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Research Article

PROTECTIVE EFFECT OF CILOSTAZOL AGAINST KETAMINE-INDUCED BIOCHEMICAL AND BEHAVIORAL PHENOTYPE OF SCHIZOPHRENIA IN MICE

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ABSTRACT

Objective: Schizophrenia (SCZ), a mental illness affecting 1% of the world population, is characterized by extensive structural and functional brain changes. It is brought on by a confluence of psychological, environmental, and hereditary variables. It is frequently coexisted with other diseases, lowering the quality of life and increasing the risk of early death. The objective of this research is to explored the potential of cilostazol (Phosphodiesterase-3 inhibitor) in ketamine (KET)-induced SCZ -like behavioral and biochemical alterations in mice.

Methods: In mice, SCZ was induced by injecting KET (30 mg/kg; i.p.) for 10 days in a row. Different behavioral parameters such as immobility time (Forced swim test), locomotor and anxiety (open field test), cognitive dysfunction (Morris water maze), social interactions, and catalepsy were examined. Histopathological and biochemical changes (lipid peroxides, glutathione [GSH], acetylcholinesterase [AChE] activity) were also examined. Cilostazol (25 and 50 mg/kg; p.o.) as a test and clozapine (7.5 mg/kg p.o.), as a standard drug were used in this investigation. Tukey's multiple comparison test and one-way analysis of variance were used for statistical analysis of all the findings. $p<0.050$ was regarded as statistically significant.

Results: Significant ($p<0.05$) behavioral changes have been observed following 28 days of KET treatment (increased immobility time, impaired locomotor and anxiety-like behaviors, cognitive dysfunction, social interactions, and catalepsy). Increased oxidative stress (higher lipid peroxides and decreased GSH), AChE activity, and histopathological changes were also noted significantly in KET -treated mice. Cilostazol and clozapine treatment significantly ($p<0.05$) corrected the histological changes, biochemical alterations, and behavioral problems.

Conclusion: As per the behavioral, histopathological, and biochemical outcomes, we can draw a conclusion that cilostazol may provide neurodefensive effects against KET -induced SCZ in mice.

Keywords: Social withdrawal, Phosphodiesterase, Oxidative stress, Cognition, Immobility time, Catalepsy, Lipid peroxidation.

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INTRODUCTION

A significant mental illness called schizophrenia's (SCZ) lifetime prevalence affecting 1% of the world population [1]. It is extremely expensive for healthcare systems and can have disastrous effects on patients as well as caregivers [2]. According to the epidemiology, SCZ is quite common and typically coexists with other diseases, which lowers quality of life and increases the risk of early death [3,4]. SCZ is brought on by a confluence of psychological, environmental, and genetic variables. This psychological disorder is characterized by extensive structural and functional brain changes, the etiology of which is still poorly known [5]. Positive, negative, and cognitive are the three main types of symptoms that are associated with SCZ [6]. Positive symptoms are the most easily includes hallucinations, suspiciousness, and delusions [7,8]. Deficiencies in attention, avolition, alogia, and anhedonia are examples of mental or emotional functioning deficiencies that characterize negative symptoms [9]. Cognitive symptoms include difficulties in concentrating and recalling things, poor working memory and executive function, as well as disordered speech or ideas [10].

Ketamine (KET) is a dissociative anesthetic, and antagonize N-methyl-D-aspartate (NMDA) receptors [11]. Specifically, KET causes negative symptoms such as reduced emotional response and sociality in addition to positive effects such as delusions and perception abnormalities [12]. In mice, SCZ was induced by injecting KET for 10 days (30 mg/kg; i.p.) in a row. Phosphodiesterases (PDEs) are produced by 11 different PDE families and splice variants (PDE1 to PDE11) in mammals, which are expressed in various regions such as the brain and spinal cord [13]. Cilostazol is a selective PDE-3 inhibitor and alleviates depleted antioxidant status, nitrite content, cholinergic dysfunction, and cerebral inflammation in addition to regulating motor coordination, mood, learning, and memory [14,15]. PDE inhibitors are presently being studied as potential antidementia antidepressants, memory enhancers, and anti-psychotics, medications [16-18]. On the other hand, there are no evidence regarding the possible therapeutic use of the PDE-3 inhibitor (cilostazol) in the management of SCZ. On the basis of the above discussion, it may be hypothesized that PDE-3 inhibition may provide beneficial effects against KET-provoked SCZ in mice.

MATERIALS AND METHODS

Experimental animal

The Swiss albino mice used for the experiments were male 25–30 g animals. The animals were housed at 25±2°C and 55±5% humidity in polypropylene cages in an adequate ventilated room with a light/dark cycle of 12:12. Water and standard pellet meal were given to the animals on an as-needed basis. One week before the tests started, the animals were acclimated to the lab environment. The Institutional Animal Ethics Committee regulations were followed to ensure that experimental animals did not suffer throughout the process.

Chemical/reagents

Cilostazol taken from Lupin Ltd, India. The rest of the chemicals/reagents of standard analytical grade were arranged from standard vendors.

Experimental protocol

Before being used, all medications of conventional analytical quality and pharmacological fresh solutions have been prepared. Based on reports that have already been published, dosages and a dosing regimen were chosen. To investigate PDE-3 receptor modulator in KET-induced SCZ, seven groups in all had been used in this research and each group having 6 animals.

Group-I- normal: Mouse was administrated with a dose of 0.9% of normal NaCl (10 mL/kg; i.p) for 28 days. Group II- Vehicle: Each mouse was treated with a dose of 0.5% carboxymethylcellulose for 28 days. Group III- Drug perse: Each mouse was administered with a dose of the treatment drug cilostazol (50 mg/kg; p.o) for 28 days. Group IV- KET: Each mouse was administered with KET a dose (30 mg/kg i.p.) for 10 consecutive days followed by behaviors and biochemical assessment on 28 days. Group V- KET+cilostazol (25 mg/kg; p.o.): Mouse was treated with KET for 10 consecutive days along with the cilostazol from the 10^{th} to 28^{th} day. Group VI- KET+cilostazol (50 mg/kg; p.o.): Each mouse was treated with KET for 10 consecutive days along with the cilostazol from the 10^{th} to 28^{th} day. Group VII- KET + clozapine (7.5 mg/kg p.o.): Each mouse was administrated with a dose of KET along with the standard drug clozapine from the 10^{th} day to 28^{th} day.

Behavioral assessment

Forced swim test (FST)

Each mouse was made to swim in a circular, open glass chamber measuring 15 cm diameter and 25 cm height, and maintained a steady temperature of 25±1°C and filled with fresh water up to that height. Animals could not maintain themselves at this level of water by using their tails or hind feet to contact the chamber's side walls or bottom. After each animal had FST, the water in the chamber was replaced because used water changes an animal's behavior. The animal swims for the first 2 min with strong motions. During the last 4 min of the 6-min swimming session, the immobility period was noted and then examined [19,20].

Open field test (OFT)

Rodents' exploratory skills, anxiety levels, and locomotor activity were measured using open-field tests. The OFT apparatus was made of non-porous, white, high-density plastic, and its square chamber was 50 cm wide, 38 cm high, and 50 cm long. Mice were positioned individually at the center of the field and permitted to wander about freely for 5 min after becoming accustomed to one another. A number of variables were noted, including the amount of time spent in the middle square and the overall distance traveled within the chamber. To get rid of any fragrance traces left by the previous subject mouse, the chamber was cleaned with 95% ethanol before being used and before any more experiments [21].

Morris water maze test (MWM)

MWM tests were utilized to evaluate memory and learning. Mice were led to an underwater platform (10 cm) that was immersed in a circular pool filled with water (45 cm in height and 150 cm in circumference) and colored with white colorant. Two cotton strands were used to create four even quadrants in a chamber, with a hidden underwater platform held 1 cm below the water. The subaquatic platform remained constant throughout the exercise phases, with a single mouse subjected to four daily tests with varying sinking locations. Animals were tracked on an immersed platform for 2 min, staying for 20 seconds. If unsuccessful, mice were directed to the stand and allowed to sit for 20 s. The 4^{th} test day's escape latency time (ELT) was used to measure learning, with Q4 being a target section in all acquisition trials. Mice were removed from the underwater stand and given 2 min to inspect the chamber. Four trials were done from different quadrants, with spending time in the target quadrant indicating memory. The experiment was maintained at a fixed location in the working area, and after evaluating ELT, animals underwent global cerebral ischemia and reperfusion, followed by a retrieval test on the 5^{th} day [22,23].

Social withdrawal test

A rectangular, three-chamber box made up the equipment for the social withdrawal test. Every compartment was 19 by 45 cm and had an open middle portion that permits unrestricted access to every chamber. The separating walls were composed of transparent plexiglas. Two identical wire-cup-shaped containers that could accommodate a single mouse each had detachable lids. One of these is positioned vertically inside the device in each side chamber, holding the inexperienced or unfamiliar mouse. Each container was made of tiny metal wires that allow air to circulate between the cylinder's interior and exterior without obstructing direct physical contact between an animal inside and an animal outside. General lighting in the room, observation, and follow-up factors were recorded. Used 70% ethanol to clean every chamber after every experiment [24].

Catalepsy bar test

The height of the bar test was 12 cm. Mice were positioned carefully, with their hind limbs on the apparatus's floor and their forelimbs on the bar. A stopwatch was used to time how long it took the mice to remove both of their paws off the bar so that the automated measurements could be compared. Before injection, baseline readings using the test and standard drugs were obtained [25].

Biochemical estimation

The animal's brain was removed cautiously. The sample was mixed in a normal phosphate buffer (0.10 M) employing PT 1600 E Polytron homogenizer at 4°C temp. The aliquots were isolated for biochemistry [22].

Assessment of thiobarbituric acid reactive species (TBARS)

The technique reported by Ohkawa in 1979 was used to assess TBARS spectrophotometrically (ultraviolet [UV]-1800 ENG 240V at 532 nm). After pipetting out the supernatant in a tube, 0.20 mL of sodium dodecyl sulfate (8.1%), 1.50 mL of acetic acid (30% of pH 3.5), and 1.50 mL of 0.8% thiobarbituric acid were mixed together. After adding distilled water to keep the content to 4 mL, the mixture was incubated for 1 h at 95°C before being allowed to cool. After cooling, distilled water (1 mL) and (5 mL of a 15:1 v/v) butanol-pyridine combination were combined. For 10 min, these tubes were rotated at 4000 g. It was mentioned that the emerging pinkish color was absorbent [22,26].

Assessment of reduced glutathione (GSH) contents

Decreased GSH was quantified using the methodology Beutler (1963) published. Using a spectrophotometer, absorbance was assessed at 412 nm. To the supernatant, 10% w/v trichloroacetic acid has been added in a 1:1 ratio. 1/2 mL of supernatant was used to add 2.0 mL of sodium hydrogen phosphate (0.30 M) and 0.25 mL of 0.001 M of (5, 5-dithiobis (2-nitro-benzoic acid-[DTNB]) in 1% w/v of sodium citrate]. A standard plot was made using GSH that has been reduced (10–100 μM) [27].

AChE assay in brain

Spectrophotometric measurements of the AChE were made using Ellman techniques at 420 nm. Thiocholine took on a yellow hue when it interacted with dithiobisnitro-benzoate. I filled a 25 mL flask with the 0.5 mL of supernatant. For making dilutions, DTNB solution was added. A total of 4.0 mL were divided into two different test tubes. In a single tube, the eserine solution was blended. Both tubes were then filled with 1.0 mL of the substrate solution. The blank tube was taken to contain eserine drops [28,29].

Valuation of total protein

Cerebral protein was quantified at 750 nm by UV-1800 (Shimadzu Corporation, Japan), utilizing Lowry's technique [30,31]. 0.150 mL (Supernatant)+1000 μL (Lowry's reagent) was held (15 min). 500 μL (Folin's phenol) was blended, shaken strongly, and subsequently, it was kept alone (½ h). The blue-purplish complex appeared by the collision of phenol and tyrosine with Folin chemicals and sodium tungstate molybdate [22].

Histopathological examination

Hematoxylin and Eosin (H and E) staining

Sections of histology can be diagnosed using (H and E) staining, a popular technique used by pathologists that combines the two dyes. It takes sodium iodate to oxidize hematoxylin into hematein. Purple staining of nuclei, mitochondria, and ribosomes is caused by hemalums, a combination of hematein and aluminum alum. After the stain is blued, it turns that color. Neuronal damage, pyknotic, and shrunken cell was evaluated using H and E staining. The slices were viewed at a magnification of ×400 using a polarized light microscope [22,32].

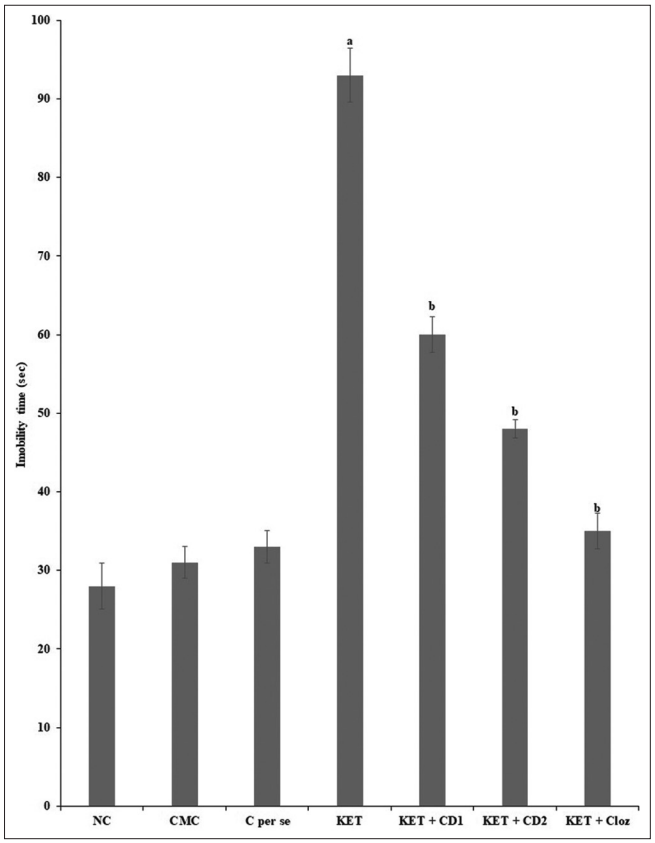

Fig. 1: Effect of cilostazol on immobility time. FST: Forced swim test, NC: Normal saline (0.9% NaCl), CMC: 0.5% carboxy methyl-cellulose, CTZ: cilostazol, KET: ketamine, Cloz: Clozapine, D1: Dose 1, D2: Dose 2

Statistical analysis

The findings were presented in the form of mean±standard error of means. Tukey's multiple comparison test and one-way analysis of variance were used for statistical analysis of all the findings. A value of $p<0.050$ was considered to be significant in terms of statistics. Version 5 of Graph Pad Prism was utilized for all statistical analyses.

RESULTS

Effect of cilostazol on immobility time

In the FST test, longer periods of immobility (depressive behavior), were observed in KET -SCZ mice. A remarkable ($F [6, 35]=108.18$, $^{a}p<0.050$ in contrast to control; $^{b}p<0.05$ in contrast to KET treated animals) immobility time was shown to have decreased in cilostazol and clozapine treated animals as compared to SCZ animals alone (Fig. 1).

Effect of cilostazol on anxiety and locomotory behaviors

In OFT trials, locomotor activity was significantly high in the KET group, in comparison to control ($p<0.05$), and treatment with cilostazol and clozapine significantly declines line crossing ($F [6, 35]=58.913$, $p<0.05$) and locomotor ($F [6, 35]=29.83$, $p<0.05$] movement as compared to the KET. On the other hand, KET injection led to a significant decline in time spent in the center for SCZ mice compared to control ($p<0.05$). Further, cilostazol and clozapine reversed the anxiogenic behaviors in mice as provoked by KET ($p<0.05$) as compared to the SCZ mice (Fig. 2a and b).

Effect of cilostazol on ELT and time spent in target quadrant (TSTQ)

Comparing day 1 ELT, MWM trials' higher day 4 ELT is interpreted as a failure in learning or acquisition. On the 6th day, however, a decreased TSTQ is indicative of memory impairment (Gupta *et al*., 2024). The control group showed a remarkable ($p<0.05$) declined in day 4 ELT in contrast to the 1st day of ELT, as well as a noteworthy increase in the 5th day TSTQ (in the Q4 quadrant) compared to the other Q1, Q2, and Q3 quadrants, that represent normal learning and memory. KET (30 mg kg^{-1}; i.p) treated mice showed a considerable drop in TSTQ on

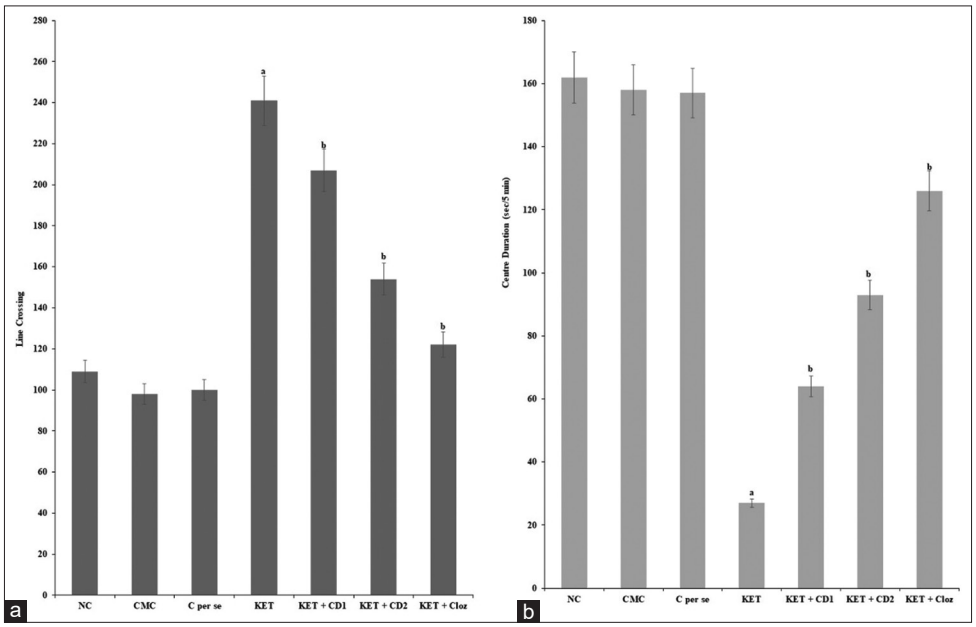

Fig. 2: (a and b) Effect of cilostazol on anxiety and locomotory behaviors. OFT: open field test, NC: Normal saline (0.9% NaCl), CMC: 0.5% carboxy methyl-cellulose, CTZ: Cilostazol, KET: Ketamine, Cloz: Clozapine, D1: Dose 1, D2: Dose 2

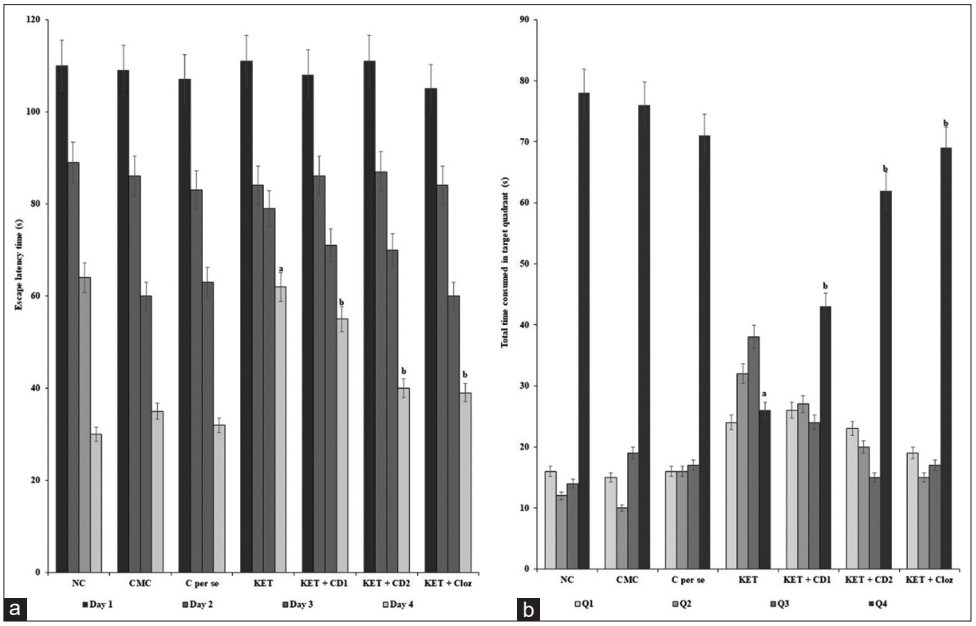

Fig. 3: (a and b) Effect of cilostazol on ELT and TSTQ. ELT: Escape latency time, TSTQ: Time spent in target quadrant, NC: Normal saline (0.9% NaCl), CMC: 0.5 % carboxy methyl-cellulose, CTZ: Cilostazol, KET: Ketamine, Cloz; Clozapine, D1: Dose 1, D2: Dose 2

day five and a marked increase in ELT on day four, in contrast to control mice showing diminished memory and learning. Cilostazol (25 and 50 mg/kg, p.o) and clozapine (7.5 mg/kg; p.o) treatment considerably reduced 4th day ELT (F [6, 35]=53.79, p<0.05), along with elevated TSTQ on the fifth day (in Q4) (F [6, 35]=98.60, p<0.050), in contrast to KET animals, indicating the mitigation of KET -SCZ) provoked a reduction in cognitive function (Fig. 3a and b).

Effect of cilostazol on social withdrawal

In the social withdrawal test, KET significantly (F [6, 35]=103.45, p<0.05) decreased the number of interactions with other animals in comparison to control (p<0.05), whereas treatment with cilostazol and clozapine remarkably (p<0.05) improved the number of interactions with other animals in contrast to KET treated mice (Fig. 4).

Effect of cilostazol on catalepsy

(Fig. 5) Exhibits the effect of cilostazol on cataleptic behavior as assessed by descent latency (DL) from the bar. The administration of cilostazol (25 and 50 mg/kg, p.o) failed to enhance the DL of the experimental mice relative to control animals, which suggests that the anti-psychotic properties of cilostazol are absent from extrapyramidal side effects. However, clozapine (7.5 mg/kg; p.o) administered to animals exhibited marked (p<0.05) rise in DL of the bar test in contrast to animals used as controls. This confirms that clozapine has anti-psychotic ability.

Effect of cilostazol on oxidative stress

According to the (Fig. 6a), the increased concentration of TBARS was noticed in the KET group. The levels of TBARS in KET-treated animals were significantly (F [6, 35]=83.623, p<0.05) greater than that of control animals. Cilostazol and mice treated with clozapine exhibited a considerable (p<0.05) reduction in TBARS levels in relation to KET-administered animals. Fig. 6b demonstrated that the GSH content was markedly (F [6, 35]=51.253, p<0.050) decreased in KET-treated animals in contrast to control animals. Treatment with cilostazol and clozapine considerably (p<0.05) increased GSH content in comparison to the KET group.

Effect of cilostazol on AChE activity

Activity of AChE was considerably (F [6, 35]=101.35, p<0.05) greater in KET-treated mice in contrast to control animals; whereas treatment with cilostazol and clozapine considerably (p<0.05) reduced (p<0.050) AChE activity in contrast to the KET group (Fig. 7).

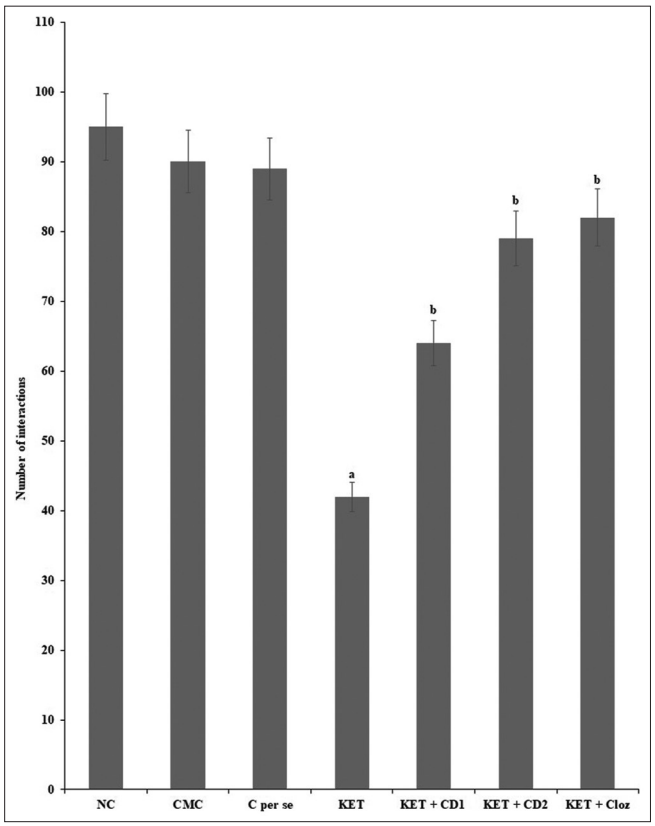

Fig. 4: Effect of cilostazol on social withdrawal. NC: Normal saline (0.9% NaCl), CMC: 0.5% carboxy methyl-cellulose, CTZ: Cilostazol, KET: Ketamine, Cloz: Clozapine, D1: Dose 1, D2: Dose 2

Effect of cilostazol on histopathology

In comparison to the normal structural design of the control animal, the cortex of the KET-administered animals had critical vacuolation and dead cells exhibits nuclear pyknosis and lessened cell bodies. The administration of cilostazol and clozapine along with KET, diminished the incidence of such alterations in the cerebral cortex of

the experimental animals and exhibited the near to normal structural design in contrast with the KET-induced SCZ in mice (Fig. 8).

Representative photographs of H and E stained (magnification ×400) in the cerebral cortex area. Neuroprotective effects of cilostazol on neuronal death induced by KET administration. Normal neurons in the cortex, exhibited regulatory round and bright blue nuclei, whereas dying or dead cells showed pyknotic nuclei and shrunken cell bodies. Arrows signify the pyknotic and shrunken neuronal cells, uneven shaped and tangled cells.

(a) KET group: dead cells showed shrunken cell bodies and nuclear pyknosis
(b) KET+cilostazol dose 1: Moderate shrunken cell bodies and nuclear pyknosis in comparison to KET
(c) KET+cilostazol dose 2: Numbers of dead cells are decreased with mild shrunken cell bodies and nuclear pyknosis in comparison to KET group
(d) KET+clozapine: neurons are appeared normal in the cortex, exhibited regulatory round and bright blue nuclei.

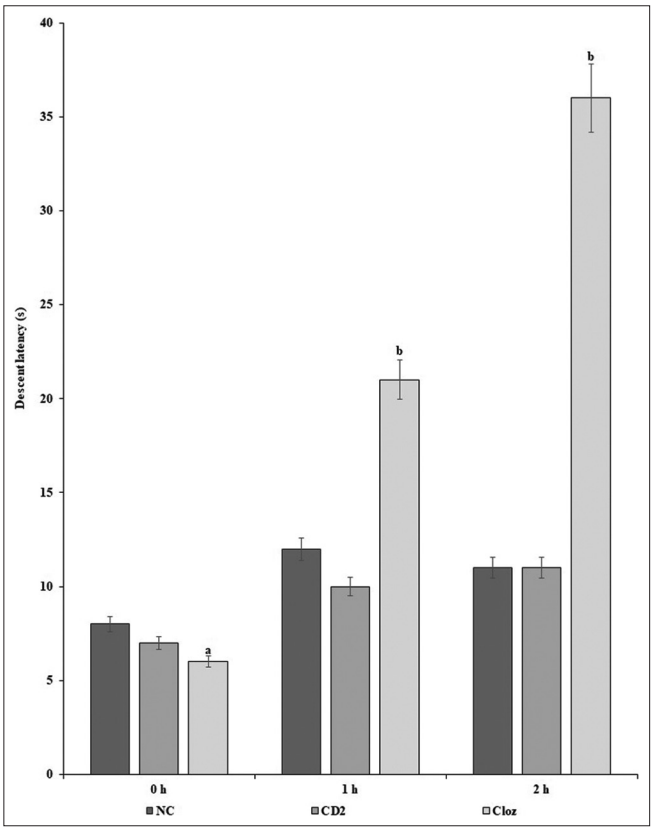

Fig. 5: Effect of cilostazol on catalepsy. NC: Normal saline (0.9% NaCl), C: Cilostazol, Cloz: Clozapine, D2: Dose 2

DISCUSSION

SCZ is an ongoing neurological disease that strikes people of all ages by changing their capacity for clear perception, anticipation, and behavior. Unluckily, current therapies for SCZ do not cure the condition and are linked to undesirable negative consequences. It encourages researchers to find new targets for the treatment of SCZ [33]. Targeting the NMDA receptor, also known as a glutamate receptor, is a more recent method of treating SCZ that is linked to the disease's etiology [34]. Since there is proof that inflammation and oxidative stress may contribute to the development of SCZ, the drug development that focuses on inflammation and oxidative paradigm is another area of study [35,36]. Here, we examine the impact of cilostazol (PDE-3 inhibitor), which may provide protection against (KET)-induced SCZ-like symptoms in mice. In this study, immobility time (depressive behavior) was assessed by FST, locomotion, and anxiety-like behaviors by open field test. In addition, MWM and social withdrawal tests were also used to test spatial learning and social interaction in mice. The mice that were given KET showed signs of increased immobility duration, impaired locomotor and anxiety behaviors, social isolation, and cognitive decline. Reduced GSH content, elevated AChE activity, and TBARS in the brain were also noticed in KET mediated SCZ-like phenotype in mice. The present findings confirm that the KET-treated mice exhibit behavioral, and biochemical alterations along with an increase in oxidative damage in the brain [37]. Treatment with cilostazol (25 and 50 mg/kg; p.o.) was successful in healing KET-induced oxidative damage (lower lipid peroxides, and enhanced GSH activities), above behavioral and histopathological alterations in mice. The results of this investigation validate that cilostazol treatment in KET-induced experimental SCZ decreases social interaction,

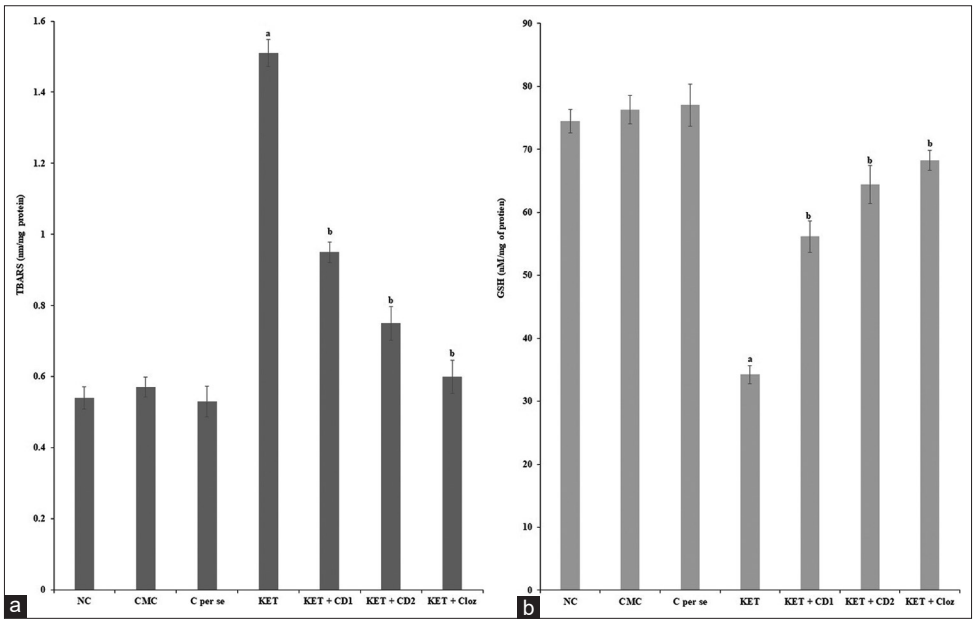

Fig. 6: (a and b) Effect of cilostazol on oxidative stress. TBARS: Thiobarbituric acid reactive substances, GSH: Glutathione, NC: Normal saline (0.9% NaCl), CMC: 0.5% carboxy methyl-cellulose, CTZ: Cilostazol, KET: Ketamine, Cloz: Clozapine, D1: Dose 1, D2: Dose 2

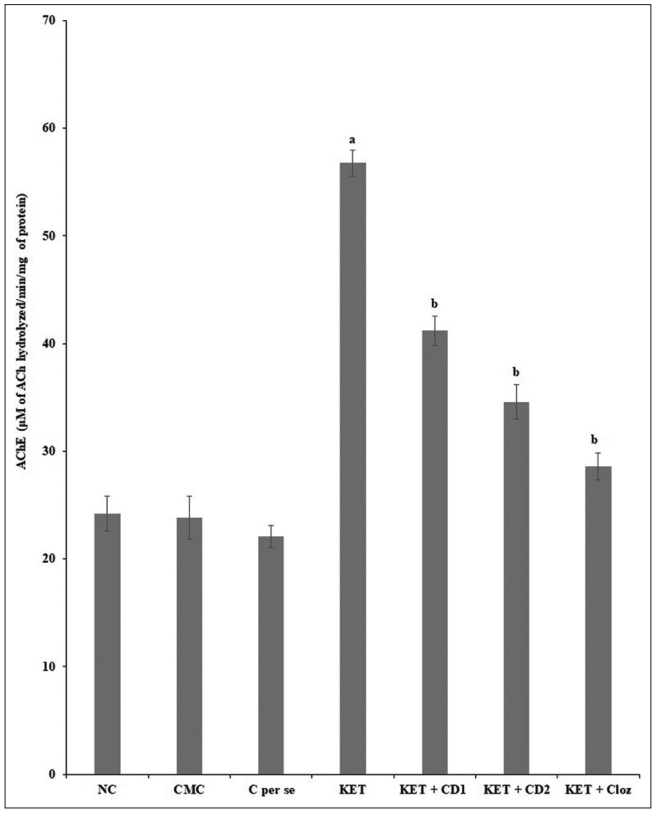

Fig. 7: Effect of cilostazol on AChE activity. AChE: Acetylcholinesterase, ACh: Acetylcholine, NC: Normal saline (0.9% NaCl), CMC: 0.5% Carboxy methyl-cellulose, CTZ: Cilostazol, KET: Ketamine, Cloz: Clozapine, D1: Dose 1, D2: Dose 2

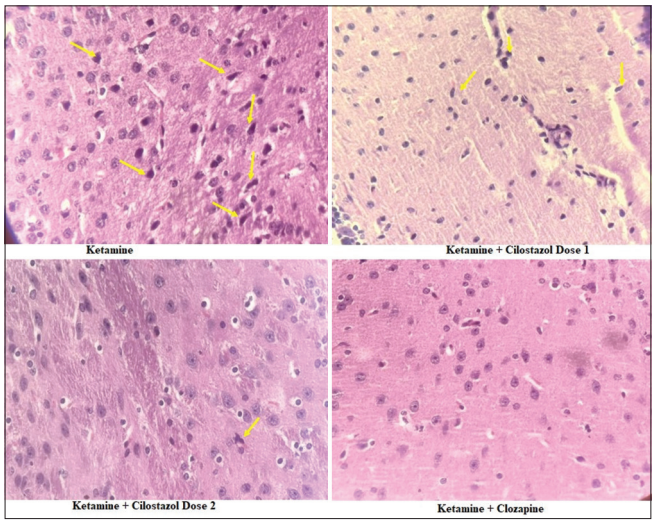

Fig. 8: Effect of cilostazol on histopathology

hyperlocomotion, and impairment of spatial working memory through modulation of the antioxidant system in the brains of experimental mice in addition to normalization of cholinergic neurotransmission. Mice were given KET, an NMDA receptor antagonist and D2 receptor agonist, to mimic the symptoms of SCZ. KET works by increasing oxidative stress, decreasing antioxidant enzyme function, and disrupting neurotransmitter levels [38]. In experimental animals, KET administration results in unpleasant symptoms (social withdrawal) and cognitive abnormalities, including memory and learning problems [39]. KET causes glutamatergic dysfunction and enhanced the activity of the amygdala in the brain, which was strongly associated with decline anxiety and social interaction-like symptoms [40]. Other study also confirms that KET also suppressed brain-derived neurotrophic factor (BDNF) and released inflammatory cytokines in the cortex, striatum, and hippocampus, which may link with cognitive deficits and other neurobehavior abnormalities in animals [30]. Many reports suggest that KET mediated SCZ like phenotype may be due to an increased cascade of oxido-nitrosative stress, depletion of the endogenous antioxidant system, upregulating inflammatory cytokines production, and inducible nitric oxide synthase [30,31,41]. KET also increases proinflammatory cytokines (interleukins) in the cerebellum, which leads to behavioral abnormalities such as SCZ [42]. KET enhanced oxidative stress through inhibiting the production of GSH along with increased TBARS concentrations in different parts of the brain (cortex, limbic system, and hippocampus) [30]. In fact, too much oxidative insult may lead to chronic inflammation changes in the brain [43]. According to this study, cellular or molecular damage may arise from an imbalance between the production of free radicals and the antioxidant defense system. In KET-induced SCZ, it was found that prolonged microglial activation and its dysfunction may cause neuronal apoptosis and damage [44]. KET was reported to provoke apoptosis in neuronal cells by promoting mitochondrial impairment, which may be due to reactive oxygen species (ROS) generation, mitochondrial swelling, and development of caspase-3 activation, and the cytochrome-c and other apoptogenic proteins are released from the mitochondria [45]. Hence, on the basis of the above discussion KET was able to provoke SCZ-like behavioral and biochemical alterations which may be due to increased cholinergic dysfunctions, increased oxidative stress, inflammation, and impaired mitochondrial-mediated brain apoptotic cascade.

PDE inhibitors may be an effective strategy for affecting mood, memory, and learning-related second messengers [13]. Cilostazol, a strong cyclic adenosine monophosphate (cAMP) PDE inhibitor, raises (cAMP), which causes substantial vasodilation, boosting blood flow, and has anti-inflammatory benefits [46]. Cilostazol minimizes cerebral infarction and offers neuroprotection against cerebral ischemia [47], and Huntington's via modulating CREB signaling [48]. According to various investigations, cilostazol possesses a variety of pharmacological actions, including anti-oxidative, anti-inflammatory, and anti-apoptotic qualities [49-51]. Cilostazol enhanced the histology and behavioral conditions of rats. It contributes in lowering hippocampus AChE content and amyloid-beta protein levels. In behavioral tests, cilostazol has potential to enhance memory and learning abilities impaired by heavy metal via modulating the cAMP/CREB cascade [51]. Cyclic nucleotides (cAMP) are suggested to participate in neuronal synaptic plasticity and memory functions. PDE3 inhibitor is a potential target for improving cognition by limiting the degradation of cAMP and cyclic guanosine monophosphate [52]. In the MWM test, cilostazol increased probe time, indicating improved cognitive function [16]. It improves motor functions in patient with peripheral arterial disease [53]. Cilostazol modulated cerebral glutamatergic, and dopamine neurotransmission, which helps to manage motor functions [54]. An increasing number of research suggests that cilostazol possesses an antioxidant action (radical scavenger) that reduces $O2^{\bullet}-$ and thereby removes of $OH^{\bullet}$. Cilostazol pretreatment diminished lipid peroxidation, which may be due to decline in ROS production [55]. Furthermore, cilostazol eliminates ROS production, lowers mitochondrial swelling, and maintains mitochondrial integrity [56]. Cilostazol was known to offer neuroprotection by preventing hippocampal injury and neuronal loss. It helps to diminished microglial activation, which also helps to prevent apoptosis [48]. Prolonged microglial stimulation was well reported to be involved in SCZ-linked neuronal apoptosis [44]. In other reports, cilostazol was also found to decline caspase-3, nuclear factor kappa B, and BDNF expressions, which are related to neuronal death and damage [16]. Hence, the shielding consequences of cilostazol in this study versus impaired cognition, motor uncoordination, and neural demises are the outcome of its antioxidant, anti-inflammation, and anti-AChE profile.

CONCLUSION

As per the outcomes of this investigation and the abovementioned discussion, it has been confirmed that cilostazol (25 and 50 mg/kg; p.o.). Remarkably enhances the neuroprotective impact in KET-treated experimental mice. These findings confirm that cilostazol improves cognition, depressive and anxiety behaviors, and social interaction, along with improved cerebral biochemical alterations in KET-induced experimental SCZ in mice.

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AUTHOR'S CONTRIBUTION

Each author actively contributed to the idea and design, collection of the data, or the analysis and, interpretation of the findings. All authors also made substantial contributions in drafting the article and reviewing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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