Chronic Light-Distorted Glutamate-Cortisol Signaling, Behavioral and Histological Markers, and Induced Oxidative Stress and Dementia

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Title:

Chronic light distorted Glutamate-Cortisol signaling, behavioral, histological markers, and oxidative stress resulting dementia and amelioration by Melatonin

Authors:

Priyanka Sarena[1], Ashish Sharma[1], Maiko T. Urmera[2], Murtaza M Tambuwala[3], Alaa AA Aljabali[4], Dinesh Kumar Chellappan[5], Kamal Dua[6], Rajeev Taliyan[7,] and Rohit Goyal[1]*

Affiliation:

[1]Department of Neuropharmacology, School of Pharmaceutical Sciences, Shoolini University, Solan, 173 212, HP, India

[2]Institute on Aging and Centre for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA, E-mail: maikohs@kuhp.kyoto-u.ac.jp

[3]School of Pharmacy and Pharmaceutical Sciences, Ulster University, Coleraine, County, Londonderry, BT52 1SA, Northern Ireland, United Kingdom, E-mail: m.tambuwala@ulster.ac.uk

[4]Faculty of Pharmacy, Department of Pharmaceutical Sciences, Yarmouk University, Irbid 21163, Jordan, E-mail: alaaj@yu.edu.jo

[5]Department of Life Sciences, School of Pharmacy, International Medical University, Bukit Jalil, Kuala Lumpur, 57000, Malaysia, E-mail: dinesh_kumar@imu.edu.my

[6]Discipline of Pharmacy, Graduate School of Health, University of Technology Sydney, Ultimo, NSW, 2007, Australia, E-mail: kamal.dua@uts.edu.au

[7]Department of Pharmacy, Birla Institute of Technology Science, Pilani, Rajasthan, 333301, India; E-mail: taliyanraja@gmail.com

*Corresponding author:

Rohit Goyal, Professor, School of Pharmaceutical Sciences, Shoolini University, Post Box 9, Solan, 173212, HP, India; Mobile: +91-98160-62679; E-mail: rohitgoyal@shooliniuniversity.com.

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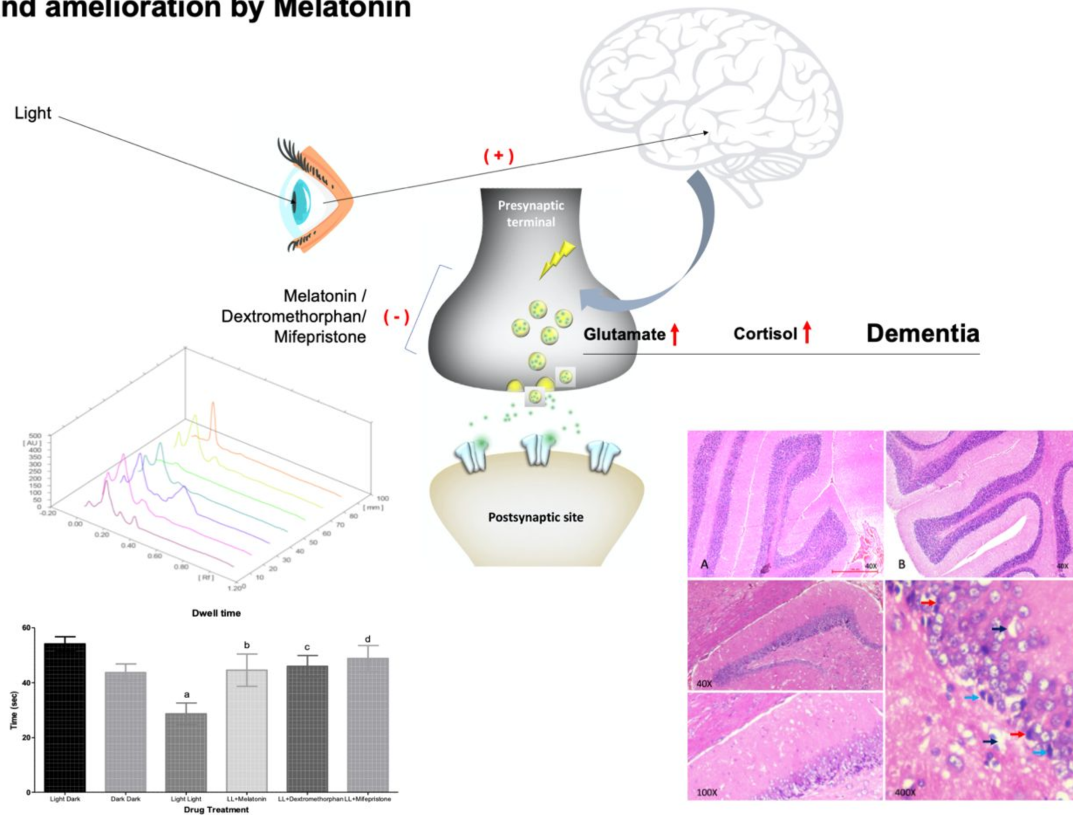

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Abstract

The present work aimed to investigate induction of circadian rhythm dysfunction and dementia upon chronic exposure to light-light, and its reversal by Melatonin in Wistar rats. Animals were undergone different light-dark conditions viz. light/dark (DL), light/light (LL), and dark/dark (DD) in respective groups for four months. Melatonin 0.5 mg/kg *s.c.*, dextromethorphan 100 µg/200g *s.c.*, and mifepristone 50 µg/200g *s.c.* were given once a day. Chronic LL and DD conditions significantly increased brain glutamate and cortisol levels. LL period caused a deficit in spacial memory, working memory, decision making, and exploration of novel objects, compared to LD animals. A significant ($p<0.05$) change in neuropathological observations in the hippocampus, CA1, CA2, and CA3; cortex; and cerebellum regions (40X, 100X, and 400X) were observed in the histological study. Induced oxidative stress in brain tissue was also observed by estimating tissue glutathione and TBARS levels. Dextromethorphan (NMDA antagonist), Mifepristone (corticosterone antagonist), and melatonin significantly ($p<0.05$) reversed the pathological states caused due to LL. The histological features in the hippocampus, cortex, and cerebellum region revealed inflammatory cells, vacuolation, and pyknotic cells which were rescued by antagonizing NMDA or cortisol or melatonin treatment significantly. It may be concluded that continuous exposure to light-light conditions produced an imbalance between neuronal excitotoxicity, and stress hormone leading to cognitive dysfunction and neuropathology.

Keywords:

Circadian; Melatonin; Cortisol; Dementia; Glutamate.

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Introduction

Circadian Rhythm (CR) is an endogenous and entrainable biological process in animals, plants, and fungi that shows oscillations of about 24 h (Edgar et al., 2012). A rhythm recorded daily, weekly, and annually under chronobiology is referred to as CR, a biological temporal rhythm (Vitaterna et al., 2001). Zeitgebers derived from the German word meaning "time giver" entrain CR, which may be photic (light-induced) or non-photic (feeding time, temperature, physical activity-induced) (Bass, 2012). Shift workers experience an increased risk of memory-related problems, impaired sleep, and cardiovascular problems due to circadian rhythm disruption (Ferri et al., 2016). A recent report from our previous investigation revealed that CR disruption induced by chronic light-light conditions precipitates a neurodegenerative state resulting in dementia (Sharma et al., 2020; Sharma & Goyal, 2016).

The biological clock's function is nearly reported in every cell and tissue, including enucleated cells like red blood cells (Buhr and Takahashi, 2013). Suprachiasmatic nuclei (SCN) is identified as a primary circadian clock in mammals and comprised of a pair of distinctive cells in the ventral hypothalamus. The light signals transmitted from the retina incident to the surface of SCN through glutamatergic signals in the retinohypothalamic tract (RHT) (Bedont and Blackshaw, 2015). The mammalian retina performs the function of vision using "classical" photoreceptors, "rods," and "cones and is also functionalized with the specialized photosensitive ganglion cells projecting directly to the SCN (Do and Yau, 2015). Melanopsin, a photopigment found in Ganglionic cells spans directly to RHT leading to the SCN (Welsh et al., 2010). A circadian rhythm is observed in circulating cortisol and melatonin levels (Fatima et al., 2016). In the subjective morning, the rising cortisol levels drive an awakening response that peaks post meridian (Pruessner et al., 1997); in contrast, rising melatonin levels during the evening induce sleep and peak around midnight (Yonei et al., 2010). Excitotoxicity is the pathological process that occurs at neuronal synapses due to excessive stimulation caused by glutamate and calcium influx and induced depolarization (Esposito et al., 2013). This results in altered and disorganized neuronal firing, signaling, and localized vulnerability consistent with neurodegenerative disorders such as Alzheimer's disease (AD) (Dong et al., 2009). Photic Zeitgebers in day-to-day life may cause electrochemical excitotoxicity, and the resultant stress disrupts the coordination and homogeneity of nervous signaling across the brain regions.

In our previous investigation, light-light intervention to a rodent class, Wistar rats for the chronic period was able to modulate the level of *per2, PRX1,* and PRX-SO2/3 gene expression, markers of CR disruption in SCN brain region, associated with neurodegeneration, and increased level amyloid b, *BACE1* and Mgat3 transcript with an altered expression of *Sirt1* and *Prokr2* in the hippocampus region. A pathology of AD phenotype with behavioral disturbances of dementia and cognitive disorientation was concluded (Sharma et al., 2021). An extension to previous novel findings, we aimed to investigate the role of chronic light-induced excitatory signaling caused by glutamate, cortisol, and the reciprocating role of melatonin in-memory performance in Wistar rats.

Results and discussion

Daily physiological and behavioral rhythms are fundamental features of human organization. A daily predictable environment that changes and also drives the rhythms of sleep, endocrine secretion, autonomic function, energy metabolism, and function of the vital organs (Morre-Ede et al., 1984). Cortisol secretion and induced mammalian body temperature increase before

morning awakening so that the body can coordinate to meet the metabolic demands in the active sense of the body (Ebling, 1996). The present study emphasized that continuous exposure to light leads to memory dysfunction, biochemical and histological changes due to excess glutamate release and excitotoxicity.

Glutamate is an excitatory neurotransmitter found in the central nervous system and is implicated in the primary visual pathways. The master clock of the body, SCN receives photic Zeitgieber's through a monosynaptic projection from the retina. Glutamate in SCN initiates a neuronal signal transduction cascade that ultimately leads to a phase shift in the circadian system (Michel et al., 2002). The level of glutamate increased significantly ($p<0.05$) in LL group rats, and melatonin (0.5 mg/kg) was able to decrease it. Inhibition of glutamate action by blocking the NMDA receptor by dextromethorphan (100 µg/200 g) was observed. A similar effect was also noted while a decreased level of glutamate was found in blocking glucocorticoid receptors using Mifepristone in LL rats (Fig 1). Cortisol is identified as a stress hormone and is released in the bloodstream in a hyper-excited form upon stress. In the present finding, the level of serum cortisol was found to be significantly ($p<0.05$) increased in LL group rats in comparison to LD rats. Treatment with melatonin, dextromethorphan, and mifepristone caused a decrease in cortisol when compared to the LL group (Fig 2). Melatonin treatment was found to be neuroprotective by decreasing the level of glutamate in the hippocampus (Alghamdi, 2017).

Continuous exposure and long-term exposure to light induce behavioral and biochemical changes in rats (Tapia-Osorio et al., 2013; Sharma and Goyal, 2020). In a recent study by Sharma and Goyal (2020) from the same laboratory, it was observed and reported by authors that chronic exposure of LL condition cause massive alteration in glutamate, GABA, and serotonin levels along with altered state clock genes. In a behavioral study using Morris water maze, a task of learning to swim in the water tank and to escape to the platform under the water was developed (Morris, 1984). The ability to locate the hidden platform efficiently under the situation will depend on the configuration settings for the cues outside the tank. Learning is identified with the attainment of shorter latency to escape and path length to find the platform. Although rodents can find the platform using non-spatial cues. It is reported to be an efficient spatial strategy in young animals to escape after a few days of trial. In the present study, contextual learning and memory retention in rats after circadian rhythm disruption was assessed on Morris water maze. A significant ($p<0.05$) decrease in dwell time in animals underwent to LL condition (Fig 3), increased latency to entry (Fig 4), and frequency of entries in the target quadrant (Fig 5) were noted, compared to LD and DD groups. Treatment with melatonin, dextromethorphan, and a glucocorticoid receptor antagonist mifepristone significantly ($p<0.05$) restored the memory retention scores, compared to the LL group. The spontaneous alternation, a determinant of working memory, was measured on a Y-maze. Rats could freely move in all the enclosed arms for 5 min, and their working memory was assessed with spontaneous alternations. A significant ($p<0.05$) decrease in Spontaneous alternations was noted in LL group in comparison to LD group. Treatment with melatonin has been noted to improve the performance of animals maintained on chronic LL condition, as evidenced by decreasing alternations. Antagonizing NMDA receptors using dextromethorphan and glucocorticoid receptors using mifepristone respectively in different groups evidenced the significant recovery in working memory, and mifepristone significantly recovered ($p<0.05$) the

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3 working memory deficits compared to the LL rats (Fig 6). A similar finding in the development of recognition memory was observed in NORT in the rats. A significant (p<0.05) decrease in discrimination index was observed in the LL group in comparison to the LD group. Treatments with melatonin, dextromethorphan, and mifepristone in respective groups significantly recovered (p<0.05) the recognition memory deficits in comparison to the LL rats (Fig 7). All animals of different drug-treated groups showed no significant differences in the ambulatory scores in a closed field test (photoactometer). The possibility of locomotor activity of the animals influencing the memory impairment was ruled out (Fig 8).

Histopathological changes in the brain specimens from hippocampus, cortex, and cerebellum regions treated with hematoxylin and eosin dyes from different groups were examined under the microscope. Chronic LL condition for four months to the rats caused significant changes in histological features as evidenced by darkly stained nuclei, inflammatory cells, and vacuolation in hippocampus CA1, CA2, and CA3 region significantly under 40x, 100x, and 400x resolution, compared to LD brains. DD rat brain also showed moderate vacuolation with fewer dark nuclei. In individual groups, histological characteristics were improved in brain tissues from rats treated with melatonin, dextromethorphan, and mifepristone, showing significant restoration from chronic neuronal degeneration (Fig 9). The brain cortex region at 100x and 400x revealed significant marks of darkly stained nuclei, pyknotic cells, and vacuolations upon LL exposure, compared to prolonged LD and DD period, whereas the protective effect of melatonin was observed in histology (Fig 10). The neuronal signaling of glutamate and glucocorticoids may be identified upon treatment of dextromethorphan and mifepristone, respectively, with significant restoration in histology. No substantial change in histology was observed in the brain cerebellum region at 40x upon exposure to chronic LL and DD period and even after drug treatments (Fig 11).

Chronic exposure to LL and DD conditions in different groups for two months is reported to cause dysregulation in neuronal signaling mediators, which may further induce oxidative stress in brain regions (Sharma and Goyal, 2020). This may be identified with decreased glutathione and raised lipid peroxidation levels in brain tissue. A significant oxidative stress (p<0.05) was noted with reduced brain tissue GSH and increased TBARS levels in rats who underwent LL and DD chronic conditions. Melatonin (0.5 mg/kg) alleviated the oxidative state caused in the brain by reversing the level of GSH and TBARS, compared to the LL group. The antioxidant effect of melatonin in rodents has been documented in various studies and brain oxidative stress, providing neuroprotective action (Joshi et al., 2014) (Figs 12 and 13).

Glucocorticoid binds to glucocorticoid receptor in the basolateral amygdala, which activates cyclic AMP and leads to the activation of protein kinases, stimulation of memory consolidation in the hippocampus, and other regions of the brain (McGaugh et al. 2002). Excess release of cortisol leads to the destruction of memory, as is indicated in the behavioral test. Cortisol and melatonin rhythm are greatly influenced during each animal's altered light and dark state, as observed in control animals. Melatonin treatment antagonizes the effect of excitotoxicity caused by cortisol and associated factors stimulated by light for a chronic period. Melatonin has been reported to provide neuroprotective effects in rodents and improve memory and cognition in circadian dystonia (Cassone et al., 1986). The present findings signify that the circuitry of SCN to the neurons showing circadian rhythm disruption due to increased excitotoxicity by cortisol-induced hyper-stimulatory activity at the pre-synapses by glutamate

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3 is confirmed by the effect of mifepristone as improved memory and cognition.
4 Dextromethorphan acts as an NMDA receptor antagonist and mifepristone acts as a
5 glucocorticoid receptor antagonist found useful in restoring memory, which may further be
6 confirmed by extensive studies.

Conclusion

The study's findings revealed that continuous exposure to light exacerbates glutamate, cortisol, memory dysfunction, and histopathological changes in the animal brain. We may conclude that constant exposure to light causes an imbalance between neuronal excitotoxicity and stress hormone leading to cognitive deficits and neuropathology.

Material and methods

Drugs and animals

Melatonin from West Coast Pharmaceutical Works Ltd., Ahmedabad, Gujarat, India; dextromethorphan [an antagonist of N-methyl-D-aspartate receptor] from Ultratech Pharmaceutical Ltd., Baddi, HP, India; and Mifepristone (glucocorticoid receptor antagonist) from Dr. Morepen Limited, New Delhi, India were procured (Cassone et al., 1986; Tortella et al., 1997; Telleria et al., 1999). All reagents and chemicals used in the work were of analytical grade.

Experimental Animals

Animals, Adult Wistar rats (male), bodyweight 180–220 g, were procured from a registered establishment and housed in the animal house facility of the institution. They were maintained under controlled 25±2°C room temperature, 55±5% relative humidity, and provided with food and water *ad libitum*. Each experiment was duly approved by Institutional animal ethical committee (IAEC) vide protocol no. IAEC/SU/17/22. The experiment was conducted following the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA).

Chronic light and dark model for Circadian rhythm dysfunction

Animals were habituated to 12:12 hr light and dark cycles for two weeks, acclimatized to the laboratory environment, and divided into six groups, each comprising n=8. The animals were subjected to the exposure of different light and dark conditions maintained at light/dark (DL), light/light (LL), and dark/dark (DD) conditions for four months with two months of drug treatment in the latter part. The intensity of light in LD and LL rooms was maintained at 200 Lux at the base of the cage. The drug treatment, melatonin at 0.5mg/kg *s.c.* (dissolved 0.9% of ethanol in normal saline), dextromethorphan 100 μg/ 200g *s.c.*, and mifepristone 50 μg/200g *s.c.* were given once a day. The investigator of the memory performance assays was blinded to the trials.

Morris water maze (MWM) for contextual/ spatial memory

MWM was performed to assess the behavioral performance of rodents for contextual or special memory. In the test, water maintained at room temperature 25±2oC was contained in a flat bottom container painted in black. Animals have been undergone training for five days, consisting of two acquisition trials starting from each extreme (north, south, east, and west). The target for each acquisition trial was to find a submerged platform and time is noted. Subsequently, the animal was allowed to spend some time for 30 sec on the platform. In the situation, where an animal is unable to find a platform for 180 sec, subsequent directions through hand may be given. The animal may be given time 10 s to spend on the platform before

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3 retrial. Each trial for an animal was split by a period of two hours. Each animal was randomly started in the north, east, south, and west extreme facing the wall in each quadrant during a training day's session. Testing was performed two times a day at the same time interval. During training, dwell time for each animal in each quadrant and time to find the platform (latency) were noted for each trial. In a probe trial on 6th day, the animals were subjected to swimming for 180 s with prior removal of the platform. In this trial, the performance of the animals was tracked for retainment of contextual and spatial memory for platform location. Latency and time duration to each of the quadrants including target quadrant and dwell time were used as retention indices.

Y-maze for spontaneous alternation working memory

Y-maze behavioral assessment is used to track the performance of animals for working memory by measuring a willingness to explore a new environment. Animals opt preference investigating a new arm and return to previously visited one. All three arms are made identical and require no artificial motivators like food rewards. Each successive entry to an individual arm has been taken as an account of the number of alternations. The observation was presented as percentage alternation which was calculated from triplet sets.

Novel object recognition test (NORT)

NORT is an ideal assessment for discriminative learning. Animals were habituated by giving exposure to an open field environment for up to five min time. Further, the animals were familiarized with two identical objects under immediate exposure. Animals respond to objects with sniffing, touching, and exploratory behavior. The time for exploration of both objects up to 10 sec. In a test session, animals were subjected to exploring a new object of different sizes and shapes along with the objects used during the familiarization step max up to 10 min. Duration of time noted on each of the objects by each animal was noted (Leger et al., 2013).

Spontaneous locomotor activity test using Photoactometer

The animals were weighed and numbered accordingly. The equipment was first turned on, the photocells were checked for accurate recording. The animal was kept at the center of the location and each of the activities was recorded for 10 min. The basal activity score of all the animals was recorded. Photoactometer maze was used to determine the basic locomotion in each animal during the trial.

Brain homogenate

Animals were sacrificed, the whole brain was isolated and rinsed with ice-cold saline. The brain tissue was homogenized using phosphate buffer (pH 7.4), centrifuged at 800 g for 5 min at 4°C and the nuclear debris was separated. The supernatant obtained was subjected to centrifugation at 10,500 g for 20 min and post mitochondrial supernatant was separated. The estimation of glutamate, serum cortisol, TBARS, and reduced glutathione (GSH) levels was done in brain tissue homogenate as briefly explained below.

Estimation of glutamate level

An aluminum Silica gel 60 F254 high-performance thin-layer chromatography (HPTLC) pre-coated plates procured from Merck Life Science Private Limited, Mumbai, India was used. A standard sample solution of L-glutamic acid was prepared in 0.1 N HCl in 80% ethanol at conc. 1 mg/ml. A sample application in a volume of 2 μl of each solution to afford 10-100 ng per spot was done. For visualization of the spot, a ninhydrin solution was prepared by dissolving 200 mg of ninhydrin in acetone. 1 ml of pyridine was also mixed into the solution and a volume

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3 up to 100 ml was made up to with acetone. Tissue from Hippocampal region was suspended in 0.1 N HCl in 80% ethanol, homogenized, and centrifuged at 4,500 rpm for 20 min at 25°C. A clear supernatant obtained was subjected to the assessment of glutamate level. An applicator-Linomat V, Scanner-Camag TLC scanner III, and Software-winCATS version 1.3.4 from CAMAG, Muttenz, Switzerland were used for HPTLC study. n-butanol: glacial acetic acid: water (22:3:5 v/v/v) was used as mobile phase to run the chromatogram using a developing chamber of twin trough glass (20×10). The other conditions to the development include ascending development mode, time of chamber saturation up to 45min, temp. 25ºC and 55-65% relative humidity. Linearity, specificity, sensitivity, accuracy, precision, and repeatability have been duly validated in each method. Sample development on a pre-coated plate was sprayed using a reagent, ninhydrin (0.2%), and kept in an oven maintained at 60-70° for 4-5 min. The spots observed under the chromatogram were scanned at 550 nm wavelength using a scanner and were analyzed. A calibration curve of standard Glutamic acid was performed.

Estimations of serum cortisol level

Serum cortisol was assessed using a competitive binding immunoenzymatic solid-phase *chemiluminescence* immunoassay. Cortisol represents a competitive binding towards its alkaline phosphatase conjugate for specific antibodies. A conjugate of cortisol-alkaline phosphatase, cortisol antibody, and paramagnetic particles coated with a goat anti-rabbit capture antibody was added to a reaction vessel. Some materials were bound to a solid phase in a magnetic field after incubation whereas remainder is washed off. Lumi-Phos 530, a chemiluminescent substrate was added for assessment of light generated using a luminometer. A standard calibration curve using cortisol was developed and used for calculation.

Estimation of lipid peroxidation

Lipid peroxidation is indicative of cell wall rupture following necrosis and is assessed by the method of Wills (1966). Briefly, brain homogenate (500 µl) was mixed into an equal volume of 0.1 M Tris buffer and incubated at 37oC for 2 hrs. To the solution, 1 ml of Trichloroacetic acid (10%) was added and subjected to centrifugation for 10 min at 3,000 rpm. To the supernatant isolated 1 ml of thiobarbituric acid (0.67%) in distilled water was added and heated on a water bath for 15 min. The final solution was cooled under tap water and eluted by adding 1 ml of distilled water. The sample was analyzed at wavelength 532 nm using a U.V. spectrophotometer and TBARS was calculated as nm/mg of tissue.

Estimation of GSH (reduced)

Glutathione reduced was assessed by the method of Ellman (1959). Briefly, a tissue homogenate (1 ml) was added to an equal volume of TCA solution (20%) comprising ethylenediaminetetraacetic acid (1 mM). An incubation to the sample at 25oC for 5 min was subjected and was centrifuged for 10 min at 2000 rpm. A solution of 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) reagent (0.1 nM) 1.8 ml was added to 200 µl of supernatant and absorbance was performed on 412 nm.

Brain tissue histological assessments

For the histological study, brain tissues from the cortex, hippocampus, and cerebellum regions were excised out and preserved on a 10% formalin solution. The tissue was processed as per standard histological procedures, sectioned up to 3-5-micron using a microtome, and stained using Hematoxylin and Eosin. After fixation to a glass slide, the sections were examined under

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a trinocular microscope and photomicrograph at different resolutions 40x, 100x, and 400x for brain tissue architecture and inflammatory cells.

Statistical analysis

Data obtained was presented as mean±standard deviation (S.D.) and statistical analysis one-way and two-way ANOVA followed by Bonferroni's multiple comparison test was performed using GraphPad Prism5 software. *P-value* <0.05 value was taken as a statistical significant.

Abbreviations

CR: Circadian Rhythm; SCN: suprachiasmatic nuclei; Prokr2: prokineticin-2-receptor-2; HPTLC: high-performance thin-layer chromatography; AD: TTFL: transcription-translation negative feedback loop; Alzheimer's disease; GSH: glutathione; NMDA: N-Methyl-D-aspartate receptor; Mgat3: monoacylglycerol acyltransferase-3; NORT: novel object recognition test; Aβ: beta-amyloid; PBS: phosphate-buffered saline; Per2: period-2; PK2: prokineticin-2; PRX-SO2/3: sulfinylated peroxiredoxins; PRX1: peroxiredoxin-1; Sirt1: sirtuin-1; MDA: malondialdehyde; ZT: zeitgeber time; Bace1: β-site amyloid precursor protein cleaving enzyme-1.

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Conflict of interests

None declared.

Authors contributions:

Rohit Goyal (RG), Ashish Sharma (AS), Rajeev Taliyan (RT), Maiko T. Urmera (MTU) have drafted the proposed study. RG, PS, and AS performed the study. RG, PS, Murtaza M Tambuwal (MMT), Alaa AA Aljabali (AAA), Dinesh Kumar Chellappan (DKC), Kamal Dua (KD) conceptualized the experimental part and performed the interpretation of the findings. Drafting of whole text was performed by all the authors moreover, filing of histology in pictures was performed by RG and PS.

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References:

1. Alghamdi, B.S., The neuroprotective role of melatonin in neurological disorders. *J Neurosci Res* **2018**, 96(7): 1136–1149.
2. Bass, J., Circadian topology of metabolism. Nature **2012**, 491(7424): 348-356.
3. Bedont, J.L.; Blackshaw, S., Constructing the suprachiasmatic nucleus: a watchmaker's perspective on the central clockworks, *Front Syst Neurosci* **2015**, 9: 74.
4. Buhr, E.D.; Takahashi, J.S., Molecular components of the mammalian circadian clock, *Handb Exp Pharmacol* **2013**, 217: 3-27.
5. Cassone, V.M.; Chesworth, M.J.; Armstrong, S.M., Dose-dependent entrainment of rat circadian rhythms by daily injection of melatonin. *Journal of biological rhythms* **1986**, 1(3): 219-229.
6. Do, M.T.H.; Yau, K., Intrinsically Photosensitive Retinal Ganglion Cells, *Physiol Rev* **2015**, 90(4): 1547-1581.
7. Dong, X.X.; Wang, Y.; Qin, Z.H., Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacologica Sinica* **2009**, 30(4): 379-387.
8. Ebling, F.J.,The role of glutamate in the photic regulation of the suprachiasmatic nucleus. *Progress in neurobiology* **1996**, 50(2-3): 109-132.
9. Edgar, R.S.; Edward, W.; Zhao, Y; Van, O.G.; Olmedo, M.; Qin, X.; Xu, Y.; Pan, M.; Valekunja, U.K., Peroxiredoxins are conserved markers of circadian rhythms. *Nature* **2012**, 485: 459-464.
10. Ellman, G.L., Tissue sulfhydryl groups. *Arch Biochem Biophys* **1959**, 82(1): 70-77. doi:10.1016/0003-9861(59)90090-6.
11. Esposito, Z.; Belli, L.; Toniolo, S.; Sancesario, G.; Bianconi, C.; Martorana, A., Amyloid β, glutamate, excitotoxicity in Alzheimer's disease: are we on the right track?. *CNS Neuroscience & Therapeutics* **2013**, 19(8): 549-555.
12. Fatima, G.; Sharma, V.P.; Verma, N.S., Circadian Variations in Melatonin and Cortisol in Patients with Cervical Spinal Cord Injury, *Spinal Cord* **2016**, 54(5): 364-7.
13. Ferri, P.; Guadi, M.; Marcheselli, L.; Balduzzi, S.; Magnani, D.; Di Lorenzo, R., The impact of shift work on the psychological and physical health of nurses in a general hospital: a comparison between rotating night shifts and day shifts. *Risk management and healthcare policy* **2016**, 9: 203.
14. Joshi, N.; Biswas, J.; Nath, C.; Singh, S., Promising role of melatonin as neuroprotectant in neurodegenerative pathology. *Molecular Neurobiology* **2015**, 52(1): 330-340.
15. Leger, M.; Quiedeville, A.; Bouet, V.; Haelewyn, B.; Boulouard, M.; Schumann-Bard, P.; Freret, T.; Object recognition test in mice. *Nat Protoc* **2013**, 8(12): 2531-2537. doi:10.1038/nprot.2013.155.
16. McGaugh, J.L.; Roozendaal, B., Role of adrenal stress hormones in forming lasting memories in the brain. *Current opinion in neurobiology* **2002**, 12(2): 205-210.
17. Michel, S.; Itri, J.; Colwell, C.S., Excitatory mechanisms in the suprachiasmatic nucleus: the role of AMPA/KA glutamate receptors. *Journal of Neurophysiology* **2002**, 88(2): 817-828.

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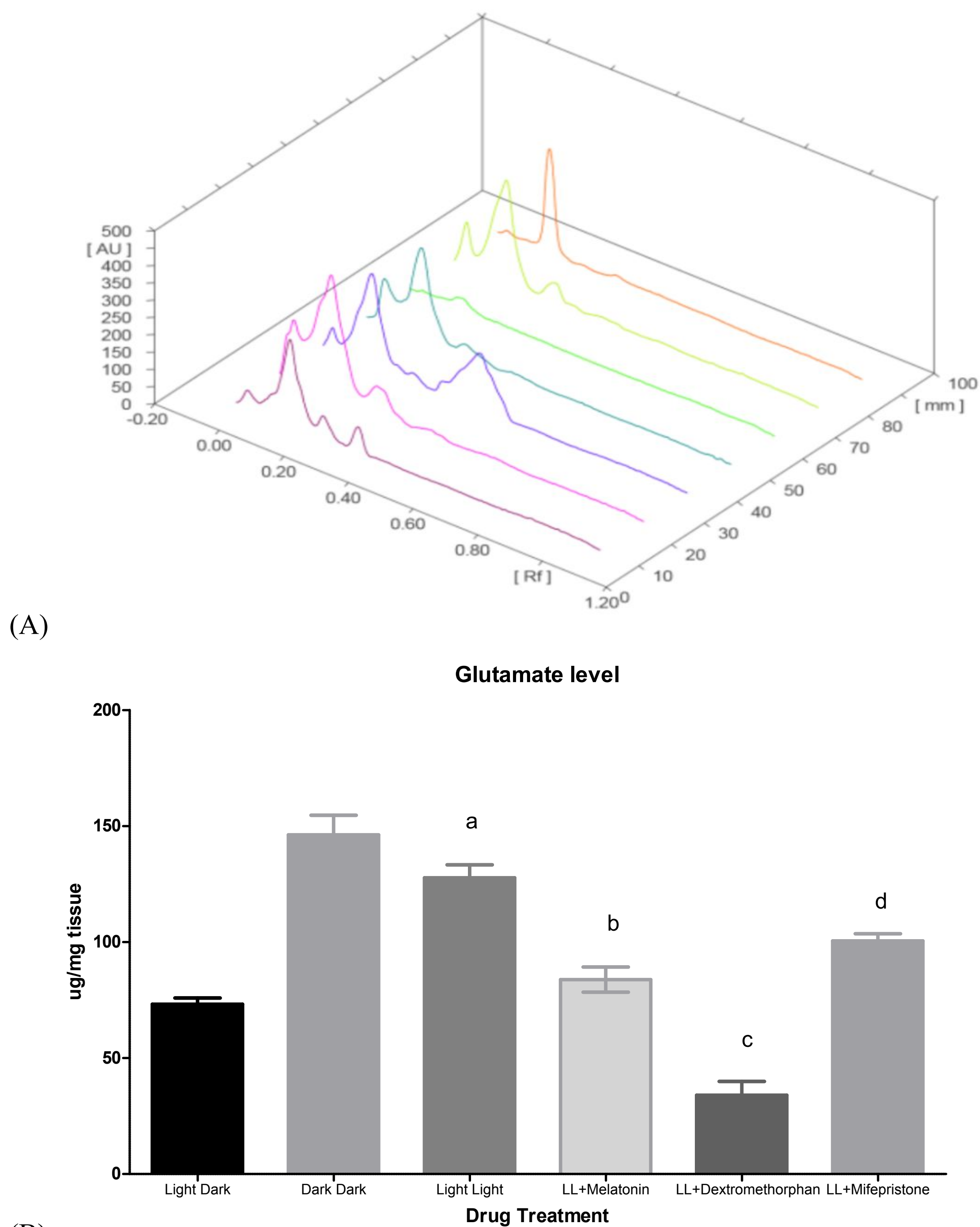
18. Morre-Ede, M.C.; Becker, C.E., Circadian Time Keeping in Health and Disease. *Journal of Occupational and Environmental Medicine* **1984**, 26(3): 232.
19. Morris, R., Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of neuroscience methods* **1984**, 11(1): 47-60.
20. Pruessner, J.C.; Wolf, O.T.; Hellhammer, D.H.; Buske-Kirschbaum, A.; Von Auer, K.; Jobst, S.; Kirschbaum, C., Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life sciences* **1997**, 61(26): 2539-2549.
21. Sharma, A.; Goyal, R., Do Circadian Rhythms Draw the Patterns of Sustained Mental Vigor and Ailment? *Drug Development Research* **2016**, 77(8): 469-473.
22. Sharma, A.; Goyal, R., Long-term exposure to constant light induces dementia, oxidative stress and promotes aggregation of sub-pathological Aβ42 in Wistar rats. *Pharmacology Biochemistry and Behavior* **2020**, 172892.
23. Sharma, A.; Mohammad, A.; Saini, A.K.; Goyal, R., Neuroprotective Effects of Fluoxetine on Molecular Markers of Circadian Rhythm, Cognitive Deficits, Oxidative Damage, and Biomarkers of Alzheimer’s Disease-Like Pathology Induced under Chronic Constant Light Regime in Wistar Rats, *ACS Chemical Neuroscience* **2021**, 12(12), 2233-2246.
24. Sharma, A.; Sethi, G.; Tambuwala, M.M.; Aljabali, A.A.; Chellappan, D.K.; Dua, K.; Goyal, R., Circadian rhythm disruption and Alzheimer's disease: The dynamics of a vicious cycle. *Current Neuropharmacology* **2020**, 18.
25. Tapia-Osorio, A.; Salgado-Delgado, R.; Angeles-Castellanos, M.; Escobar, C., Disruption of circadian rhythms due to chronic constant light leads to depressive and anxiety-like behaviors in the rat. *Behavioural brain research* **2013**, 252: 1-9.
26. Telleria, C.M.; Mezzadri, M.R.; Deis, R.P., Fertility impairment after mifepristone treatment to rats at proestrus: Actions on the hypothalamic-hypophyseal-ovarian axis. *Contraception* **1997**, 56(4): 267-274.
27. Tortella, F.C.; Britton, P.; Williams, A.; Lu X.C.M.; Newman, A.H., Neuroprotection (focal ischemia) and neurotoxicity (electroencephalographic) studies in rats with AHN649, a 3-amino analog of dextromethorphan and low-affinity N-methyl-D-aspartate antagonist. *Journal of Pharmacology and Experimental Therapeutics* **1999**, 291(1): 399-408.
28. Vitaterna, M.S.; Takahashi, S.; Turek, F., Overview of circadian rhythms. *Alcohol Research and Health* **2001**, 25: 85-93.
29. Welsh, K.; Takahashi, J.S.; Kay S.A., Suprachiasmatic nucleus: cell autonomy and network properties. ***Annual review of physiology 2010***, 72: 551-577.
30. Wills, E.D. Mechanisms of lipid peroxide formation in animal tissues. The Biochemical journal **1966**, 99(3): 667-676. doi:10.1042/bj0990667
31. Yonei, Y.; Hattori, A.; Tsutsui; K.; Okawa, M.; Ishizuka, B., Effects of melatonin: basics studies and clinical applications. Anti-Aging Med **2010**, 7: 85-91.

Figures



(B)

Fig 1. Effects of drug treatment on brain glutamate levels. (A) Densitogram of glutamate in hippocampi; (B) Quantitation of glutamate levels; Bars represent mean±SD; $^{a}p<0.05$ vs Light/Dark; $^{b}p<0.05$ vs Light/Light; $^{c}p<0.05$ vs Light/Light; $^{d}p<0.05$ vs Light/Light

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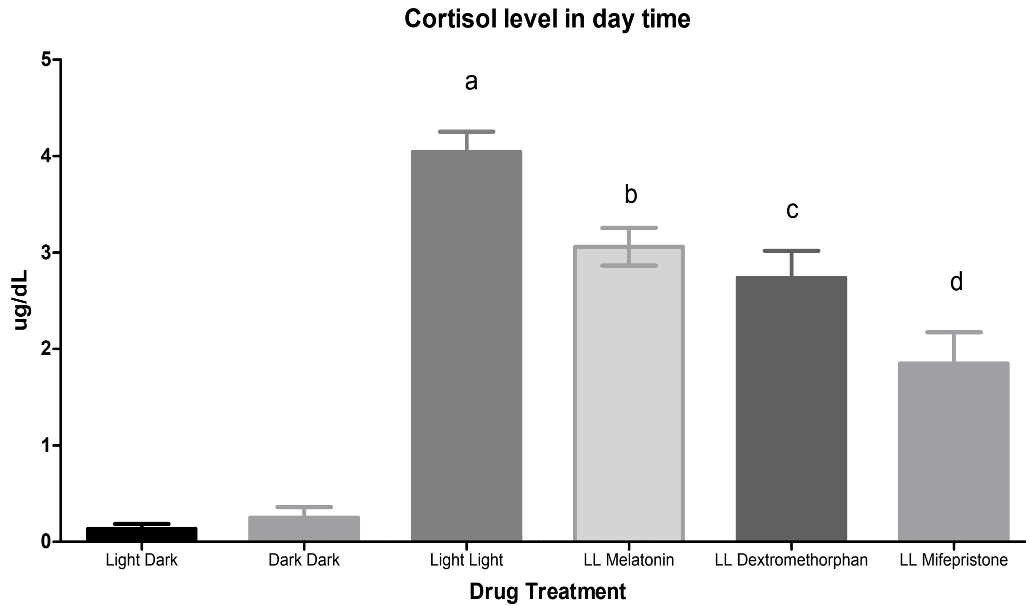

Fig 2. Effects of drug treatment on blood cortisol levels; Bars represent mean±SD. [a] $p<0.05$ vs Light/Dark; [b] $p<0.05$ vs Light/Light; [c] $p<0.05$ vs Light/Light; [d] $p<0.05$ vs Light/Light

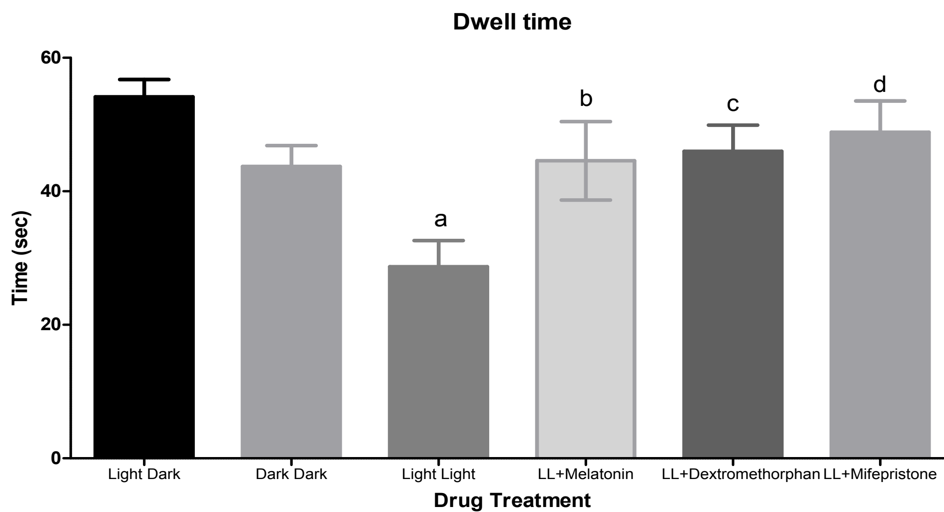

Fig 3. Time spent in the target quadrant for memory retention in Morris water maze; Bars represent mean±SD. [a] $p<0.05$ vs Light/Dark; [b] $p<0.05$ vs Light/Light; [c] $p<0.05$ vs Light/Light; [d] $p<0.05$ vs Light/Light.

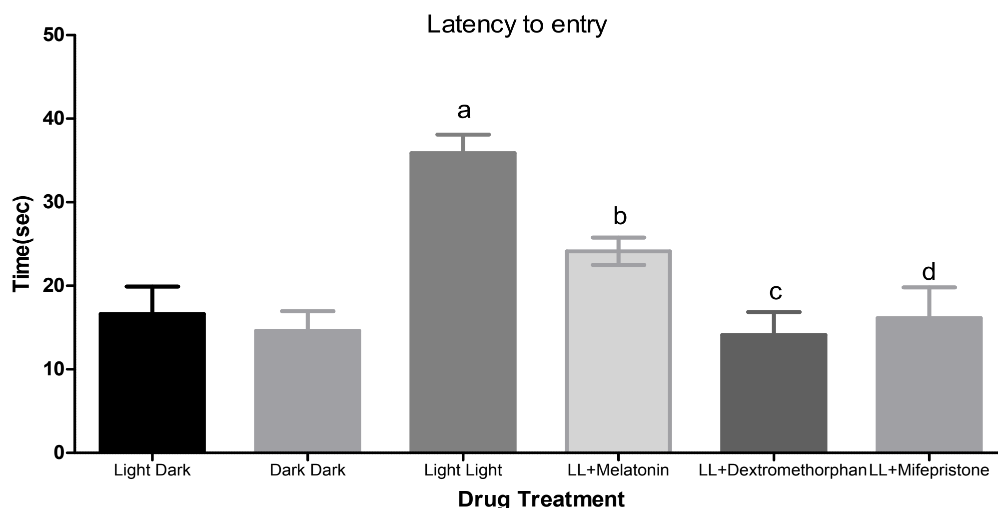

Fig 4. Latency to entry in target quadrant for memory retention in Morris water maze; Bars represent mean±SD. [a]p<0.05 vs Light/Dark; [b]p<0.05 vs Light/Light; [c]p<0.05 vs Light/Light; [d]p<0.05 vs Light/Light.

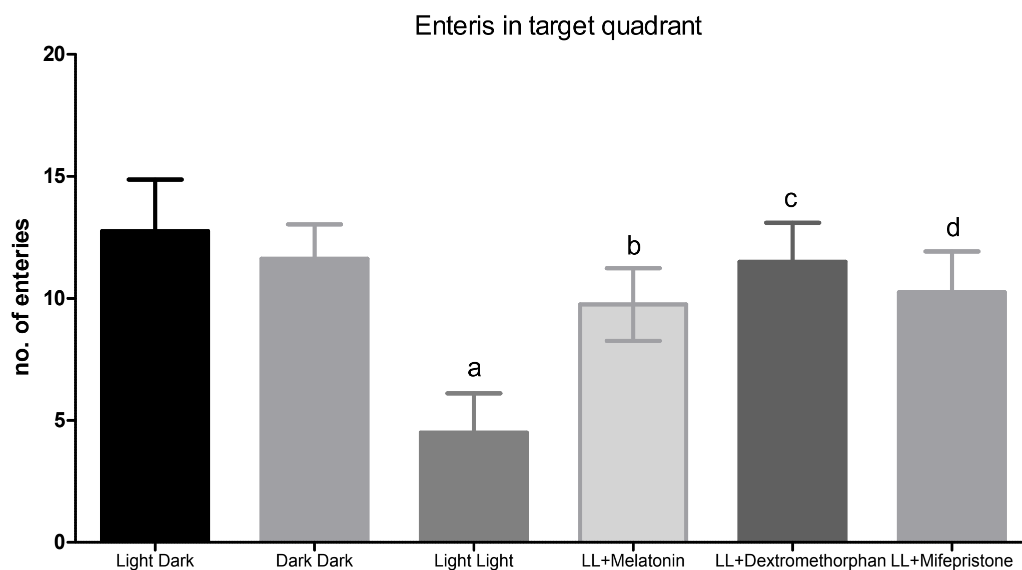

Fig 5. Number of entries in the target quadrant for memory retention in Morris water maze; Bars represent mean±SD. [a]p<0.05 vs Light/Dark; [b]p<0.05 vs Light/Light; [c]p<0.05 vs Light/Light; [d]p<0.05 vs Light/Light.

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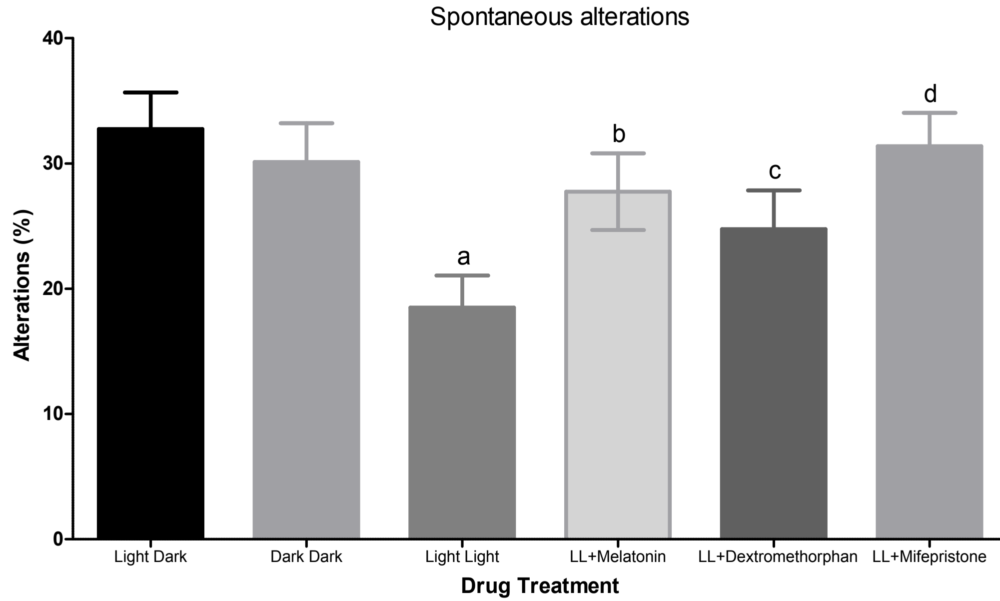


Fig 6. Spontaneous alterations in percentage on Y maze; Bars represent mean±SD. [a]$p<0.05$ vs Light/Dark; [b]$p<0.05$ vs Light/Light; [c]$p<0.05$ vs Light/Light; [d]$p<0.05$ vs Light/Light.

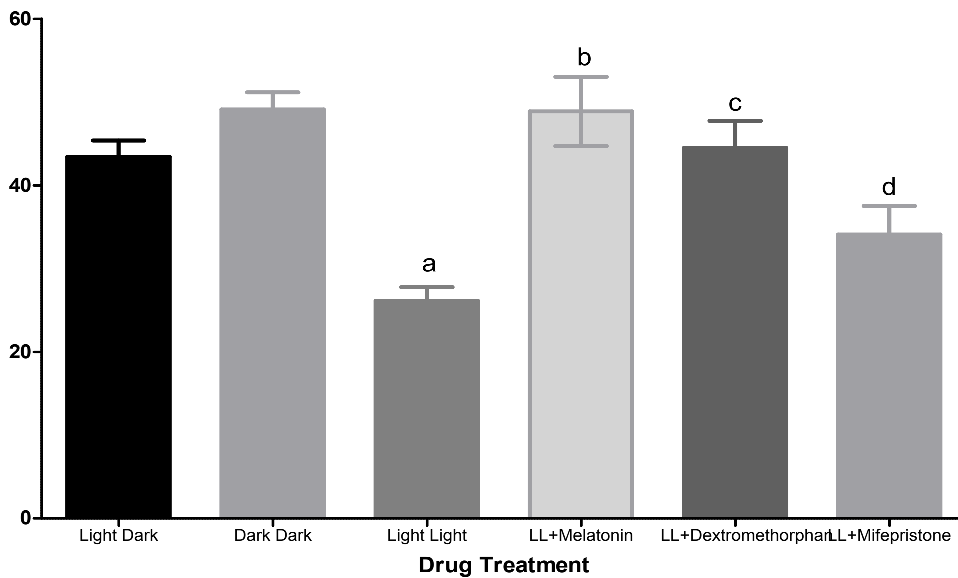


Fig 7. Effect of drug treatment on discrimination index in NORT; Bars represent Mean±SD. [a]$p<0.05$ vs Light/Dark; [b]$p<0.05$ vs Light/Light; [c]$p<0.05$ vs Light/Light; [d]$p<0.05$ vs Light/Light.

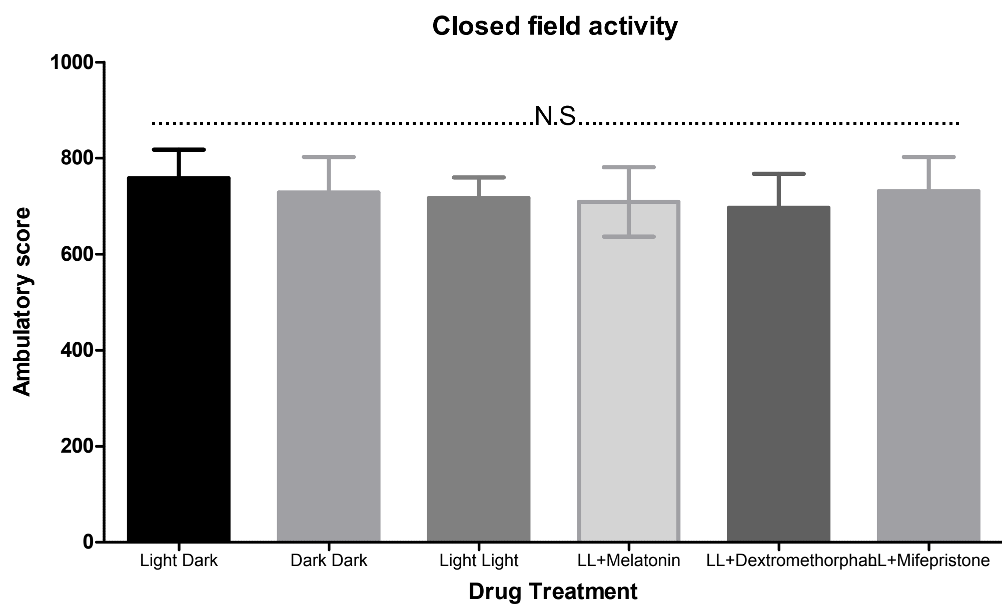
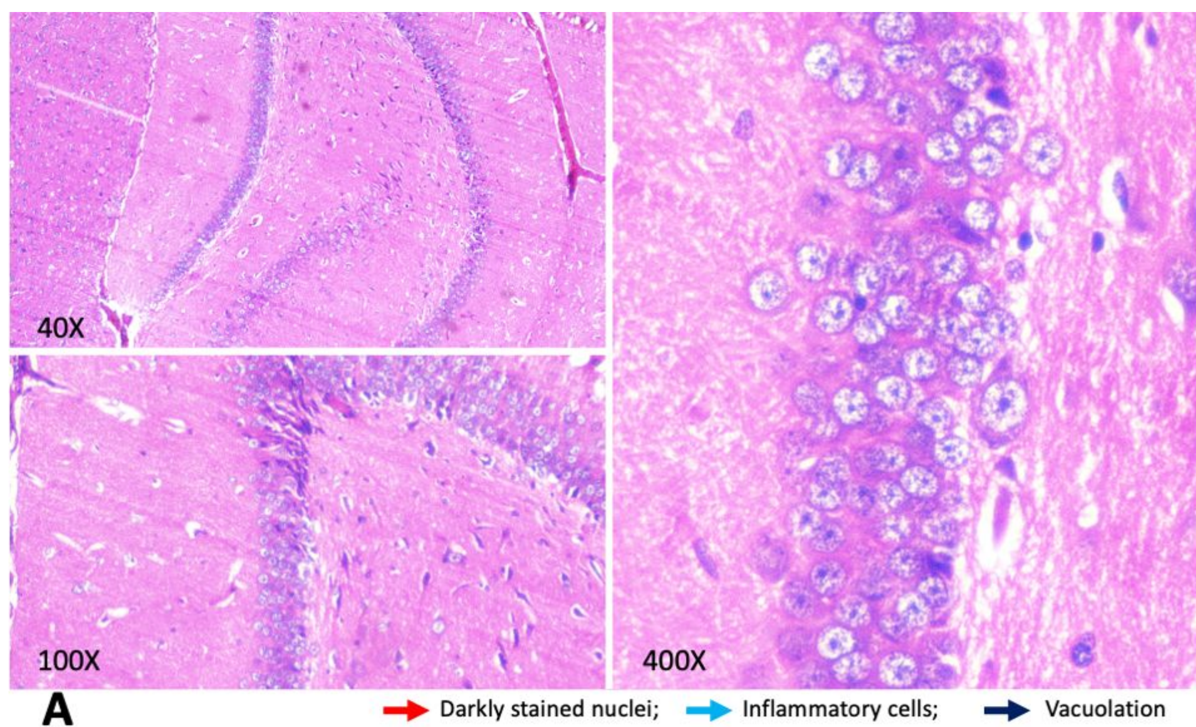


Fig 8. Ambulatory scores in Photoactometer during the experiment period; Bars represent Mean±SD; N.S.: Non-significant.



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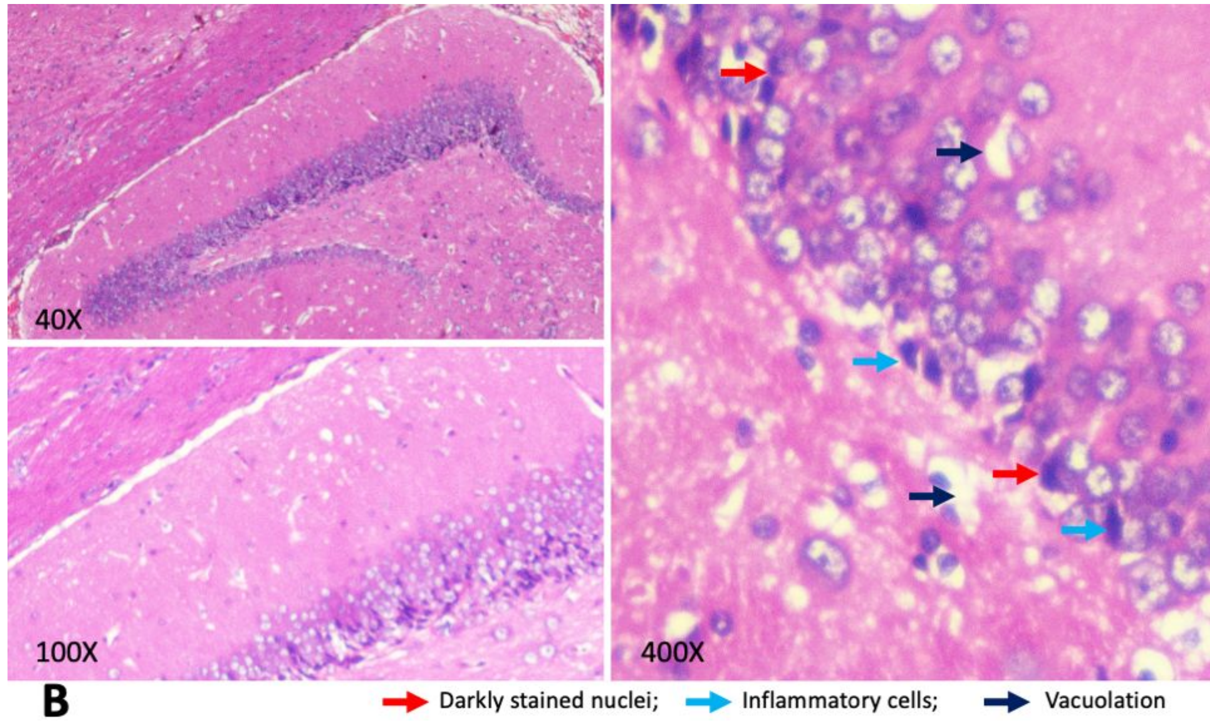

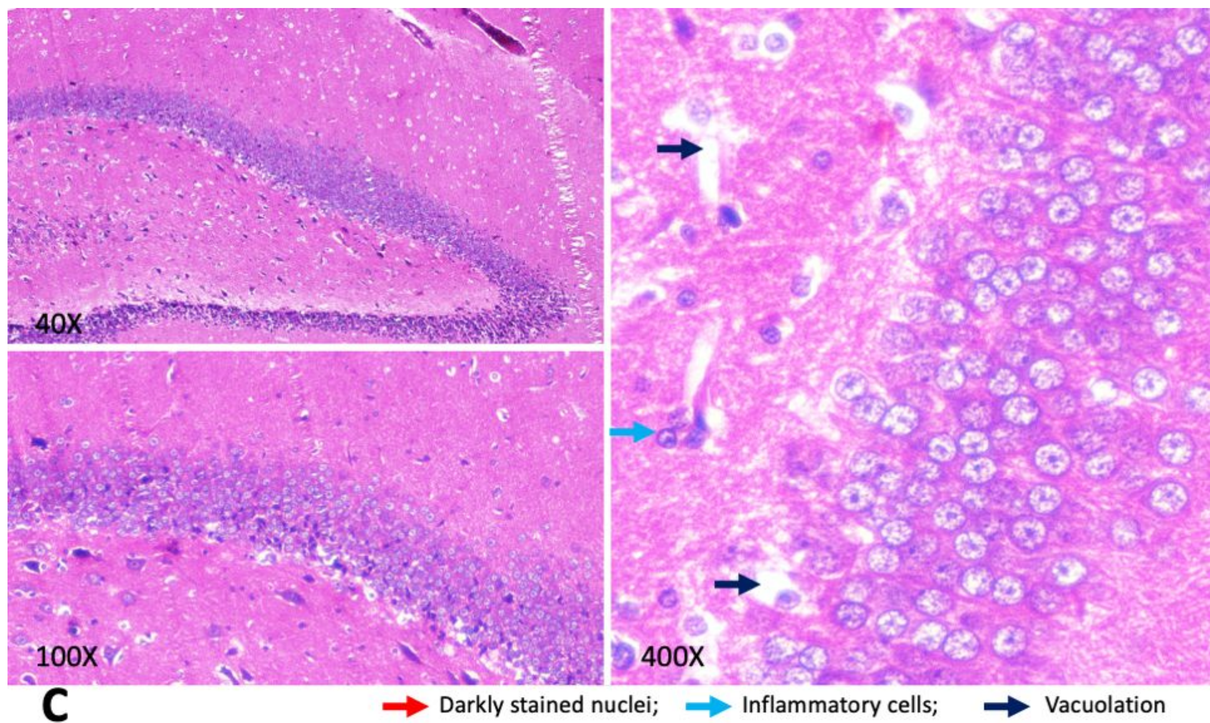

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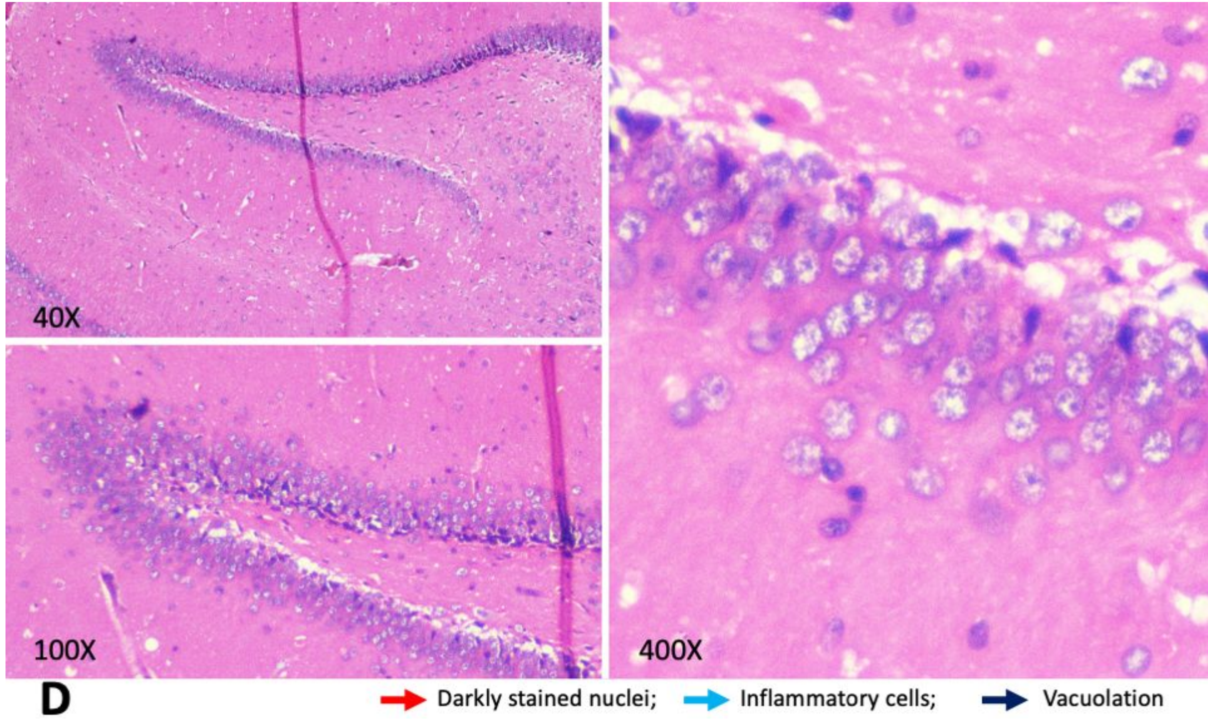

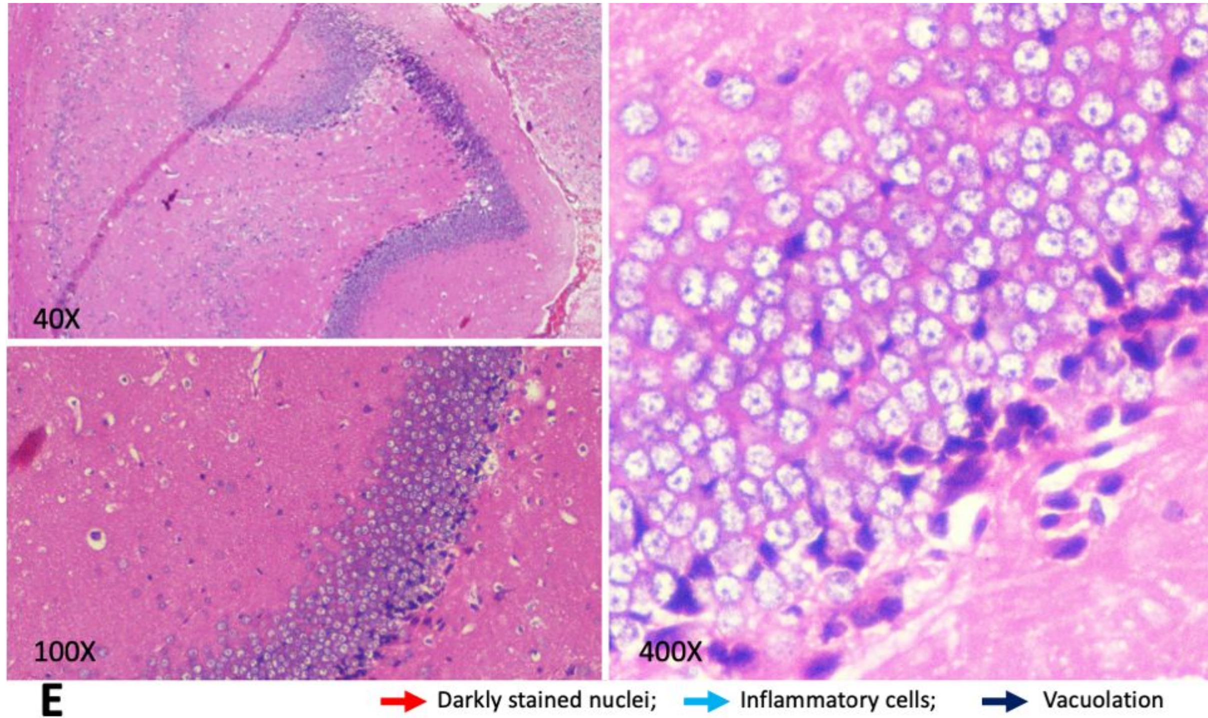

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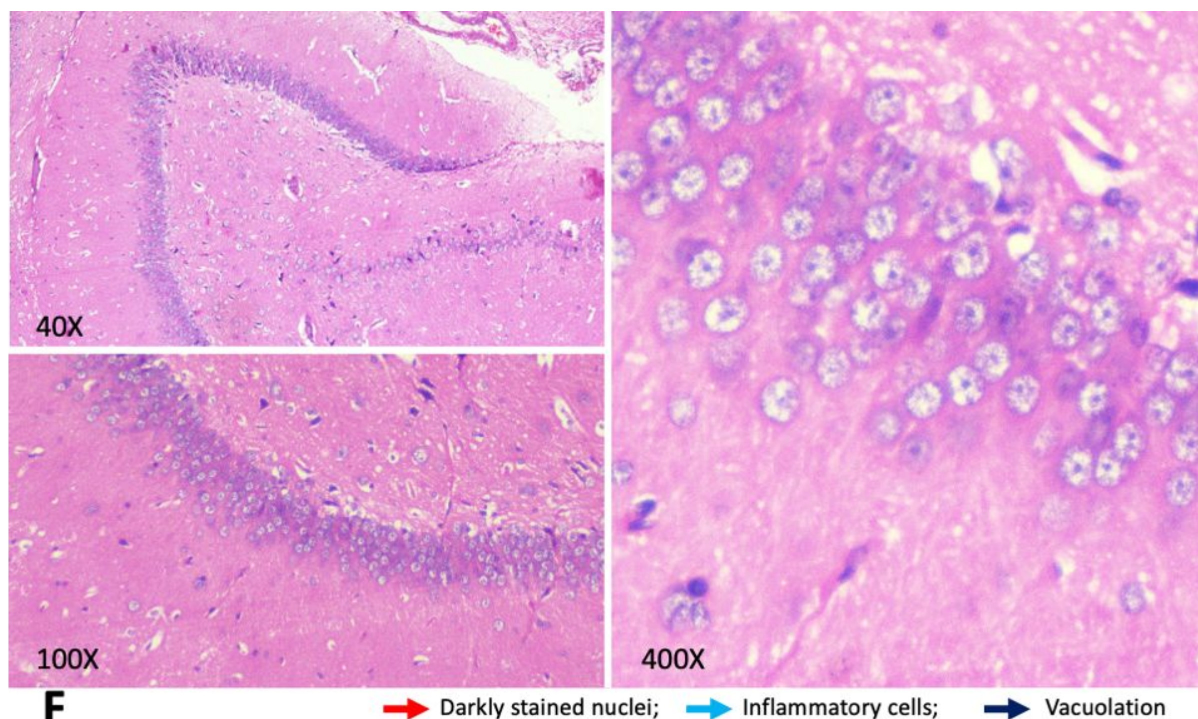

Fig 9. Representative images of brain hippocampus, CA1, CA2, and CA3 region (40X, 100X, and 400X); A) LD: Rat brain showing normal histological characteristics upon LD; B) LL: Rat brain showing darkly stained nuclei, inflammatory cells, and vacuolation significantly; C) DD: Rat brain showing vacuolation with less dark nuclei; D) LL+melatonin: Rat brain showing improvement in the histopathological structure of the brain tissue; E) LL+dextrotromethorphan: Rat brain showing improvement in the histopathological structure of the brain tissue; F) LL+mifepristone: Rat brain showing improvement in the histopathological structure of the brain tissue.

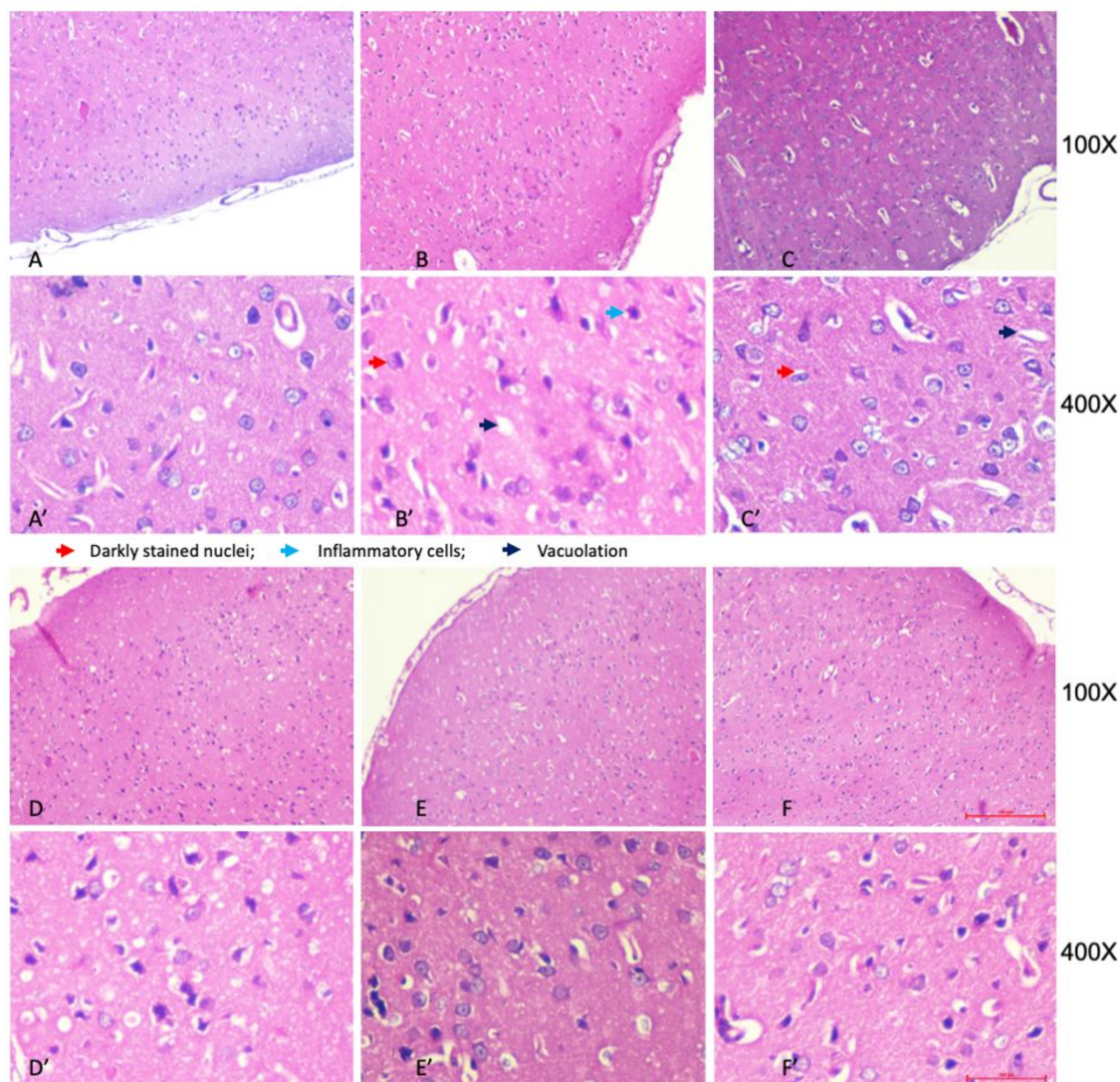

Fig 10. Representative images of brain cortex region (100X and 400X); A & A') LD: Rat brain showing normal histological structure; B & B') LL: Rat brain showing darkly stained nuclei, pyknotic cells, and vacuolations; C & C') DD: Rat brain showing pyknotic cells and vacuolations; D & D') LL+melatonin; E & E') LL+dextrotromethorphan; and F & F') LL+mifepristone: Rat brain showing significant restoration in histopathological structures.

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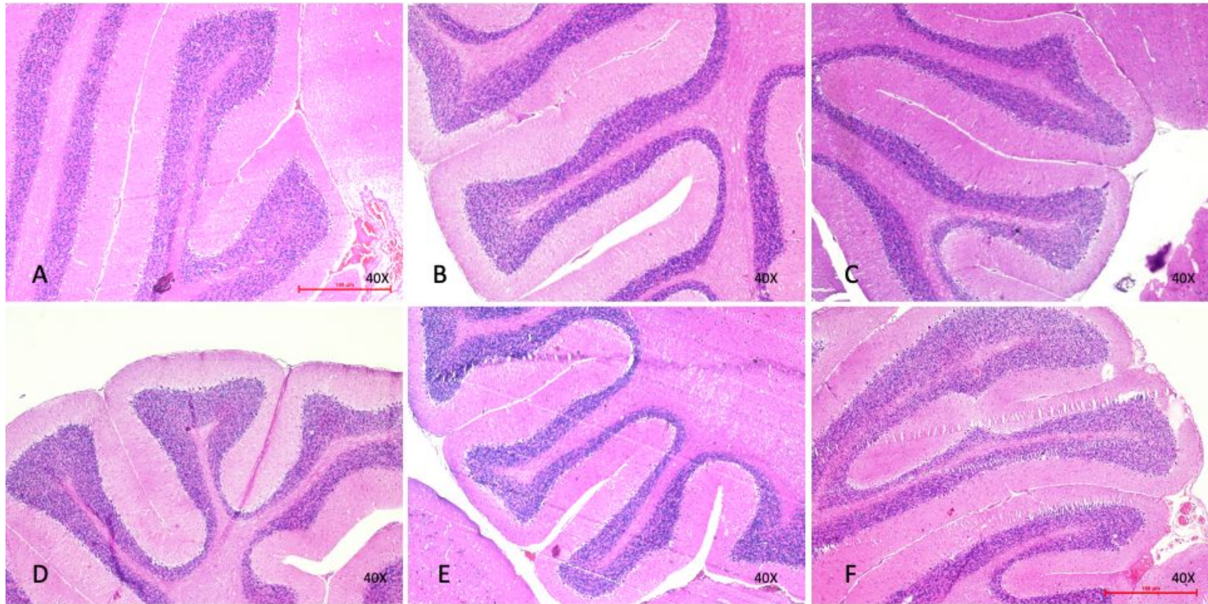

Fig 11. Representative images of brain cerebellum region (40X). A) LD rat brain; B) LL rat brain; C) DD rat brain; D) LL+melatonin; E) LL+dextrotromethorphan; F) LL+mifepristone; There is no evidence showing restoration in histopathological structures.

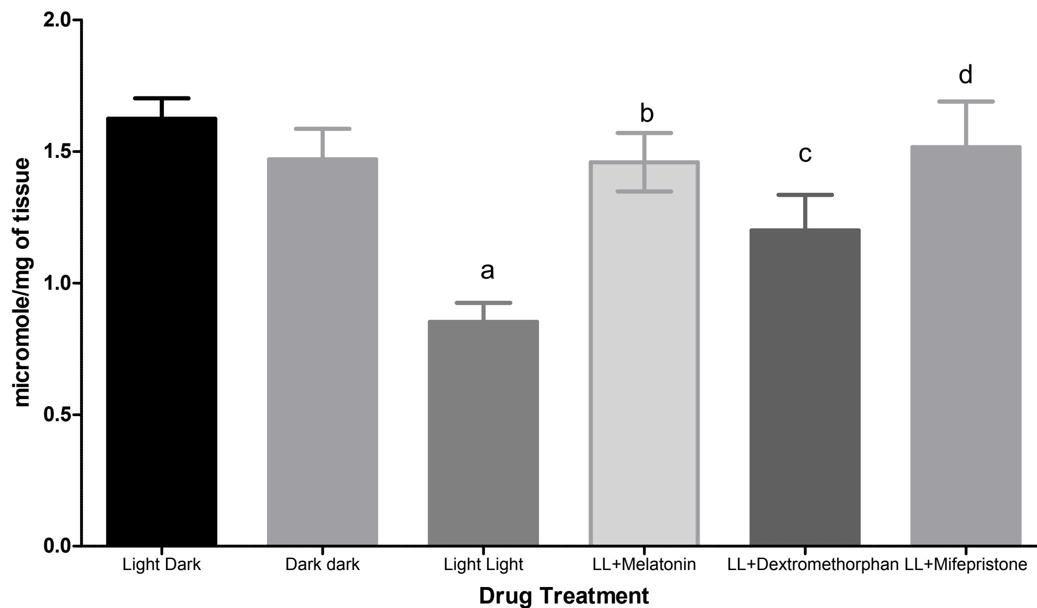

Fig 12. Effect of drug treatment on brain GSH. Bars represent mean±SD. [a]$p<0.05$ vs Light/Dark; [b]$p<0.05$ vs Light/Light; [c]$p<0.05$ vs Light/Light; [d]$p<0.05$ vs Light/Light.

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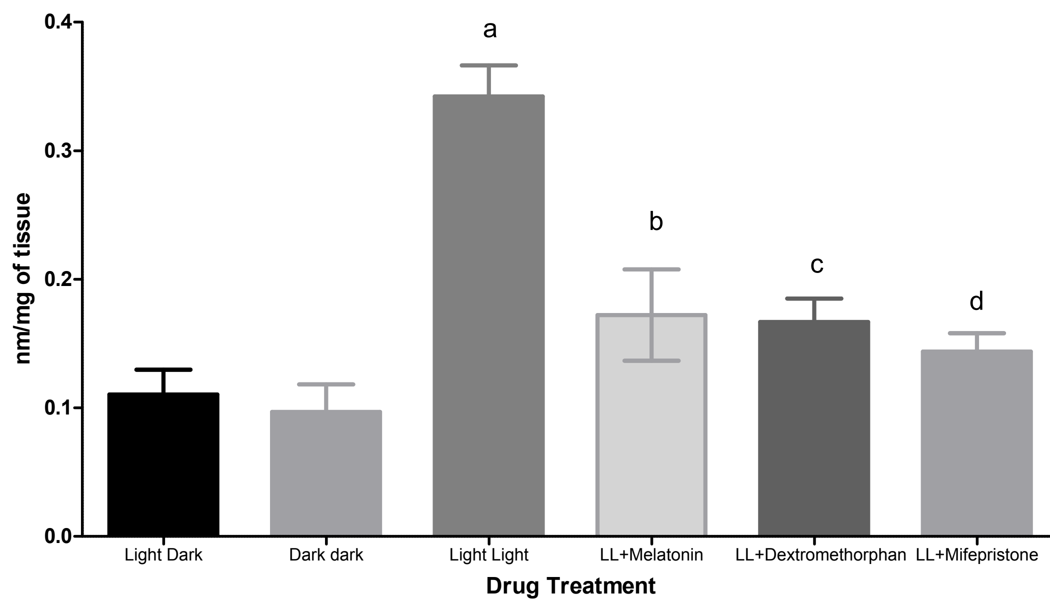

Fig 13. Effects of drug treatment on brain lipid peroxidation level. Bars represent mean±SD. [a]p<0.05 vs Light/Dark; [b]p<0.05 vs Light/Light; [c]p<0.05 vs Light/Light; [d]p<0.05 vs Light/Light.