3):267-71. doi: 10.1016/j.psychres.2006.07.011

[48]. Liu F, Guo X, Wu R, Ou J, Zheng Y, Zhang B *et al.* Minocycline supplementation for treatment of negative symptoms in early-phase schizophrenia: a double blind, randomized, controlled trial. Schizophr Res. 2014; 153(1-3):169-76. doi: 10.1016/j.schres.2014.01.011

[49]. Saeedi Saravi SS, Amirkhanloo R, Arefidoust A, Yaftian R, Saeedi Saravi SS, Shokrzadeh M *et al.* On the effect of minocycline on the depressive-like behavior of mice repeatedly exposed to malathion: interaction between nitric oxide and cholinergic system. Metab Brain Dis. 2016; 31(3):549-61. doi: 10.1007/s11011-015-9764-z

[50]. Lewis DA, Lieberman JA. Catching up on schizophrenia: natural history and neurobiology. Neuron. 2000; 28(2):325-34. doi: 10.1016/s0896-6273(00)00111-2

[51]. Sharma AC and Kulkarni SK. Evaluation of learning and memory mechanisms employing elevated plus-maze in rats and mice. Prog Neuropsychopharmacol Biol Psychiatry. 1992; 16(1):117-25.

[52]. Parle M, Dhingra D, Kulkarni SK. Memory-strengthening activity of Glycyrrhiza glabra in exteroceptive and interoceptive behavioral models. J Med Food. 2004; 7(4):462-6.

[53]. Mahadik SP, Evans D, Lal H. Oxidative stress and role of antioxidant and omega-3 essential fatty acid supplementation in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2001; 25(3):463-93.

[54]. Naderi Y, Sabetkasaei M, Parvardeh S, Zanjani TM. Neuroprotective effect of minocycline on cognitive impairments induced by transient cerebral ischemia/reperfusion through its anti-inflammatory and anti-oxidant properties in male rat. Brain Res Bull. 2017; 131:207-13. doi: 10.1016/j.brainresbull.2017.04.010

[55]. Antonio RC, Ceron CS, Rizzi E, Coelho EB, Tanus-Santos JE, Gerlach RF. Antioxidant effect of doxycycline decreases MMP activity and blood pressure in SHR. Mol Cell Biochem. 2014; 386(1-2):99-105. doi: 10.1007/s11010-013-1848-7

[56]. Monte AS, De Souza GC, McIntyre RS, Soczynska JK, Dos Santos JV, Cordeiro RC *et al.* Prevention and reversal of ketamine-induced schizophrenia related behavior by minocycline in mice: Possible involvement of antioxidant and nitrergic pathways. J Psychopharmacol. 2013; 27(11):1032-43. doi: 10.1177/0269881113503506

[57]. Sarter M and Bruno JP. Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. Brain Res Brain Res Rev. 1997; 23(1-2):28-46.

[58]. Samochocki M, Hoffle A, Fehrenbacher A, Jostock R, Ludwig J, Christner C *et al.* Galantamine is an allosterically potentiating ligand of neuronal nicotinic but not of muscarinic acetylcholine receptors. J Pharmacol Exp Ther. 2003; 305(3):1024-36. doi: 10.1124/jpet.102.045773

[59]. Misiak B, Stanczykiewicz B, Kotowicz K, Rybakowski JK, Samochowiec J, Frydecka D. Cytokines and C-reactive protein alterations with respect to cognitive impairment in

schizophrenia and bipolar disorder: A systematic review. Schizophr Res. 2018; 192:16-29. doi: 10.1016/j.schres.2017.04.015

[60]. Zhang Y, Sha R, Wang K, Li H, Yan B, Zhou N. Protective effects of tetrahydropalmatine against ketamine-induced learning and memory injury via antioxidative, anti-inflammatory and anti-apoptotic mechanisms in mice. Mol Med Rep. 2018; 17(5):6873-80. doi: 10.3892/mmr.2018.8700

[61]. Sharma V, Goyal A, Sharma N, Ganti SS. Amelioration of intracerebroventricular Streptozotocin induced Cognitive Dysfunction by Minocycline: A Behavioral study. Journal of Pharmacy Research. 2010; 3(5):938-42.

[62]. Boozalis T, Teixeira AL, Cho RY, Okusaga O. C-Reactive Protein Correlates with Negative Symptoms in Patients with Schizophrenia. Front Public Health. 2018; 5:360. doi: 10.3389/fpubh.2017.00360

[63]. Fernandes BS, Steiner J, Bernstein HG, Dodd S, Pasco JA, Dean OM *et al.* C-reactive protein is increased in schizophrenia but is not altered by antipsychotics: meta-analysis and implications. Mol Psychiatry. 2016; 21(4):554-64. doi: 10.1038/mp.2015.87

[64]. Miller BJ, Culpepper N, Rapaport MH. C-reactive protein levels in schizophrenia: a review and meta-+analysis. Clin Schizophr Relat Psychoses. 2014; 7(4):223-30. doi: 10.3371/CSRP.MICU.020813

[65]. Zhu J, Hu W, Zhou Y, Qiao J, Chang X, Tong Z. Serum high-sensitivity C-reactive protein levels are positively associated with cognitive impairments in patients with first-episode schizophrenia. Compr Psychiatry. 2019; 94:152118. doi: 10.1016/j.comppsych.2019.152118

[66]. Sun J, Li F, Chen J, Xu J. Effect of ketamine on NF-kappa B activity and TNF-alpha production in endotoxin-treated rats. Ann Clin Lab Sci. 2004; 34(2):181-6.

[67]. Di Caprio R, Lembo S, Di Costanzo L, Balato A, Monfrecola G. Anti-inflammatory properties of low and high doxycycline doses: an in vitro study. Mediators Inflamm. 2015; 2015:329418. doi: 10.1155/2015/329418

[68]. Chen X, Xiong Z, Li Z, Yang Y, Zheng Z, Li Y *et al*. Minocycline as adjunct therapy for a male patient with deficit schizophrenia. Neuropsychiatr Dis Treat. 2018; 14:2697-2701. doi: 10.2147/NDT.S179658

[69]. Kloppenburg M, Brinkman BM, De Rooij-Dijk HH, Miltenburg AM, Daha MR, Breedveld FC *et al.* The tetracycline derivative minocycline differentially affects cytokine production by monocytes and T lymphocytes. Antimicrob Agents Chemother. 1996; 40(4):934-40.

[70]. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci. 1997; 17(8):2921-7.

[71]. Lai CC, Lee LJ, Yin HS. Behavioral and neurochemical changes induced by repetitive combined treatments of ketamine and amphetamine in mice. Neurochem Res. 2014; 39(11):2180-8. doi: 10.1007/s11064-014-1419-7

[72]. Singh S, Rani P, Khanna D. Exploring Genetic, Pharmacological, Behavioral and Neurodevelopmental Animal Models for Obsessive Compulsive Disorder. Journal of Science and Technological Researches. 2021 December; 3(4):16-23. doi: 10.51514/JSTR.3.4.2021.16-23.

[73]. Malhosia, A., Singh, N., & Singh, S. M. (2022). Determination of Therapeutic Effect of Fenugreek Seed on the Patients Suffering from Type II Diabetes Mellitus. Issues and Developments in Medicine and Medical Research Vol. 4, 120-127.

[74]. R. Kumar, N. Singh, GK Tripathi, SP Singh, A Govindan, M. Chavali (2022), A Survey on Smart Sensors Drivers and Gas Detection Technologies, Nanomedicine & Nanotechnology Open Access 7 (1), 1-8.

[75]. Welberg LA, Kinkead B, Thrivikraman K, Huerkamp MJ, Nemeroff CB, Plotsky PM. Ketamine-xylazine-acepromazine anesthesia and postoperative recovery in rats. J Am Assoc Lab Anim Sci. 2006; 45(2):13-20.