Tramadol rescues visual cortex gliosis and modulates ocular lipido-inflammatory responses in a rat model of paradoxical sleep deprivation

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Research Article

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Abstract

Individually, sleep deprivation and sub-chronic tramadol exposure have been reported to impair visual processes, however the underlying mechanisms of their combined effects are largely unknown. Thus, this study investigated the role of tramadol hydrochloride on lipid-immune activities in the ocular tissue and visual cortex of sleep-deprived periadolescent rats. Sixty female periadolescent Wistar rats were either sleep-deprived with or without tramadol treatment. Following euthanasia, brain and whole eye tissues were collected for biochemical and immunohistochemical assays. Results revealed impaired ocular tissue lipid profile following sleep deprivation (SD). Sleep deprivation also induced lipid peroxidation; upregulated apolipoprotein E (ApoE), and nuclear factor kappa B (NF-κB) 1 levels in the ocular tissue. Furthermore, chronic SD exposure triggered gliosis with marked increase in astrocyte and microglia counts in the visual cortex. However, treatment with tramadol restored ocular tissue lipid function markers, downregulated ocular tissue NF-κB levels, as well as ameliorated sleep deprivation-induced gliosis in the visual cortex. Taken together, this study demonstrates the role of tramadol in improving inflammatory processes and lipid homeostasis in the visual system by modulating ocular tissue ApoE and NF-κB signalling, and attenuating gliosis in the visual cortex of sleep-deprived rats.

Introduction

The eye which is a special sense organ made up of three tunics; an outer fibrous layer of connective tissue which incorporates structures such as the cornea and sclera, a middle vascular layer composed of structures such as the iris, ciliary body and choroid and the final innermost layer of the retina (Remington and Goodwin 2021). The retina of the eye, like the brain and spinal cord, is considered as a part of the CNS and as such is naturally rich in lipids (Pende et al. 2020). The neural tissue of the retina, by complex biochemical processes is responsible for the generation of neural signals from light rays, these signals exit via the optic nerve and are relayed onto areas of the brain for processing. it achieves this by the means of the visual pathway which includes the retina, optic nerve, optic chiasm, optic tract, lateral geniculate nucleus (LGN), optic radiations and striate cortex. The central visual system is located within the central nervous system and can be said to encompass all the structures within the CNS which can be said to be associated with the provision of the visual sensation. It involves the primary and associate visual cortices along with the visual pathway. The primary visual cortex (Brodmann's area VI) also known as the striate cortex serves as the point of termination of the visual pathway is located almost entirely on the medial surface of the occipital lobe with just a small portion extending around the posterior pole towards the lateral surface (Remington and Goodwin 2021).

The eyes of vertebrates are complex sensory organs which consist of multiple distinct tissues which are of its own unique biochemical composition, structure, and physiological function. Defects in these regions either of genetic of acquired origin is capable of compromising vision. Complex neurosensory tissue of the eye known as the retina which is composed of about six neuronal cell types organized into distinct cell layers with associated glia such as Müller cells, microglia, and astrocytes. The lipid portion of the eyes serves various functional purposes, amongst the various functions of the lipids specific to the

eye includes the anti-inflammatory and neuroprotective characteristics in the development of age-related macular degeneration (ARMD) and retinal aging (Georgiou and Prokopiou 2015). Docosahexaenoic acid (DHA) which is a long-chain polyunsaturated fatty acid making up a significant portion of the body lipids (12–20 %) of human fatty acids andmore than 30% in rodent phospholipids) is also directly involved in the photo-transduction pathway (Zárate et al. 2017). Docosahexaenoic acid enhances the ability of the photo-pigment rhodopsin to change to the active state (Murray et al. 2022). Cells of the retinal pigment epithelium (RPE) are constantly subjected to oxidative stress due to their close proximity to the surrounding choroidal capillary network, and this "docosanoid" product may protect them as well as enhance photoreceptor cell survival (Jain 2019). In the retina, lipids play a critical role, the outer segment of rods and cones contains a high concentration of phospholipids. LDL (low density lipoprotein) particles play an important role in retinal lipid delivery (Colijn et al. 2019).

Lipid homeostasis has a direct impact on the health and functioning of the neurological system. To put this in perspective, the brain constitutes roughly 60% dry weight lipid (Ralhan et al. 2021), and inflammation and lipid processing are finely intertwined to regulate homeostasis and immunity (Glass and Olefsky 2012). The role of lipid droplets in the brain has only lately received attention, despite the fact that lipid homeostasis has been extensively researched in many neuro-pathologies. The presence of lipid droplets in cells of the nervous system during early development, aging, and neuro-pathologies has been established (Ralhan et al. 2021). Small molecule co-transmission" (Nusbaum et al. 2017) has recently been proposed, which suggests that membrane compounds regulate neurotransmitter signalling independent of, and in conjunction with, transmitter receptors. Long-chain polyunsaturated fatty acid, DHA is found in the CNS including the eyes and is known to have a key role within the visual system, neurotransmission at synapses and within the brain. For the maturation of visual functions, these lipids are essential (González de San Román et al. 2018).

Sleep is a fundamental requirement to maintain a healthy lifestyle and yet, millions of people across the world are sleep deprived. Sleep/Somnolence, derived from the Latin word *somnus*, the German words *sleps, slaf*, or *schlaf*, and the Greek word *hypnos* (Chokroverty 2017), is a naturally recurring state of mind and body, characterized by altered consciousness, relatively inhibited sensory activity, reduced muscle activity and inhibition of nearly all voluntary muscles during rapid eye movement sleep, and reduced interactions with surroundings (Padmavathi et al. 2019). Sleep requirement for an average adult is approximately 7½–8 h regardless of environmental or cultural differences (Watson et al. 2015). Sleep deprivation (SD) is a general term to describe a state caused by inadequate quantity or quality of sleep, including voluntary or involuntary sleeplessness and circadian rhythm sleep disorders. Sleep disruption can also be caused by the use of stimulants or health problems, including stress or sleep-related disorders (Gaine et al. 2018). There are two types of normal human sleep: rapid eye movement sleep (REM) and non–REM (NREM) sleep, which alternate cyclically throughout a sleep episode (Devi et al. 2015). There is no precise time when sleep begins—there are gradual changes in numerous behavioural and physiologial characteristics such as electroencephalogram (EEG) rhythms, cognition, and mental processing, including reaction time (Chokroverty 2017). Sleep disorders, SD can lead to altered cellular responses, oxidative stress, and increased levels of stress hormones, such as norepinephrine and cortisol

(Li et al. 2018a), decreased wake maintenance signals, impaired attention and/or sensory system performance, decreased task-related activation, and greater lapses (vigilance failure), reduced visual processing speed, reduced distractor suppression, reduced attention selectivity in the visual cortex (Chee 2015). Reduction of aqueous tear secretion or increased evaporation of tear film induced by disruption of the lipid layer results in tear film instability and in the disruption of homeostasis at the ocular surface leading to dry eye syndrome (Lee et al. 2014).

Tramadol, (1RS;2RS)-2-[(dimethylamino)methyl]-1-(3- methoxyphenyl)-cyclohexanol hydrochloride (TrHC) is an opioid drug that, unlike classic opioids, also modulates the monoaminergic system by inhibiting noradrenergic and serotoninergic reuptake (Bravo et al. 2017). For this reason, tramadol is considered an atypical opioid. These special pharmacological characteristics have made tramadol one of the most prescribed analgesic drugs to treat moderate to severe pain (Bravo et al. 2017). The main metabolite of tramadol, O-desmethyl tramadol (M1), acts on the μ-opioid receptor as a weak agonist and acts on serotonergic and noradrenergic nociception (Miotto et al. 2017). Increased misuse potential of tramadol can be attributed to its attractive euphoric, stimulant, and calming properties (Miotto et al. 2017). Limited studies in both human and animal experiments exist which have linked the impacts of chronic sleep deprivation to impairment or disruption in visual processes. For instance, the visual system was resilient to the stress of a 65-hour sleep loss. However, after 48 hours of sleep deprivation, there was a reduction in visual efficiency (Quant 1992). Although Lee et al. (2002) did report that a 37-hour total sleep deprivation induces a significant decrease in visual discrimination, which correlated with sleepiness and fatigue in the subjects (Lee 2002), the biological mechanisms have so far not been adequately explored. Our present study therefore examined the impacts of tramadol administration on ocular lipid and inflammation profiles as well as glial responses (astrogliosis and microgliosis) in the visual cortex of periadolescent rats subjected to and without paradoxical sleep deprivation.

Materials And Methods

Animals

Sixty (60) female Wistar rats weighing 80 – 100 g obtained from ABUAD Animal Research Centre were housed in standard plastic cages at room temperature under a 12/12-h light-dark cycle with rat chow and water *ad libitum.* All experimental procedures were conducted in line with the National Institute of Health Guide for Care and Use of Laboratory Animals (Nih et al. 2011). Animals discomfort and pain were highly minimized. The study was approved by the Research Committee on Animal Use and Experimentation of Afe Babalola University, Nigeria (AB/EC/21/02/08).

Experimental design

At the end of the one-week acclimatization, rats were weighed and randomly assigned into six (6) groups and treated as follows:

i. Sleep deprivation (SD) group was exposed to the multiple platform-over-water paradigm (MPOWP) for 45 days (8 h/day), allowed free access to rat chow and drinking water.
ii. Control (CTRL) group received 0.5 mL of normal saline for 45 days with *ad libitum* to food and water.
iii. Tramadol continuous (TMD-C) group received oral administration of tramadol (Micro Organics Pharmaceuticals, India) at a dose of 50 mg/kg for 45 days.
iv. Tramadol withdrawal (TMD-W) group received oral administration of tramadol at a dose of 50 mg/kg up until the 30th day.
v. The Sleep Deprivation plus Tramadol-Continuous (SD+TMD-C) group was exposed to the MPOWP and simultaneously treated with tramadol (50 mg/kg, orally) for 45 days.
vi. Sleep Deprivation plus Tramadol-Withdrawal (SD+TMD-W) group was exposed to the MPOWP and simultaneously treated with tramadol (50 mg/kg, orally) up until day 30.

Following all treatments, animals were euthanized and the whole eye and brain tissues harvested for analyses (see experimental timeline, Figure 1).

Induction of paradoxical sleep deprivation

Paradoxical sleep deprivation was established using the “multiple platform-over-water” paradigm as previously described with slight modifications (Edem et al. 2022), where animals were sleep deprived 8 hours/daily (9:00 hr to 17:00 hr) for 45 days. Briefly, acrylic cylinders (platforms) with a diameter of 5 cm were attached to the base of a transparent plastic container, the tank (30 cm H; 60 cm L; 25 cm) with the aid of a high-bonding, non-irritant, odour-free adhesive. A 4-cm distance was allowed between the cylinder platforms in the tank. The tanks contained 6 platforms each Afterwards, clean water was poured to 2 cm below the cylinder platforms. A rat was placed on each of the platforms. As typical of the REM stage (paradoxical) sleep, a rat that sleeps and enters the REM stage will undergo muscle atonia which will culminate in it falling into the water at the base of the tank, and because of its natural aversion to water will jerk itself back onto the cylinder platforms. This ‘falling off and jerking back up’ experimentally models the so-called paradoxical or REM-sleep deprivation paradigm.

Euthanasia and tissue collection

At the end of the experiment, the rats were euthanized with a single dose of ketamine hydrochloride (100mg/kg, (Overmyer et al. 2015); and the whole eyes and brain issues were harvested and preserved appropriately for biochemical and immunohistochemical investigations.

Biochemical assessments

Animals for biochemical studies were perfused with PBS only, and the ocular tissues collected and frozen for analyses. Frozen ocular tissues were carefully homogenized in PBS (pH 7.4). The homogenates were centrifuged for 10 min at 3000 xg (times gravity) to yield a solid residue that was discarded, and the low-

speed supernatant was kept for subsequent analyses. The supernatant obtained was used to assay for levels of ocular lipid profile markers (high-density lipoprotein, low-density lipoprotein, total cholesterol, triglycerides levels); malondialdehyde (MDA) levels (marker of lipid peroxidation); apolipoprotein E (ApoE) levels; and nuclear factor kappa B subunit 1 (NFKB1) levels.

Estimation of ocular lipid profile

Ocular tissue total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) levels were evaluated using ELISA kits (Fortress Diagnostics Limited, UK). The assay protocol for ocular lipid profile was established as follows: For total cholesterol levels (product code: BXC0261); 10 µL of DDH_2O and 10 µl of sample were into different test tubes with 1000 µl cholesterol reagent introduced into the test tubes and mixed well. The mixture was incubated at 37 °C for 5 minutes. The absorbance of sample/standard was compared to reagent blank and recorded with the endpoint remain steady for an hour. For triglyceride levels (product code: BXC0271); the same protocol was followed for triglyceride levels but using triglyceride reagent. For HDL Cholesterol (product code: BXC0422A); 100 µl of DDH_2O, 100 µl of standard R4 and 100 µl of sample were pipettes into reagent blank, standard, and sample test tubes respectively. 1ml of cholesterol reagent were added to each test tubes mixed and incubated for 5 minutes at 37 °C. The absorbance was read at 500 nm. Low-density lipoprotein level was estimated using the formula:

$$\text{LDL} = \text{total cholesterol} - \frac{\text{triglycerides}}{5.0} - \text{HDL Cholesterol (in mg/dl)}$$

Evaluation of ocular lipid peroxidation

Ocular lipid peroxidation (determined by the measure of malondialdehyde (MDA) levels in the tissue) was assayed as previously reported (Oyenihi et al. 2017) . This assay is based on the reaction of a chromogenic reagent, 2-thiobarbituric acid, with MDA. One molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid via a Knoevenagel-type condensation. The assay procedure for lipid peroxidation in ocular tissue was set up as follows: 0.5g tissue was homogenized in 2.5 mL protein precipitation reagent. The prepared standard (200µl of standard and 200µl of indicator solution) and samples (200µl of sample and 200µl of Indicator solution) were added to the glass test tubes and thoroughly mixed. The mixture was heated at 65 °C for 45 minutes then 300 µl was transferred into the microplate. For MDA content, the absorbance value was determined at 532 nm with pink colouration and expressed in µM.

Determination of ocular apolipoprotein E (ApoE) and nuclear factor kappa B subunit 1 (NFKB1) levels

Using enzyme immunosorbent assay (ELISA), ocular ApoE and NFKB1 levels were measured with the appropriate ELISA kits for NFKB1 (CAT NO ER0599; Fine Test, Hubei, China) and ApoE (CAT NO ER0353; Fine Test, Hubei, China), in accordance with the manufacturer's instructions.

Immunohistochemistry

Animals for histological studies were transcardially perfused with phosphate buffered saline (PBS, pH 7.4) to clear the blood vessels and prevent cell shrinkage, then with 10% buffer formal saline (BFS) which served as a pre-fixative. With the aid of the atlas of Rat Brain in Stereotaxic Coordinates (Franklin and Paxinos 2008), area of focus (visual cortex) on the same hemisphere across the groups, were mapped, and grossed. Grossed out brain regions were then post fixed for 24 h in 10% BFS awaiting further processing. After post fixing the tissues for 24 hours, they were cut in PBS 50 µm-thick using a vibratome (VT1200 S; Leica). Afterwards, sections were stored in cryoprotectant (30% ethylene glycol and 30%glycerol in PBS) at −20˚C. Microglial and astroglial expressions were determined by immunohistochemical staining using rabbit anti-IBA1 primary antibody (CAT NO FNab04096; Fine Test, Hubei, China) and anti-GFAP primary antibody (CAT NO FNab03427; Fine Test, Hubei, China) diluted 1:100 of blocking solution. After antigen retrieval with Tris-buffered saline (TBS; 50 mM at pH 7.4), endogenous peroxidase activity was blocked by incubation in avidin–biotin-peroxidase complex (ABC; 1:100 in TBS). Nonspecific binding sites were blocked with a solution containing 10% foetal bovine serum (FBS), 3% bovine serum albumin (BSA) and 1% Triton X100. Goat Rabbit-DAB (diaminobenzidine) (Poly-HRP) (1:200 in TBS; CAT NO IHC0007; Fine Test, Hubei, China) was applied as a secondary antibody. Staining was performed with CitriSolV, and coverslipped with dibutylphthalate polystyrene xylene (DPX). Sections were photographed using an OPTU-EDU light microscope. With the aid of ImageJ, astrocyte and microglia counts were determined from 10-15 visual cortex tissue sections, obtained at x20 magnification (Schneider et al. 2012).

Statistical analyses

Statistical analyses were conducted using GraphPad Prism software (v. 9.0) for Windows, GraphPad Software, San Diego, California USA (www.graphpad.com). A parametric one-way ANOVA test was performed and followed by a Bonferroni post-hoc test to make specific comparisons between groups; SD vs CTRL, SD vs SD + TMD, and CTRL vs TMD and all the other figures. Differences were considered statistically significant at $p < 0.05$. All reported values are mean ± standard error of the mean (S.E.M.) represented using bar charts and graphs

Results

Tramadol administration ameliorates sleep deprivation-induced ocular lipid dysfunction

Results from the lipid profile analyses reveal a significant increase in ocular total cholesterol (TC) levels in the SD group (****$p<0.0001$) and TMD-C (**$p<0.01$) when compared to CTRL. Treatment with tramadol produced significant decrease in ocular TC levels in the SD+TMD-C (**$p<0.01$) and SD+TMD-W (***$p<0.001$) groups compared to the SD group. Ocular triglyceride (TG) levels revealed a significant increase in the SD (***$p<0.001$) group compared to CTRL. Administration of tramadol produced significant decrease in the SD+TMD-C (*$p<0.05$) and SD+TMD-W (**$p<0.01$) groups when compared to the SD group. Ocular high-density lipoprotein levels were significantly decreased in the SD (****$p<0.0001$), TMD-C (***$p<0.001$) and TMD-W (***$p<0.001$) groups compared to the CTRL. Tramadol treatment

revealed a significant increase in ocular HDL levels of SD+TMD-C (*p<0.05) group compared to the SD group. Ocular low-density lipoprotein levels were significantly increased in the SD (****p<0.0001) and TMD-C (*p<0.05) groups when compared to the SD group. SD+TMD-W (***p<0.001) when compared to the SD group. Lastly, tramadol treatment revealed a significant decrease in ocular LDL levels of SD+TMD-C (**p<0.01) and SD+TMD-W (**p<0.01) groups when compared to the SD group (see Figure 2).

Tramadol treatment reverses ocular lipid peroxidation in sleep-deprived rats

Results from the evaluation of lipid peroxidation by measuring ocular MDA levels reveals a significant increase in TMD-C (****p<0.0001) and TMD-W (**p<0.01) when compared to CTRL. Treatment with tramadol produced significant increase in SD+TMD-C group (***p<0.001) and not in the SD+TMD-W group, when compared the SD group (see Figure 3).

Tramadol administration modulates ocular ApoE levels in sleep-deprived rats

Results from the ocular ApoE analysis reveals a significant increase in SD (**p<0.01) and a non-statistically significant increase TMD-C when compared to CTRL. Treatment with tramadol produced a significant decrease in SD+TMD-C (**p<0.01) and SD+TMD-W (*p<0.05) groups, when compared to the SD group (see Figure 4).

Tramadol mitigates ocular NFKB1 activation in sleep-deprived rats

Results from the ocular NFKB1 analysis reveals a highly significant increase in SD (****p<0.0001) and TMD-C (**p<0.01) when compared to CTRL while TMD-W showed a slight increase but not statistically significant. Treatment with tramadol produced a highly significant decrease in SD+TMD-C (****p<0.0001) and SD+TMD-W (**p<0.01) group, when compared the SD group (see Figure 5).

Tramadol abrogates sleep deprivation-induced astrocytic activation in the visual cortex

Immunohistochemical expression of GFAP in the visual cortex reveals varying degrees of astrocytic activation with sleep deprivation and amelioration following tramadol treatment. Astrocyte count shows significant increase in astrocyte count in the SD (**p<0.01), and TMD-C (p<0.05) groups when compared to CTRL. Treatment with tramadol significantly reduced astrocyte count in the SD+TMD-C and SD+TMD-W (**p<0.01) groups when compared to SD group (see Figure 6).

Tramadol ameliorates microgliosis following sleep deprivation in the visual cortex

IBA1 in the visual cortex reveals varying degrees of microglial activation with prominent large cell-body microglia upon sleep deprivation as against the predominance of quiescent/surveying microglia in the CTRL group. Treatment with tramadol produced attenuated the sleep deprivation-induced activation. Microglia count shows significant increase in astrocyte count in the SD group compared to CTRL

(**p<0.01). Treatment with tramadol significantly reduced astrocyte count in the SD+TMD-C group compared to SD group (*p<0.05) (see Figure 7).

Discussion

Sleep loss is considered a silent epidemic in our modern society with far-reaching effects on various health systems (Naiman 2017). Sleep is a fundamental biological requirement for all life forms as the optimal functioning of all biological pathways thrive with adequate sleep (Zhang et al. 2022). Amongst the most studied effects of sleep deprivation (SD), the cognitive system is highly impacted by the insidious effects of SD. An important element of cognition is the visual system which functions as a crucial part of achieving and sustaining cognitive expressions (Cavanagh 2011; Roelfsema and de Lange 2016). Sleep disruption has been linked to stimulant usage in an effort to compensate for the performance deficit it creates. Tramadol is one of such stimulants, an opioid which possesses unique sympathomimetic actions (Gaine et al. 2018), normally employed in the management of chronic pain. Although its full mechanisms of action remain largely inconclusive, however it possesses a weak affinity for μ-opioid receptors as well as inhibit monoaminergic reuptake within the CNS, thus producing a sympathomimetic effect and the activation of the PI3K/Akt signalling pathway which has been shown to be of neuroprotective property (Franceschini et al. 1999; Walder et al. 2001; Nagakannan et al. 2012). We therefore investigated with the present study the impacts of tramadol treatment on lipido-inflammatory responses in the visual system of sleep-deprived rats.

Apolipoprotein E (ApoE) is one of the various apolipoproteins associated with all forms of lipoproteins as it plays a role in the regulation of hepatic clearance as well as receptor ligands to specific cell surface receptors including the low-density lipoprotein (LDL) receptor family along with heparin sulphate proteoglycans (HSPGs) (Hagberg et al. 2000; Mahley and Huang 2006; Sienski et al. 2021). Depending on the biological milieu, cholesterol can be a double-edged modulator in almost all the vital organs including the cardiovascular system (CVS), where it has been well studied (de Chaves and Narayanaswami 2008). However, when it comes to the CNS including the visual system, cholesterol is still an enigma. Although some of the fundamental functions of cholesterol have been explored in the CNS, not much has been done regarding the role of cholesterol and its close mediator, ApoE, particularly in the visual system. In a study that evaluated the impact of SD on some lipid-metabolism-related genes including ApoE, SD downregulated the expression of ApoE gene in the lacrimal gland (Li et al. 2018b). Meanwhile, evaluation of ApoE levels in the whole eye tissue as was done in our study revealed an upregulation of ApoE levels in the whole tissue. This is in not in agreement with results from (Li et al. 2018b), although ApoE expression was measured in the lacrimal gland. Increased activity of ocular tissue ApoE following SD could possibly be a physiological response of the eye towards maintaining its lipids and proteins compositions by optimising lipogenesis. Perhaps with short-term or overtly prolonged SD, there could be downregulation or further upregulation of ocular ApoE levels. This is not currently understood nor explored, but tramadol treatment during SD downregulated in the current decreased ocular ApoE levels. More investigations are needed to elucidate the mechanism behind this interesting changes.

Evaluation of ocular lipid profile of the SD, as well as the TMD-exposed groups showed an alteration in profile with blunted activity of the HDL and a corresponding increase in LDL and triglyceride levels. There are inconsistent reports as to the effects of lipid dysregulation on retinopathy or maculopathy. Some of these lipid profile anomalies are characteristic of certain disease conditions such as diabetic retinopathy, age-related macular degeneration (ARMD) and Stargardt disease (Prakash et al. 2016). So far not much has been reported on the impact of SD on ocular lipid homeostasis. However, Lee et al. (2014) did show in their study the destabilizes effect of SD on ocular function evident in elevated tear hyperosmolarity and reduce tear secretion. This dysregulation can potentially contribute to the development of ocular surface diseases (Lee et al., 2014). We observed in our study that prolonged SD significantly impaired lipid function processes as seen in dysregulated lipid markers examined. The possible implication of this finding could present SD as a disruptor of the corneal molecular structural integrity essential for efficient light transmission across the cornea (Garrigue et al., 2017). The corneal tear film lipid layer which consists of lipids and proteins is critical in the maintenance of tear surface tension and ocular surface hydration within physiological limits (Garrigue et al., 2017). Impaired ocular lipid profile as reported in the current study following SD can drive ocular pathologies due to alterations in corneal lipids composition.

Conversely, treatment with tramadol during SD ameliorated sleep deprivation-induced lipid dyshomeostasis whereas the nontherapeutic administration of tramadol to unstressed animals induced hyperlipidemia evident in increased ocular triglycerides and LDLs. While the mechanism behind this outcome is unclear, our results showed decreased ocular triglyceride and LDL levels, as well as upregulated HDL compared to the untreated sleep-deprived animals. This suggests the potential ability of tramadol in improving corneal dehydration, disrupted lacrimal system, and minimising the development of ocular disorders including dry eye diseases, common with prolonged sleep loss (Chen et al., 2020, Li et al., 2018). Riad and Isaac (2018) reported histopathological retinal changes including the dominance of apoptotic cells across the different layers following tramadol administration in albino rats. They observed upon tramadol withdrawal a reversal of the histopathological changes. While the mechanisms behind these changes following tramadol withdrawal were not elucidated in their study, our results revealed significant disruption in ocular lipid profile in both the withdrawal and continuous groups. Further studies are however needed to elucidate the mechanism of action.

Oxidative stress and ocular damage drive the pathogenesis of ocular disorders such as ARMD, glaucoma and diabetic retinopathy (Fletcher 2010; Nita and Grzybowski 2016; Rivera et al. 2017). Ocular injury may generate reactive oxygen species (ROS) as well as free radicals, thus suppressing the intrinsic ocular antioxidant mechanism (Nita and Grzybowski 2016). These generated free radicals and ROS undergo oxidative reactions with lipids such as the long chain polyunsaturated fatty acids of the eye and play a role in the pathogenesis of most ocular diseases (Nagakannan et al. 2012; Njie-Mbye et al. 2013). Sleep deprivation is globally known to induce oxidative stress (Li et al. 2018a), as well as a driver of lipid peroxidation evident in unusually elevated levels of the oxidant metabolite, MDA (Edem et al. 2021). In this study, ocular lipid peroxidation with increased ocular MDA levels following SD was observed, however it was ameliorated following tramadol intervention ameliorated sleep deprivation-induced sleep lipid peroxidation. Studies have confirmed the antioxidative activity of tramadol in different conditions

including renal ischemia-reperfusion injury (Şen et al. 2020), management of COVID-19 (El-Ashmawy et al. 2021), hepatic ischemia/reperfusion injury (Mahmoud et al. 2016), myocardial ischemia-reperfusion injury (Bilir et al. 2007).

Very limited studies have directly assessed the impacts of tramadol on ocular oxidative damage, however, our finding on the lipid peroxidation-inhibiting potential of tramadol does corroborate with the above cited studies. Our findings thus suggest, sleep deprivation as a potent generator of free radicals perpetuating lipid peroxidation, potentially leading to significant reductions in vitreous and retinal volumes. This sustained oxidative disruption and a corresponding impaired or suppressed antioxidant capacity following sleep deprivation is vital in the development of ocular diseases including ARMD, cataract, glaucomas, retinopathies, etc. (Ohira et al. 2008). The anti-oxidative effect of tramadol as seen in our study was significant in the continuous group, and not in the withdrawal group (compared to the SD group). It then suggests that withdrawal of tramadol during sustained stressed/sleep-deprived states minimizes tramadol's protective potency as well as its peroxyl radicals-scavenging ability. On the other hand, findings from nontherapeutic/unstressed state use showed the oxidative stress-promoting potential of tramadol. This could be explained through its ability to induce the production of free radicals in a hitherto 'clean zone', with a subsequent dampening effect on the activities of endogenous antioxidant enzymes such as glutathione, superoxide dismutase and catalase (Mohamed and Mahmoud 2019).

Insufficient sleep can provoke pro-inflammatory responses via increased cytokine secretions (Simpson and Dinges 2007). Nuclear factor kappa B (NF-κB), a protein transcription factor serves as a regulator of innate immunity, and its signalling pathway is said to link pathogenic signals and cell danger signals in an effort to organize cell resistance to invasive pathogens (Dev et al. 2010). A plethora of studies have described the NF-κB as being at the centre of a complex network of biological signalling (Albensi, 2019). It is also considered the master regulator of evolutionarily conserved biochemical cascades (Albensi, 2019), mediator of inflammation, and a regulator of inflammasome activation via the removal of damaged mitochondria (Zhong et al. 2016). Sleep deprivation has been reported to increase the activation of NF-κB in different regions of the CNS (Brandt et al. 2004). In addition, SD may induce a multi-organ dysregulation of immune signalling activities with marked activation of NF-κB (Periasamy et al. 2015; Garbarino et al. 2021). We report an upregulation of ocular NF-κB activity following sleep deprivation as well as during nontherapeutic use of tramadol. In addition to modulating the activities of NF-κB, sleep deprivation is reportedly very efficient in hijacking the regulatory mechanisms of the NF-κB signalling pathway to promote the production of pro-inflammatory cytokines(Irwin et al. 1996) including tumour necrosis factor (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) (Shearer et al. 2001; Meier-Ewert et al. 2004). This dysregulation in immune responses can potentially influence inflammatory gene expression thus increasing the risk of inflammation-related ocular diseases such as ARMD. In the ocular system, NF-κB plays a critical role in signalling from the toll-like receptors including 2, 3, 4, 5 and 7 (Kawai and Akira 2007). These receptors are expressed in different areas of the eye, including the conjunctival, limbal, and corneal epithelial cells (Lan et al. 2012). Sleep deprivation may serve as a promoter of NF-κB activation by disrupting the activities of mitogen-activated protein kinases and peroxisome proliferator-activated receptor γ (PPARγ) (Lan et al. 2012). Different studies have reported

both the pro-inflammatory and anti-inflammatory effects of tramadol(Compton et al. 2015; Mohamed and Mahmoud 2019). The anti-inflammatory effect of tramadol has been thought to be linked to the activation of pro-inflammatory cytokines however, it was discovered that the anti-inflammatory effects of tramadol was as a result of an anti-oedema effect which followed the same mechanism of action as its analgesia (Buccellati et al., 2000). These suggest a dual functionality of tramadol depending on the status of biological milieu.

The visual cortex forms the CNS division of the visual system, with the striate cortex serving as the terminal point of the visual pathway at the medial and lateral surfaces of the occipital lobe. The primary and secondary visual areas which correspond with areas 17 and 18 has their lamination in form of a six-layered pattern which differ at the level of its subdivisions most evident at the IV layer (Gabbott and Stewart 1987; Leuba and Garey 1989). Glial cells (including microglia and astrocytes) make up the majority of the non-neuronal cells that respond to assault to the brain tissue. Astrocyte-derived adenosine acts on A1 receptors located at the neural synapses serve to induce the sleep drive (Halassa et al. 2009; Frank 2019). The activities of the A1 receptor signalling have also been linked with the regulation of sleep homeostasis and in the attenuation of the cognitive deficit that occurs as a result of sleep deprivation (Halassa et al. 2009). In this study, through the immunohistochemical expression of GFAP, it could be observed that sleep deprivation triggered significant astrocyte activation within the visual cortex which comes as no surprise giving the intricate role this glia plays in sleep homeostasis. This was also the case when unstressed animals were administered tramadol. Tramadol treatment during chronic sleep deprivation as seen in our study revealed a significant decrease in astrocytic activation. This agrees with previous studies which established the ability of tramadol in downregulating astrogliosis (Leuba and Garey 1989; Sakakiyama et al. 2014; Tewari et al. 2015). In the pathogenesis of ocular conditions such as glaucoma, ocular oxidative stress has been identified as an activator of astrogliosis (Prasanna et al. 2011), including retinal astrogliosis, and this goes on to impair ganglion cell survival (Livne-Bar et al. 2013). Since astroglial calcium signalling changes dynamically in vigilance as well as sleep-deprived states (Ingiosi et al. 2020), it could then be assumed that sleep deprivation impairs sleep homeostasis and numerous other biological processes that depend on it. Tramadol treatment may therefore act by modulating the intracellular calcium signalling thereby inhibiting astrogliosis during chronic SD (Ingiosi et al. 2020).

Microglia, the resident defence cells of the CNS form the other half of an intricately designed glial partnership, involved in the innate response of the body to the sleep loss. Sleep-deprived state whether chronic or acute has been linked to being a pro-inflammatory state in the absence of an orthodox anti-inflammatory response due to injury or infection (Shearer et al. 2001). Combined with the enhanced blood brain permeability of this state, it is difficult to link the pro-inflammatory state of sleep deprivation with the activity levels of microglia (Bellesi et al. 2017). The mechanisms by which SD results in activation of microglia (as seen in our results) is similar with that of astrocytes involving the MERTK and C3 receptors with an active transition of the microglia from its resting sentinel form to its active ramified state (Bellesi et al. 2017; Frank 2019; Vainchtein and Molofsky 2020). Prolonged sleep loss or deprivation impairs cortical synaptic plasticity with enhanced microglial phagocytosis (Bellesi et al. 2017) with potential to

induce transsynaptic degeneration (TSD) in the visual cortex and the retina (Sharma et al. 2022). The obvious therapeutic action of tramadol following its administration during chronic sleep deprivation as reported in our study is possibly associated with its ability to resolve sleep deprivation-induced proinflammatory responses that drive microgliosis and neurodegeneration. While this could pass as sheer assumption, the exact mechanisms behind the 'anti-inflammatory' effects of tramadol are largely unclear, even as it has been identified that, as an opioid, tramadol's anti-inflammatory effect is unrelated to a direct inhibitory action of prostaglandins (Buccellati et al. 2000).

Conclusion

Overall, the outcomes of this study suggest that nontherapeutic tramadol use, as well as prolonged sleep deprivation can impair visual processes by dysregulating lipid and inflammation signalling pathways in the visual system; and this can potentially contribute to the development of ocular pathologies such as dry eyes diseases, glaucomas, and even ARMD. On the other hand, our findings also demonstrate for the first time the role of tramadol in improving inflammatory processes and lipid homeostasis in the visual system by modulating ocular tissue ApoE and NF-κB signalling and attenuating visual cortex gliosis in chronically sleep-deprived rats.

Declarations

Competing interests

The authors declare none

Authors' contributions

Edem Ekpenyong Edem: conceived and designed the study, provided research materials, and supervised the study. **Emem Grace James:** conducted research, provided research materials, collected, and organized data. **Collins-Kevin Chukwudi Anyanwu:** collected data and developed the first draft of the manuscript. **Mujeeb Adekunle Adedokun:** analysed and interpreted data. **Adedamola Adediran Fafure:** conducted research and data analysis, **Elizabeth Toyin Akinluyi:** provided materials and conducted research. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Figures

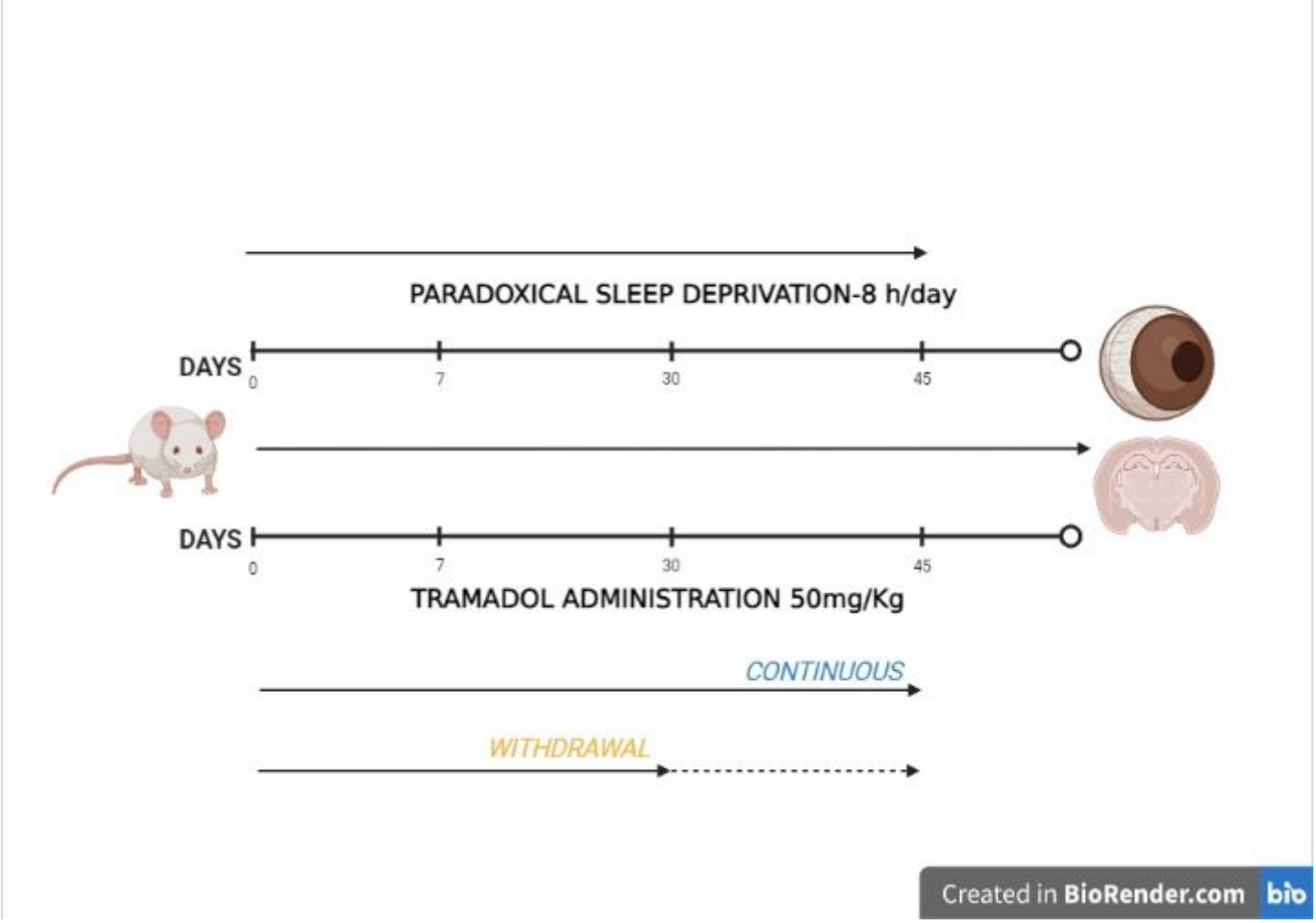

Figure 1

Experimental design

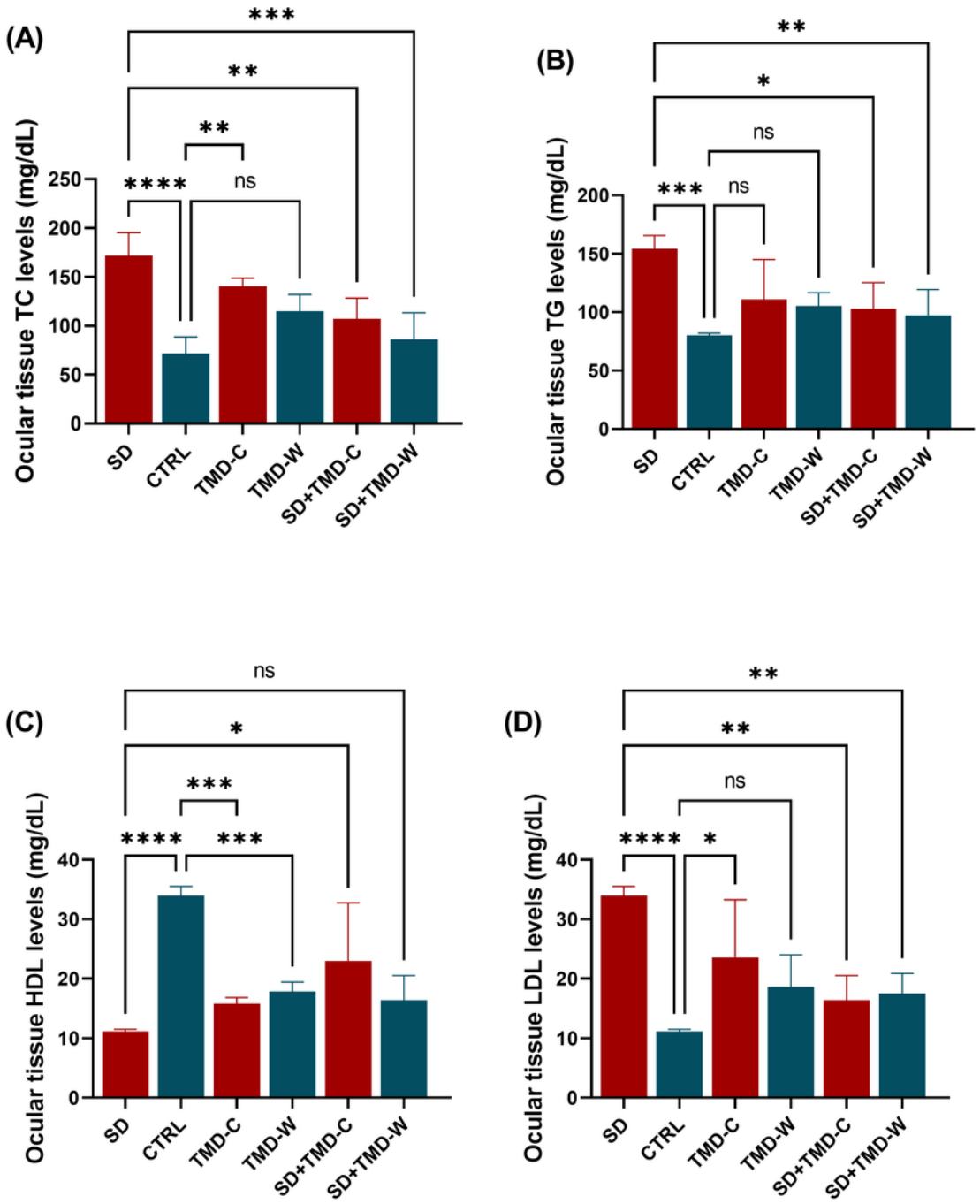

Figure 2

Effect of tramadol administration on ocular lipid-profile (A=TC; B=TG; C=HDL; D=LDL) in sleep-deprived rats. Data are expressed as ± SD; n = 4 and analysed by one-way ANOVA followed by Tukey posthoc test. *Legend: SD = Sleep Deprivation; CTRL = Control; TMD-C = Tramadol-Continuous; TMD-W = Tramadol-Withdrawal; SD+TMD-C = Sleep Deprivation plus Tramadol-Continuous; SD+TMD-W = Sleep Deprivation plus Tramadol-Withdrawal.*

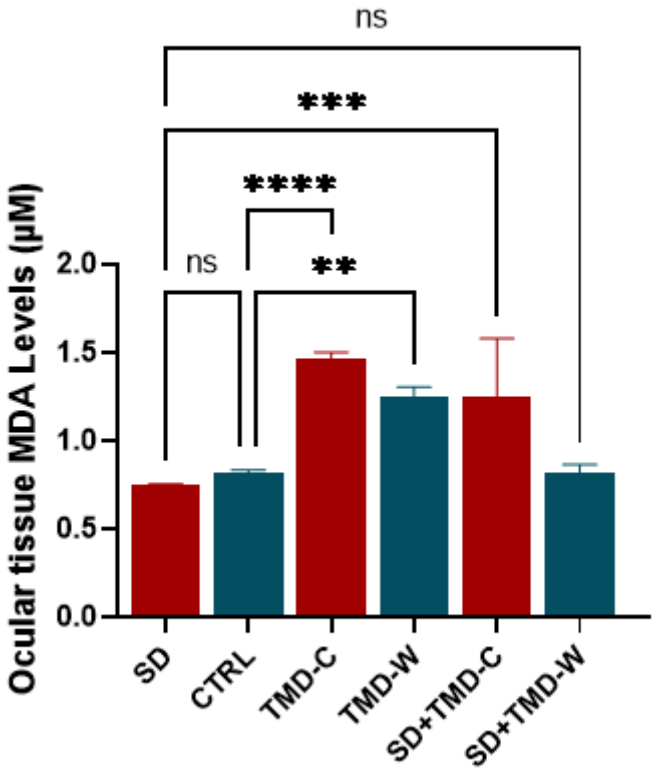

Figure 3

Effect of tramadol administration on ocular tissue MDA levels in sleep-deprived rats. Data are expressed as ± SD; n = 4 and analysed by one-way ANOVA followed by Tukey posthoc test. *Legend: SD = Sleep Deprivation; CTRL = Control; TMD-C = Tramadol-Continuous; TMD-W = Tramadol-Withdrawal; SD+TMD-C = Sleep Deprivation plus Tramadol-Continuous; SD+TMD-W = Sleep Deprivation plus Tramadol-Withdrawal.*

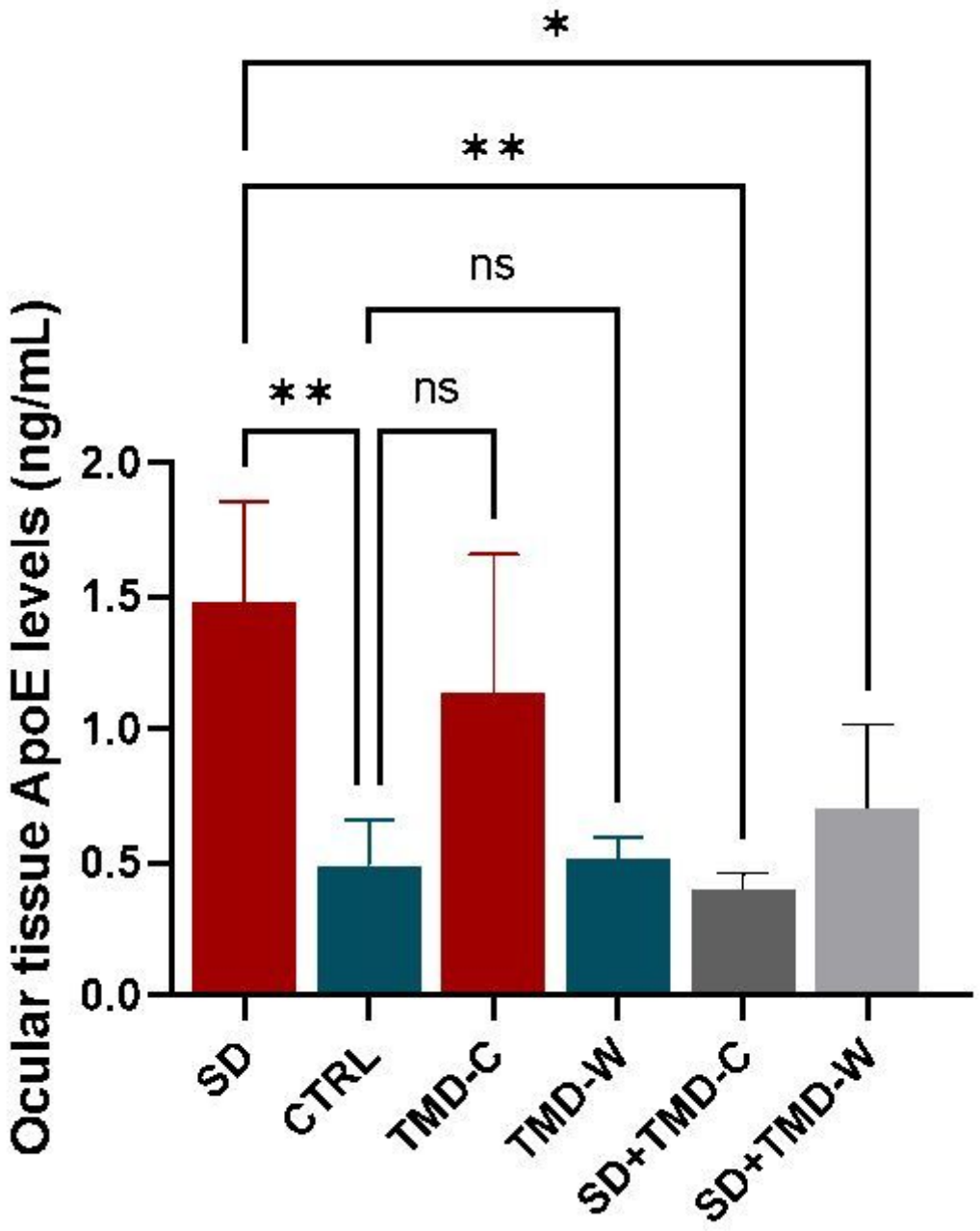

Figure 4

Effect of tramadol administration on ocular tissue ApoE levels in sleep-deprived rats. Data are expressed as ± SD; n = 4 and analysed by one-way ANOVA followed by Tukey posthoc test. *Legend: SD = Sleep Deprivation; CTRL = Control; TMD-C = Tramadol-Continuous; TMD-W = Tramadol-Withdrawal; SD+TMD-C = Sleep Deprivation plus Tramadol-Continuous; SD+TMD-W = Sleep Deprivation plus Tramadol-Withdrawal.*

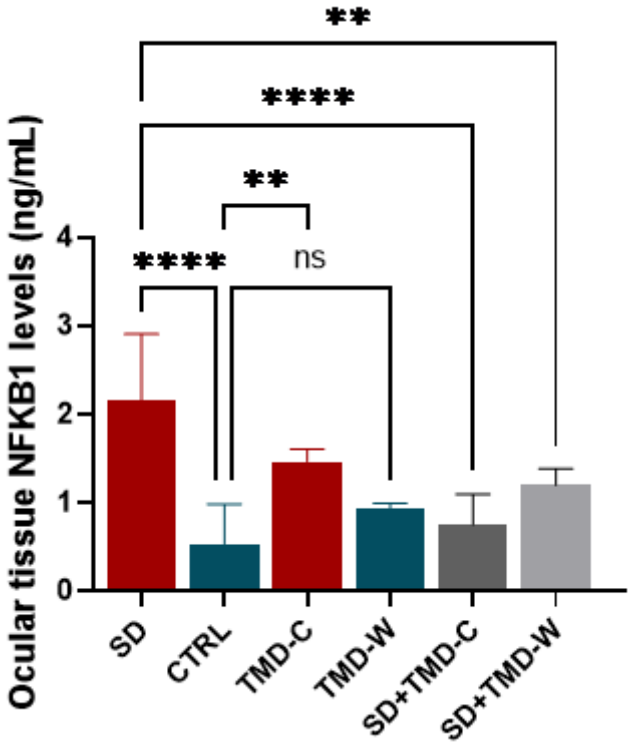

Figure 5

Effect of tramadol administration on ocular tissue NFKB1 levels in sleep-deprived rats. Data are expressed as ± SD; n = 4 and analysed by one-way ANOVA followed by Tukey posthoc test. *Legend: SD = Sleep Deprivation; CTRL = Control; TMD-C = Tramadol-Continuous; TMD-W = Tramadol-Withdrawal; SD+TMD-C = Sleep Deprivation plus Tramadol-Continuous; SD+TMD-W = Sleep Deprivation plus Tramadol-Withdrawal.*

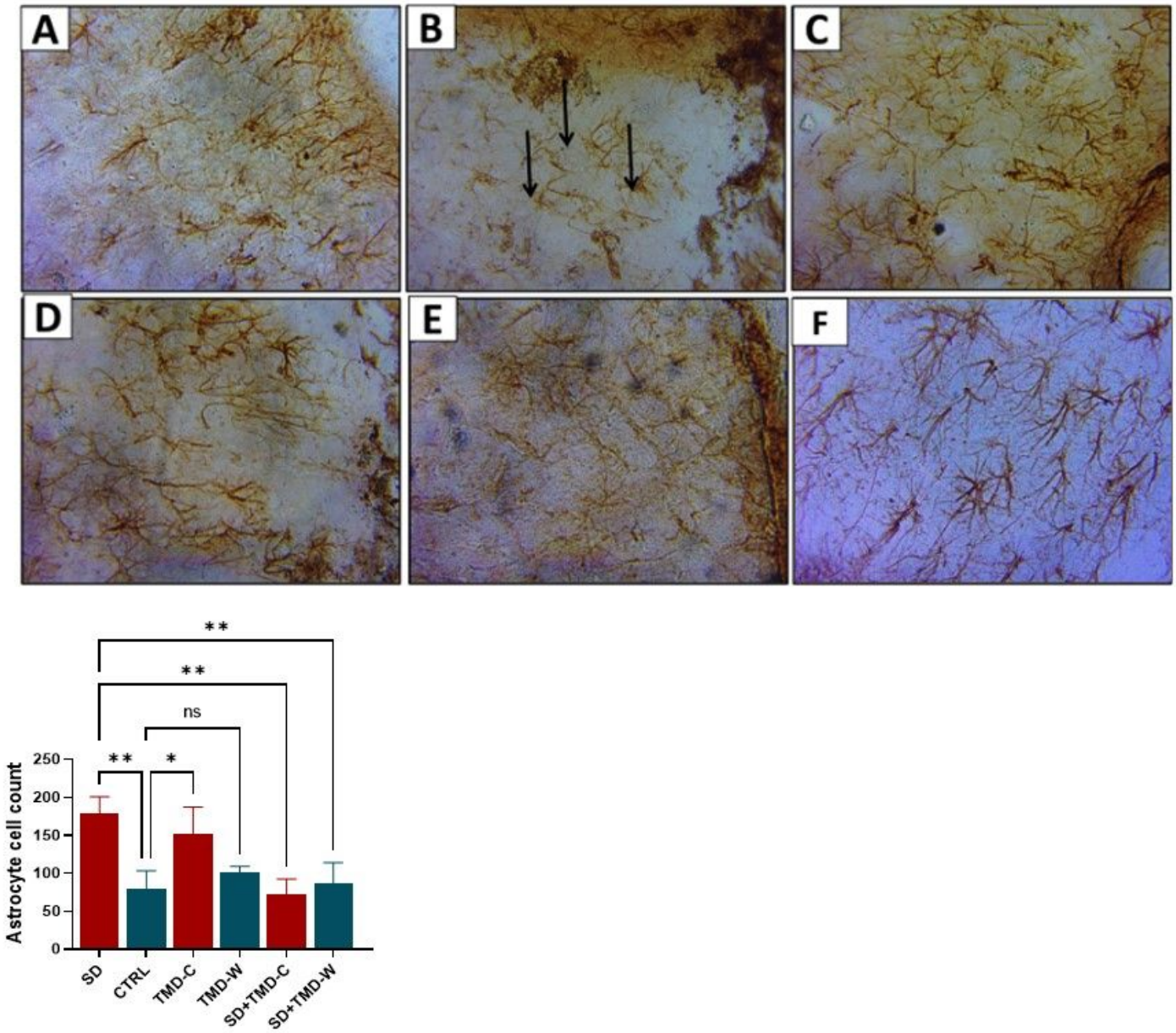

Figure 6

Effect of tramadol administration on astrocytic activity in the visual cortex of sleep-deprived rats. Mag. X800. Data are expressed as ± SD; n = 4 and analysed by one-way ANOVA followed by Tukey post-hoc test. *Legend: SD = Sleep Deprivation; CTRL = Control; TMD-C = Tramadol-Continuous; TMD-W = Tramadol-Withdrawal; SD+TMD-C = Sleep Deprivation plus Tramadol-Continuous; SD+TMD-W = Sleep Deprivation plus Tramadol-Withdrawal; Black Arrow – Astrocyte.*

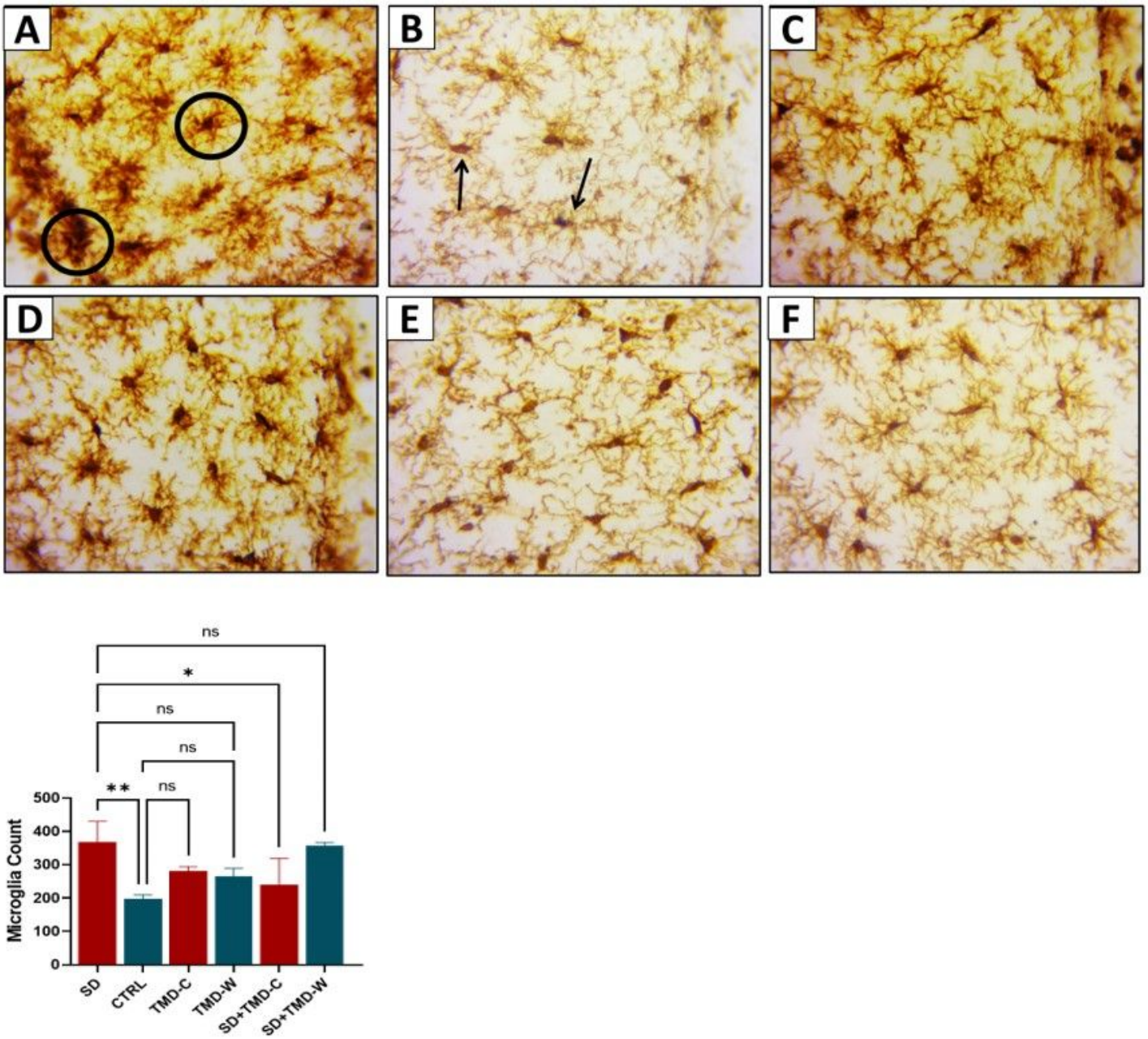

Figure 7

Effect of tramadol administration on microglial activity in the visual cortex of sleep-deprived rats. Mag. X800. Data are expressed as ± SD; n = 4 and analysed by one-way ANOVA followed by Tukey posthoc test. *Legend: SD = Sleep Deprivation; CTRL = Control; TMD-C = Tramadol-Continuous; TMD-W = Tramadol-Withdrawal; SD+TMD-C = Sleep Deprivation plus Tramadol-Continuous; SD+TMD-W = Sleep Deprivation plus Tramadol-Withdrawal; Black Arrow = Quiescent/Surveying Microglia; Black-outline Circle = Activated Microglia.*

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- floatimage1.jpeg