A Study on the Alleviating Effects of N-phthaloyl-γ-Aminobutyric Acid (P-GABA) on the Behavioral, Histopathological and Biochemical Adverse Effects of Sleep Deprivation in Zebrafish (*Danio rerio*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Sleep is defined as a reversible behavioural state of perceptual disconnection from insensitivity to the environment that facilitates the interaction of physiological and behavioural processes. Sleep Deprivation (S.D.) is defined as a decrease in sleeping duration below the recommended minimum, which has been linked to learning and memory problems.
Aim: The primary objective of this work was to determine the effect of P-GABA on metabolic parameters, behavioural changes, whole-body cortisol, and brain histology in light-induced sleep-deprived zebrafish, as well as the optimal dose of P-GABA neutralizing undesirable effects.
Methodology: The present study was conducted for ten days, consisting of three days in a row of sleep deprivation and seven days of treatment with P-GABA. The current investigation used six fishes in a group (n=6).
Group 1: Control ; Group 2: 24h Total SD ; Group 3: 48h Total SD ; Group 4: 72h Total SD ; Group 5: 24h Total SD + P-GABA (100 mg/L) ; Group 6: 48h Total SD + P-GABA (100 mg/L) ;Group 7: 72h Total SD + P-GABA (100 mg/L)

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Results: The current study provides scientific data demonstrating the positive effects of P-GABA in treating sleep deprivation and associated cognitive impairment. To test if P-GABA treatment can alleviate the cognitive and memory impairment caused by S.D., we established non-toxic concentrations and treated the zebrafish with a safe dose of 100mg/L. The use of P-GABA increased cognitive performance in the T-maze, demonstrating that it has a favourable effect in a sleep-deprivation condition. The SD group exhibited neutrophil infiltration, and this S.D fish treated with P-GABA at a concentration of 100 mg/L demonstrated a moderate reduction in neuronal cell degeneration compared to controls. The levels of biochemical parameters during sleep deprivation and treatment phase with P-GABA were checked. It was evident from the results that the SOD, CAT and GPX levels in the S.D groups were drastically decreased, whereas treatment with P-GABA could show a significant increase in the levels of biochemical parameters.

In contrast to the control group, zebrafish subjected to sleep deprivation showed enhanced AChE activity in the brain. The results of the P-GABA indicated an anti-AChE profile, which corresponds to improved memory parameters in zebrafish, as observed in the NTT and T-maze tests. When comparing the sleep-deprived fish to the control group, the MDA level, which indicates lipid peroxidation, was higher. Treatment with P-GABA considerably reduced the amount of MDA produced compared to the amount produced in sleep-deprived fish. The cortisol levels gradually increased in the single row 24h, 48h, and 72h sleep deprived groups. There was a gradual decrease in cortisol levels in the groups that received P-GABA treatment. The levels of neurotransmitters were seen to be decreased in the sleep-deprived groups when compared with the control. Upon treatment with P-GABA, the neurotransmitters were restored to near normal.

Conclusion: This study showed that P-GABA counteracts cognitive performance decrease and anxiety increase resulting from sleep deprivation through a mechanism implying mitigation of brain oxidative stress and regulation of AChE activity.

1. INTRODUCTION

It has been suggested that the master biological clock in animals is located in the suprachiasmatic nucleus (SCN) of the anterior part of the hypothalamus, which regulates physiological, biochemical, endocrine rhythms, cell division as well as visual activity, renal activity, gene regulation, blood pressure, and heart rate [1,2]. The intrinsic pacemaker activity of the SCN is regulated by environmental influences, mainly light-dark cycles, which are the most effective [3]. Recent research hypothesizes that the SCN gets photic information from the retina directly through the retinal hypothalamic tract and indirectly through the geniculohypothalamic tract from the intergeniculate leaflet thalamus [4,5]. There is evidence that night time light exposure is associated with various serious behavioural and health issues, including cancer [5]. Experiments on humans have established a relationship between night shift work (exposure to light at night) and an increased risk of breast cancer and other malignancies [6–8]. However, bright light pulses have been shown to cause phase shifts in circadian rhythms, used to treat conditions such as delayed sleep phase syndrome, seasonal affective disorder, and night work maladaptation [9-11]. Sleep is defined as a "reversible behavioural state characterized by perceptual separation from and insensitivity to the environment" that allows the interaction of physiological and behavioural processes to occur more quickly [1,5]. Sleep disturbances have been noted in 61.8% in a study cohort, a two-fold increase compared to 34.2% of the adult Indian population compared to the Chinese population [12]. Long-term potentiation (LTP) enhances synaptic plasticity and memory formation. In the context of sleep deprivation, a drop in sleeping duration below the suggested minimum has been associated with learning and memory impairments [6,7]. Zebrafish is a novel real-time model system for researching neurodegenerative illnesses and pharmaceutical discovery. According to the findings of a recent study, the neuroanatomical and neurochemical circuits of zebrafish and human brains are remarkably comparable. Physiological, emotional, and social behavioural tendencies are similar across the groups, which have also been demonstrated [13,14].

Diminished sleep can be a positive trigger for manic episodes [15]. Numerous neurotransmitter systems have been linked to behavioural changes, including dopamine (DA), serotonin (5-HT), and norepinephrine (N.E.). Dopamine (DA)

has been implicated in the pathophysiology of manic episodes since the 1970s, and alterations in dopaminergic neurotransmission have been linked to neurobiological abnormalities [16,17]. Clinical findings have established a link between dopamine and manic episodes for many years. Five-hydroxytryptamine (5-HT) is a neurotransmitter that has remained mostly unaltered throughout evolution. It regulates various physiological processes and behaviours, including cardiovascular control, pain sensitivity, eating, reproduction, cognition, impulsivity, aggression, and mood. 5-HT appears to boost wakefulness and prevent sleep through activating neurotransmitters such as AChE and noradrenaline [18,19]. Indeed, alterations in 5-HT levels have been linked to various mental diseases, including anxiety, depression, and psychosis [20]. Norepinephrine (N.E.) has long been associated with depression and manic episodes [21]. Neuroendocrine systems in the brain are thought to contribute to the pathophysiology of mood disorders.

Glutamate is the most important excitatory neurotransmitter [22,23]. Glutamate has a deleterious impact on brain physiology in circumstances of overexcitation [24,25]. Excitatory amino acid transporters (EAATs) remove the majority of glutamate from the synaptic cleft. Astrocytes are responsible for 90% of glutamate uptake [26], highlighting tripartite synapse integrity and brain function essential characteristics. The interaction of glutamate with particular membrane receptors is responsible for many neurological activities such as cognition, memory, movement, and sensation; however, excessive extracellular glutamate accumulation contributes to the progression of most neurodegenerative illnesses [27].

S.D. was induced in this study using a prolongation of the light phase followed by light pulses during the dark cycle because it has been shown to change the zebrafish's humoral innate immune system, resulting in neuroinflammation and cognitive impairment [17,18]. According to recent research, gamma-aminobutyric acid (GABA) has been identified as the fundamental neurotransmitter of the circadian system and has been implicated in the transfer of dark information to the circadian clock via afferent pathways [13-15]. Exogenously given GABA has been proven not to penetrate the blood-brain barrier [16]. Researchers overcame this by synthesizing unavailable GABA-like N-Phthaloyl GABA (P-GABA) and N-Octanoyl GABA (O-

GABA) derivatives. Both compounds act similarly to GABA, although they are more lipophilic and raise GABA levels in the brain [17]. In addition, they also have anticonvulsant and antiulcer characteristics [17-21]. Numerous GABA analogs effectively prevent a variety of stress-related symptoms in experimental studies [22]. There has been some debate about whether or not GABA affects the levels of lipid peroxidation products and antioxidants. However, this has not been thoroughly explained. The primary goal of this study was to assess the influence of P-GABA on metabolic parameters, whole-body cortisol, behaviour, and brain histology in zebrafish that had been exposed to light-induced sleep deprivation.

2. METHODOLOGY

2.1 Animals

For nine days, adult male Zebrafish (*Danio rerio*) 3 months old were acclimated. Fish were kept on a 12 h light/12 h dark cycle (150 lux intensity) with zeitgeber time (Z.T.), where lights were put on at 07:00 h and turned off at 19:00 h in a 50 L water tank (28±2°C) with six fish per tank. Fish were fed commercial fish diets (Nano fish food) three-four times a week.

The present study was conducted for ten days, consisting of three days in a row of sleep deprivation (S.D) and seven days of treatment with P-GABA. The current investigation used six fish in a group (n=6). The scheme of work is summarized in Fig. 2.

Group 1: Control
Group 2: 24h Total SD
Group 3: 48h Total SD
Group 4: 72h Total SD
Group 5: 24h Total SD + P-GABA (100mg/L)
Group 6: 48h Total SD + P-GABA (100mg/L)
Group 7: 72h Total SD + P-GABA (100mg/L)

2.2 N-Phthaloyl GABA Preparation

Otto chem India supplied GABA and phthalic anhydride. A mixture of 6.18g GABA and 8.95g finely ground phthalic anhydride was heated in an oil bath at 145-150°C for 30 minutes with stirring. The synthesized solid material was dissolved in hot methanol. According to prior reports, 20 mL of water was added to the filtrate and identified the product as previously stated [28, 29]. The synthesized P-GABA was

confirmed via UV-vis spectroscopy and its morphology was confirmed via Scanning Electron Microscopy (SEM) [Carl Zeiss, EVO 18, Germany].

2.3 Behavioral Analysis

2.3.1 Learning and memory test in a T-Maze

The T-maze is a multi-species operative task used to assess memory. The T-maze was used to explore zebrafish learning and memory activities. It was made of a transparent acrylic glass sheet shown in Fig. 1(a). The maze dimensions were 50 cm x 10 cm x 10 cm for the long arm, 20 cm x 10 cm x 10 cm for the short arm, and 10 cm x 10 cm for the start box at the stem base. The two short sides on the left and right were covered with green and red sleeves. The maze was filled to a depth of 6 cm with water, and the water temperature was kept constant at 28°C during the experiment. The time it took the fish to reach the deeper compartment was assessed as transfer latency (T.L.).

2.3.2 Light and dark test (LDT)

The light/dark preference test, as opposed to the novel tank test, investigates zebrafish exploratory behaviour in the presence of a motivational conflict between light and dark sleeves, as illustrated in Fig. 1(b). In brief, 30 minutes after the total sleep deprivation period and drug administration, the animals were transferred to the central compartment of a black and white tank (15 cm×10 cm×45 cm H×W×L) for a 3-minute acclimation period, after which the doors that delimit this compartment were removed, and the animal was free to explore the apparatus for 5 minutes.

2.4 Histology

The brains of fish were separated and fixed in Bouin's solution, then dehydrated in alcohol and included in xylene.Thin slices of 5–6 μm thickness were cut using a microtome, stained with hematoxylin-eosin and examined under a microscope for histological alterations [30].

2.5 Assay of Enzymatic Activities and Oxidative Stress Biomarkers

After collecting behavioural data, zebrafish were euthanized by 10 min immersion in cold water (2–4°C) until they stopped opercular motions and their brains were collected for biochemical parameter analysis [31]. Using a potter homogenizer, the brains were gently homogenized in cold 0.1 M potassium phosphate buffer (pH 7.4), 1.15 percent KCl. The homogenate was centrifuged at 960 g for 15 minutes. The supernatant was used to evaluate the specific activity of acetylcholinesterase (AChE), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and malondialdehyde (MDA) using the detailed methodology described by Dumitru et al. [32,33].

a)   **b)**

Fig. 1. (a) T-maze setup to assess the learning and memory; (b) Light and Dark test to assess the exploratory behaviour of zebrafish groups subjected to sleep deprivation and thereafter treated with P-GABA

2.6 Whole-body Cortisol Extraction and Analysis

The protocol for extracting cortisol from whole-body samples was adapted from [34,35]. Following the behavioural investigation, whole-body samples were taken and stored at -20°C (or lower) temperatures for biological analysis of cortisol levels. Body samples were partially defrosted, weighed, and then homogenized in 500 µL of ice-cold 1X phosphate-buffered saline (PBS) buffer. After weighing the samples (g), they were split into smaller sections on ice for efficient homogenization. The homogenizing rotor blade was cleaned with an additional 500 µL of ice-cold 1X PBS and the homogenate was collected in a 2 mL tube. The homogenizing rotor blade and probe must be cleansed with 100 percent ethanol and de-ionized H_2O between each sample. This is a critical step in minimizing sample cross-contamination. Throughout this procedure, samples were stored on ice. Each sample received 5 mL of diethyl ether. After vortexing for 1 minute, the samples were centrifuged at 3500 rpm for 5 minutes. Each sample was separated into its organic layer containing cortisol following centrifugation and deposited in a separate test tube. The procedure was performed two (or three) times throughout the trial to guarantee maximum cortisol extraction. Typically, the cortisol-containing stratum is yellowish in hue. To allow ether evaporation, samples were stored overnight in the fume hood. Alternatively, a speed vacuum centrifuge fitted with a cryotrap or evaporation to dryness under nitrogen sparge could be employed to dry the organic solvent. According to the manufacturer's instructions, cortisol concentrations were quantified using an ELISA using a human salivary cortisol test kit. Cortisol levels were standardized using the sample weight and expressed as absolute circulation cortisol concentrations.

2.7 Assays of Neurotransmitters in Brain Homogenates

2.7.1 Assays of norepinephrine and dopamine

For this test, the trihydroxyindole method was scaled down by a factor of two. To 0.02 ml of 0.4 M HCl and 0.01 ml EDTA/sodium acetate buffer (pH 6.9), 0.02 ml HCl phase was added (to the mixture 0.01 ml iodine solution was added for oxidation). After adding 0.01 ml Na_2SO_3 to 5 M NaOH, the chemical was allowed to react for 2 minutes before testing. Then, 0.01 mL of acetic acid was added to the solution and heated to 100°C for 6 minutes. After attaining room temperature, emission spectra for dopamine at 485 nm and noradrenaline at 375 nm were acquired in the microcuvette using a spectroflurophotometer.

2.7.2 Serotonin Assay

The O-phthalaldehyde method was employed to determine serotonin. OPT reagent and HCl were added to the tissue extract. The mixture was boiled at 100 °C for ten minutes to produce the fluorophore. At equilibrium, emission spectra or intensities at 470 nm were determined using a spectroflurophotometer.
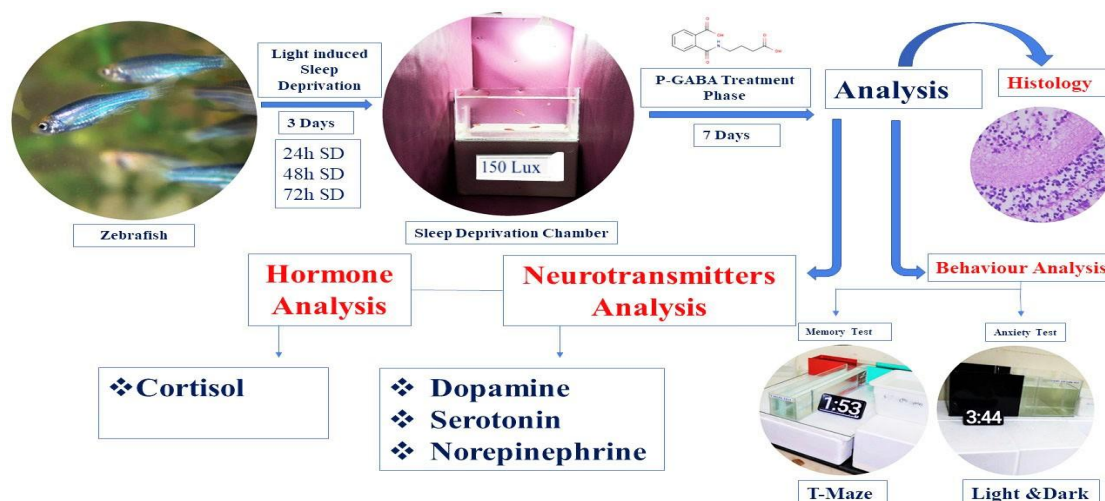

Fig. 2. Scheme of the work

2.8 Data Analysis

SPSS V23.0 (IBM SPSS, USA) software was used to analyze the data. Multiple comparisons were made using one-way ANOVA, and post hoc comparisons were made using DMRT multiple comparison tests. A *p*-value <0.05 was considered significant. The data were reported as mean±SEM.

3. RESULTS

3.1 Characterization of P-GABA

The synthesized P-GABA morphology was examined via scanning electron microscopy (SEM). The synthesized P-GABA exhibited cuboidal structural confirmation and a UV absorption peak at 266nm.

3.2 T-maze for Learning and Memory

3.2.1 Time spent in the red and green arm as a parameter

The total time spent on the green and red arm of the maze by the sleep-deprived and sleep-deprived + P-GABA treated groups has been graphically represented in Fig. 4, respectively.

3.2.2 Number of entries in the green and red arm as a parameter

The number of entries into the green and red arm: The total number of entries to the green and red arm of the T-maze by the sleep-deprived and sleep-deprived + P-GABA treated groups has been graphically represented in Fig. 5, respectively.

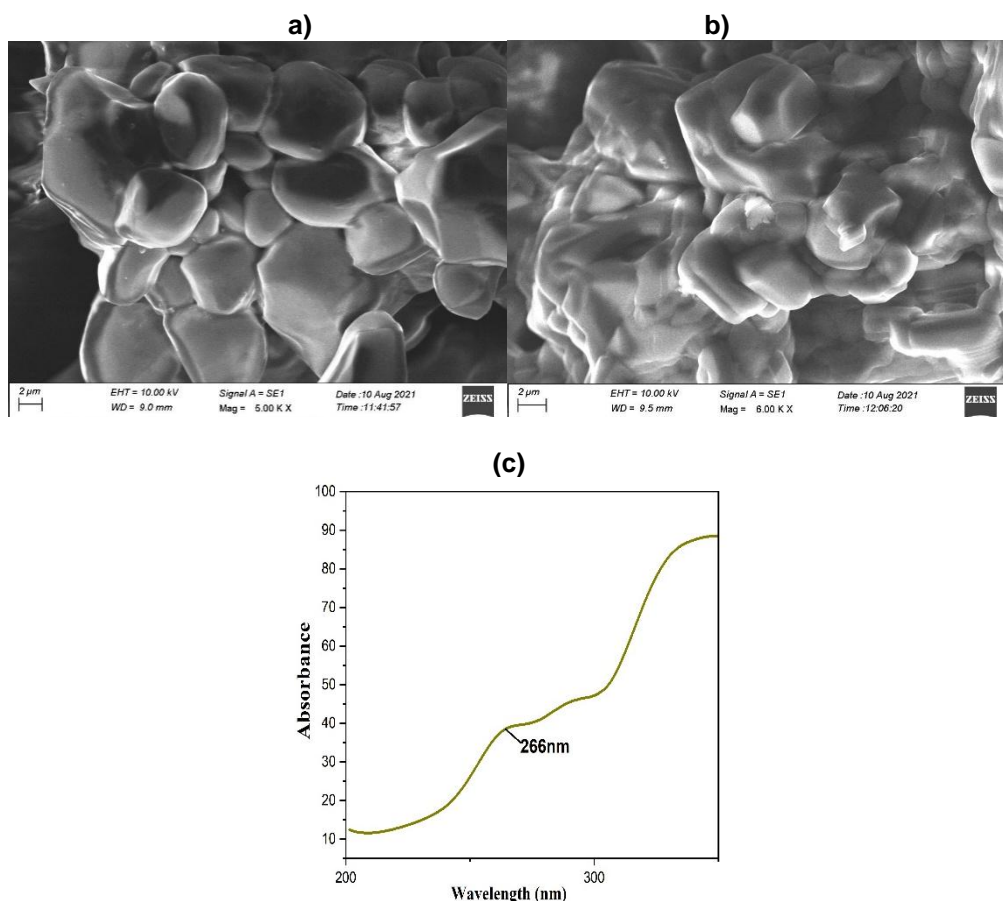a)   b)

(c)

Fig. 3 a) Shows the SEM morphology of Gamma-aminobutyric acid (GABA); b) represents the SEM morphology N-phthaloyl Gamma-aminobutyric acid (P-GABA). C) Represents the UV absorption spectrum of P-GABA
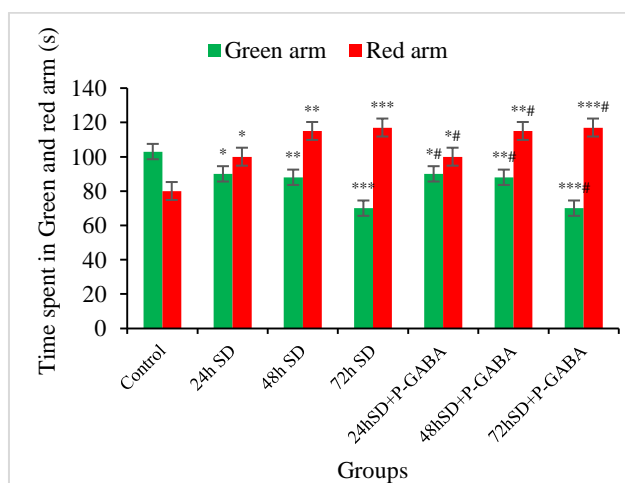
Fig. 4. Amount of time spent in the green and red arm of the T-maze by S.D and S.D+P-GABA treated groups. Values are expressed as mean±SEM and *p*<0.05 is considered significant in all groups
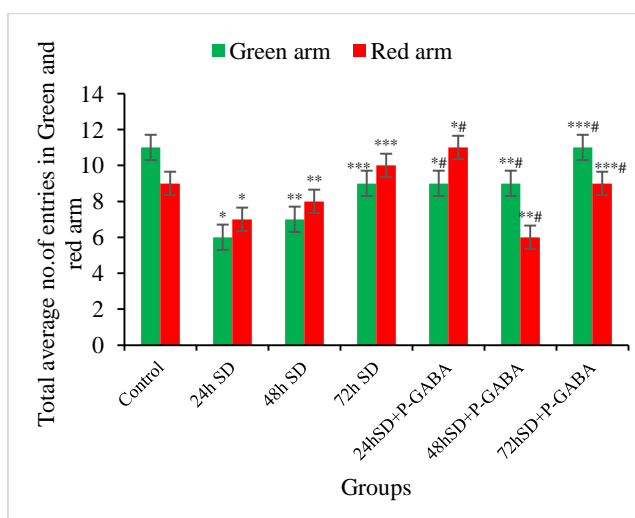

Fig. 5. The total average number of entries in the green and red arm of the T-maze by S.D and S.D+P-GABA treated groups. Values are expressed as mean±SEM and *p*<0.05 is considered significant in all groups.

3.3 Light/Dark Test for Anxiety

3.3.1 Time spent in the light and dark zone of the tank

The total time spent in the light and dark compartments of the light and dark chamber by the sleep-deprived and sleep-deprived + P-GABA treated groups has been graphically represented in Fig. 6, respectively.

3.3.2 Number of entries in the light and dark zone of the tank

The number of entries to the light and dark compartment of the light and dark chamber by

the sleep-deprived and sleep-deprived +P-GABA treated groups has been graphically represented in Fig. 7.

3.4 Histology

An investigation of the neuronal and neurophil architecture in the brain tissues of control zebrafish found no abnormalities Fig. 12(a). The SD group exhibited neutrophil infiltration and lower spine density of basal dendrites, as shown in the image below Fig. 8 (b-d). The brain of S.D. fish treated with P-GABA exhibited increased spine density of dentate gyrus granular cells and hyperplastic neurons. Additionally, SD fish

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treated with P-GABA at a concentration of 100 mg/L exhibited a moderate improvement in neuronal cell death Fig. 8(e-g).

3.5 Effect on Antioxidant Enzymatic Activities

Effects on particular activities such as SOD, CAT, and GPX, compared to the control group.Sleep deprivation, lowered SOD specific activity Fig. 9(a) in the zebrafish brain, indicating an increase in oxidative stress. The graph shows that sleep deprivation boosts the specific activity

of SOD in P-GABA treated fish administered. When zebrafish were subjected to sleep deprivation, their CAT specific activity decreased significantly Fig. 9(b) compared to the control group. However, when P-GABA was administered, CAT specific activity increased significantly in the sleep-deprived fish, indicating antioxidant properties. As a bonus, when sleep deprived groups were administered with P-GABA, the specific activity of the GPX is significantly reduced compared to the controls Fig. 9(c).
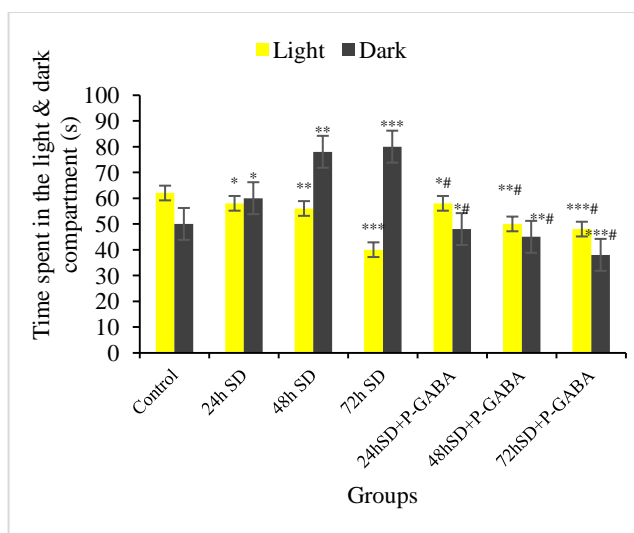

Fig. 6. Light and dark test for anxiety. The graph shows the time spent in the light and dark compartments of the S.D and S.D+P-GABA treated groups. Values are expressed as mean±SEM and *p*<0.05 is considered significant in all groups
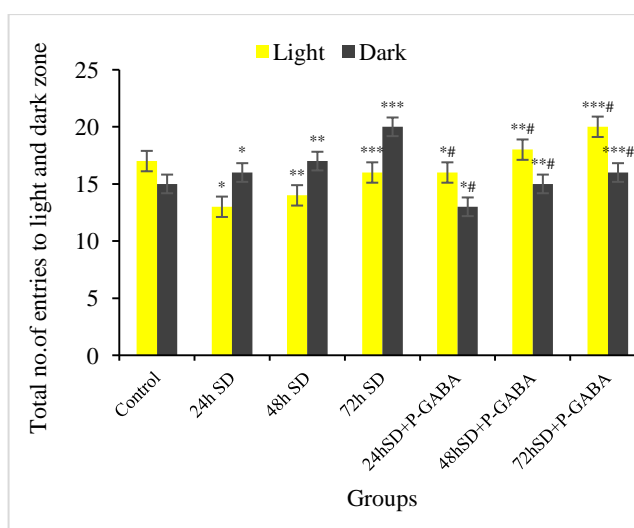

Fig. 7. Light and dark test for anxiety. This graph shows the number of light and dark compartment entries by S.D and S.D+P-GABA treated groups. Values are expressed as mean±SEM and *p*<0.05 is considered significant in all groups
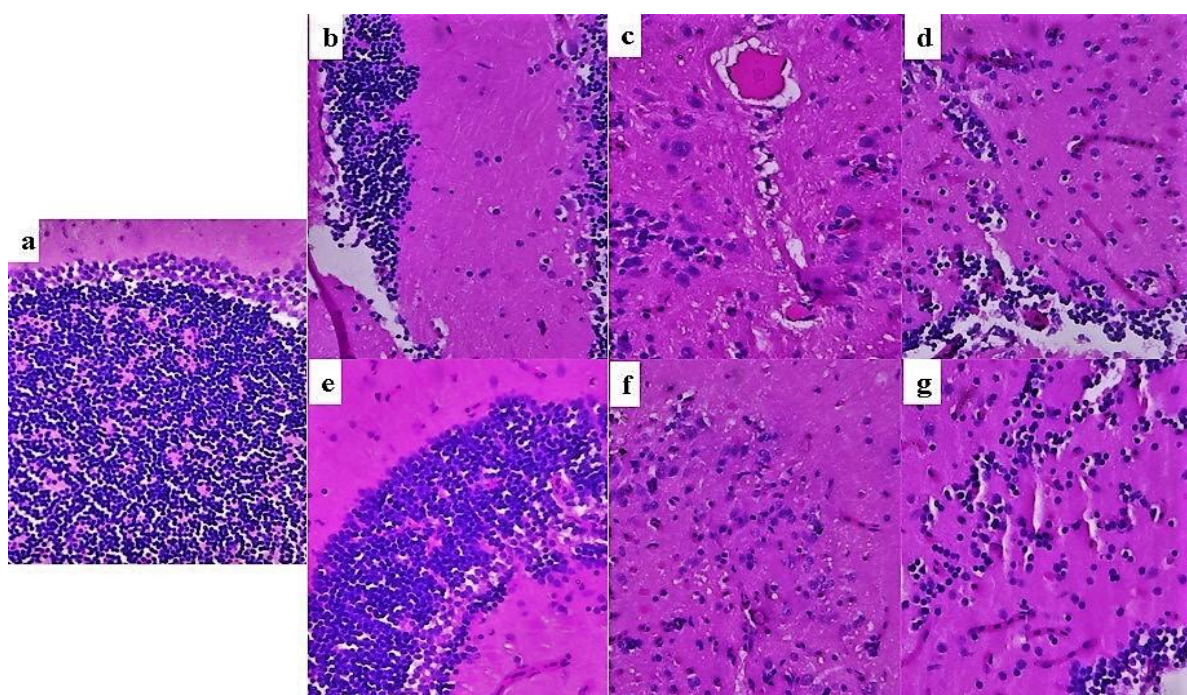
Fig. 8. Brain morphology of zebrafish subjected to S.D and S.D +P-GABA treatment. a) The neuronal and neurophil architecture in the brain tissues of control zebrafish found no abnormalities ;(b-d) The SD group exhibited neutrophil infiltration and lower spine density of basal dendrites; (e-g) P-GABA treated group exhibited increased spine density of granular cells

3.6 Effects on AChE Activity

AChE lowers the level of acetylcholine in the blood and alleviates the illness symptoms associated with the progressive loss of cholinergic function in the presence of sleep deprivation. In contrast to the control group, zebrafish subjected to sleep deprivation showed enhanced AChE activity in the brain, as demonstrated by our findings Fig. 10. The use of P-GABA dramatically lowered AChE activity when compared to the group that was sleep deprived. The results of the P-GABA study have indicated an anti-AChE profile, which corresponds to improved memory parameters in zebrafish, as observed in the NTT and T-maze tests.
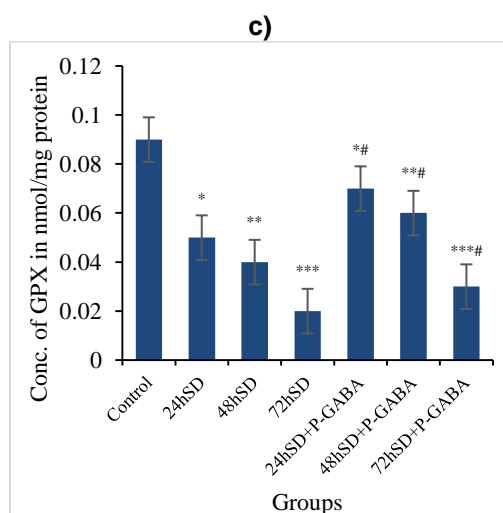
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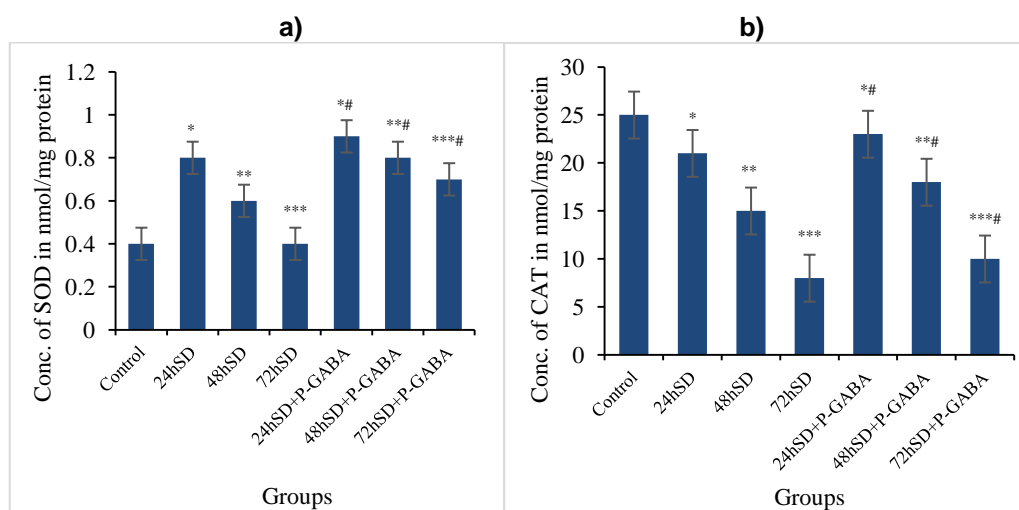
Fig. 9. Antioxidant enzyme activities in *Danio rerio* brain homogenates a) SOD (E.C No-1.15.1.1) activity (nmol/mg protein); b) CAT (E.C No-1.11.1.6) activity (nmol/mg protein); c) GPX (E.C No-1.11.1.9) activity (nmol/mg protein) in the S.D and S.D + P-GABA treated groups. Data are expressed as mean± standard error of means. Different symbols indicate the significant differences between the groups ($p< 0.05$)
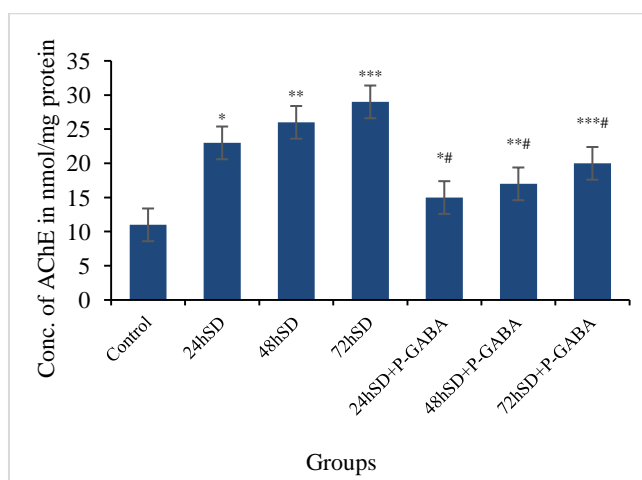

Fig. 10. AChE (E.C No-3.1.1.7) activity nmol/mg protein in S.D and S.D+P-GABA treated zebrafish brain. Data are expressed as mean± standard error of means. Different symbols indicate the significant differences between the groups ($p< 0.05$)

3.7 Effects on Malondialdehyde (MDA) Level

When comparing the sleep-deprived fish to the control group, the MDA level, which indicates lipid peroxidation, was higher. Treatment with P-GABA considerably reduced the amount of MDA produced compared to the amount produced in sleep-deprived fish Fig. 11.

3.8 Whole-body Cortisol Level

The cortisol levels gradually increased in the single row 24h,48h, and 72h sleep deprived groups. There was a gradual decrease in cortisol levels in the groups that received P-GABA treatment Fig. 12.

3.9 Effect on Neurotransmitters

Changes in the levels of neurotransmitters in the control group, 24h,48h, and 72h sleep deprivation groups, and the P-GABA treated groups have been depicted in Fig. 13.
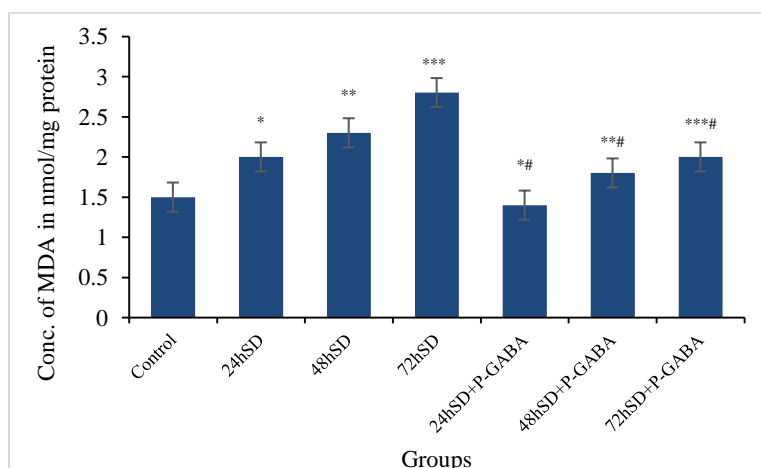
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Fig. 11. Lipid peroxidation parameter malondialdehyde (MDA) (nmol/mg protein) in S.D and S.D + P-GABA treated brain of zebrafish (*Danio rerio*). Data are expressed as mean± standard error of means. Different symbols indicate the significant differences between the groups ($p < 0.05$)

Fig. 12. The whole-body cortisol levels (ng/g) in S.D and S.D + P-GABA treated zebrafish groups. Data are expressed as mean± standard error of means. Different symbols indicate the significant differences between the groups ($p < 0.05$)

Fig. 13. The levels of neurotransmitters (ng/mg) in the brain of S.D and S.D + P-GABA treated zebrafish groups. Data are expressed as mean± standard error of means. Different symbols indicate the significant differences between the groups ($p < 0.05$)
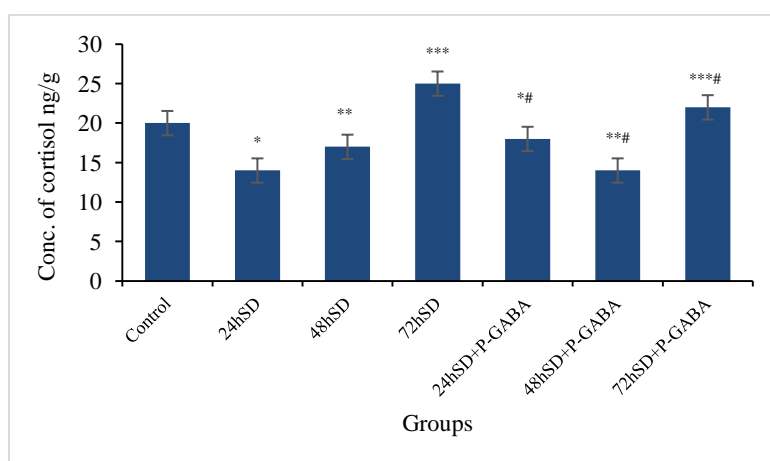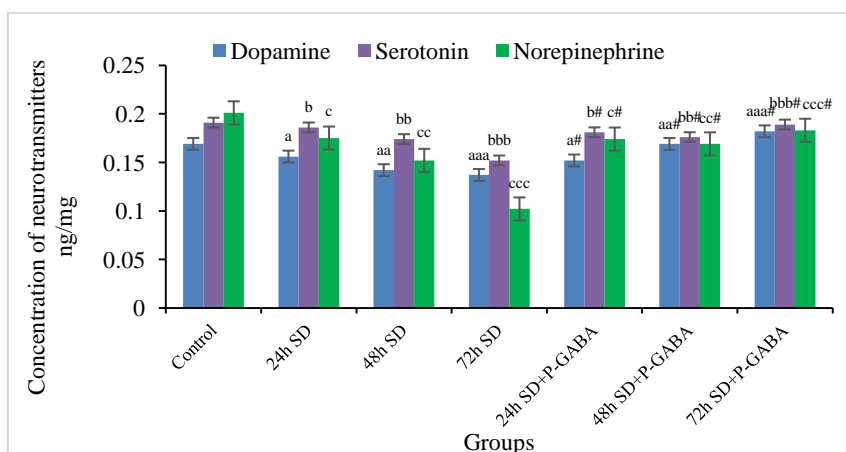
4. DISCUSSION

For the first time, evidence is presented that P-GABA has benefits in treating sleep deprivation and accompanying cognitive impairment. According to the world health organization, S.D. is one of the most significant risk factors for learning and memory impairment, and it affects approximately one out of every ten people worldwide. In specific investigations, it has been proven that the existence of a full or partial S.D. is associated with deleterious changes in cognitive performance [6,7]. A decrease in locomotor strength in both light and dark tests and higher working mistakes in the T-maze tests was observed in fish subjected to an extended light phase during the current study, indicating the induction of S.D. According to a prior study, extensive light phase exposure resulted in lower locomotor strength in light and dark tests and higher working errors in T-maze tests. During the current study, fish exposed to an extended light phase demonstrated decreased locomotor activity in light and dark tests and increased working errors in T-maze tests, indicating S.D induction. After learning a new task, the intracellular calcium (Ca^{2+}) and adenylyl cyclase activity increase, resulting in the production of the cyclic AMP (cAMP), which is produced when neurons communicate with one another [26,37]. Memory consolidation and long-term potentiation are both facilitated by the activation of the cAMP response element-binding protein (CREB), which increases the production of proteins involved in these processes [38]. Several studies have found that S.D. impairs these signalling pathways and inhibits memory formation [39]. There is substantial evidence that microglial activation promotes cognitive dysfunction and the loss of functional synapses in people with schizophrenia [40,41]. Treatment with O-GABA, a microglial activation inhibitor, has been shown to reverse cognitive impairment [42]. To test whether P-GABA treatment can alleviate the cognitive and memory impairment caused by S.D, we conducted a study establishing non-toxic P-GABA concentrations and treating zebrafish with a safe concentration of 100mg/L to determine if P-GABA treatment can alleviate the cognitive and memory impairment caused by S.D. The use of P-GABA increased cognitive performance in the T-maze, demonstrating that it has a favourable effect in a sleep-deprivation condition. To summarize, the current research indicated that P-GABA exposure had significant effects on adult zebrafish cognitive and stress-related endocrine (whole-body cortisol) responses in the

absence of other factors. In sleep-deprived zebrafish, antioxidant levels, as well as histological alterations in the brain, were measured.

Cognitive effects caused by light exposure are similar to those observed in zebrafish with a disturbed circadian rhythm [43]. P-GABA has also been shown to alleviate anxiogenic-like effects in zebrafish after exposure to a white tank and slow the progression of ageing-related cognitive deterioration [44,45]. P-GABA has been shown to have neuroprotective and antioxidant effects in zebrafish [46-48,49], suggesting that beneficial modulation of cognitive and neuroendocrine processes is shared and evolutionary conserved in both mammals and zebrafish. Aspects of the role of P-GABA in affective modulation are also evolutionarily conserved, as evidenced by the fact that it elicits substantial anxiolytic-like behaviour in zebrafish, rats and humans across a wide range of species [50-52]. Following this, zebrafish exposed to light for 48 hours and 72 hours showed heightened whole-body cortisol levels, corresponding to the elevated anxiety-like behaviour observed in this model [53]. P-GABA administration to sleep-deprived fish restored lipid peroxidation products and antioxidants to normal levels [54].

Further investigation into the specific process is required, and further study is needed now. In several studies, GABA agonists and antagonists are employed to investigate the nature of circadian rhythms and their mechanisms of action [55]. The presence of GABA in the suprachiasmatic nucleus and the lateral geniculate nucleus suggests that GABA is involved in regulating circadian rhythms [13]. We hypothesize that P-GABA treatment could modify the features of biochemical cycles, most likely by regulating the transmission in different regions nuclei across the brain. P-GABA therapy considerably reduced this rapid reduction in the sleep-deprived fish, indicating that the fish has the immediate antioxidant capacity [56]. This suggests that P-GABA has neuroprotective capabilities against oxidative stress, which is compatible with the previously documented antioxidant characteristics of GABA [57]. Previous research has indicated that the administration of GABA analogues has been demonstrated to protect against alloxan-induced hepatic and renal oxidative stress significantly [36]. The researchers have discovered that GABA possesses antioxidant properties against stomach injury in rats caused by 100 percent

ethanol consumption [57]. According to the literature, P-GABA can prevent brain oxidative damage by restoring the antioxidant enzyme activities [51]. Our findings are compatible with this hypothesis.

This study reveals that P-GABA reverses the anxiogenic-like cortisol-increasing effects of light exposure in zebrafish, indicating that this hormone has a favourable influence on stress-related neuroendocrine responses in this species. P-GABA administration has also been shown to diminish plasma cortisol levels in rainbow trout (*Oncorhynchus mykiss*) after stress, and it has been shown to lower blood corticosterone levels and anxiety-like behaviour rodent chronic immobilization stress models [58,59]. Furthermore, P-GABA has been shown to reduce the behavioural and neuroendocrine repercussions of persistent stress in mice, confirming the evolutionarily conserved interaction between this hormone and the stress axis [60-62]. Finally, our zebrafish findings support human studies that have linked P-GABA to altered cortisol signalling, those showing that P-GABA consumption lowers cortisol response to adrenocorticotropic hormone (ACTH) in humans [63]. In contrast, mouse neonatal maternal deprivation stress reduces the levels of melatonin in the pineal and hypothalamus regions [64]. In contrast, when the zebrafish were exposed to a standard 14-hour light/10-hour dark cycle, P-GABA did not affect cortisol levels in either the treated or the untreated control groups. This suggests that sleep deprivation under light exposure impairs normal brain function, corrected by P-GABA supplementation.

Increased 5-HT release during S.D. occurs independently of the presence of stress [65]. According to the literature, 5-HT abnormalities are frequently connected with mood ailments such as bipolar illness and contribute significantly to psychosis, where the stimulation of this neurotransmitter modifies the response behaviour to hallucinogens [66]. Stress-related sleep disorders are thought to be caused by a 5-HT deficiency, which may partly explain that 5-HT is implicated in suppressing sleep. 5-HT release assays demonstrate its ability to promote wakefulness in the brain. After 24, 48 and 72 hours of S.D., 5-HT levels in the brain were abnormally decreased. After treatment with P-GABA, the levels of 5-HT significantly increased in the 72h S.D. treatment group, whereas the 24h and 48h group showed results near normal to the control.

Norepinephrine (N.E.) has a wide range of actions, including the modulation of alertness. The production of N.E. by brain stem nuclei aids in developing arousal in situations that necessitate intense concentration or sympathetic nervous system activity. N.E. concentrations in the plasma and cerebrospinal fluid (CSF) are higher [67]. N.E. levels are inversely related to the severity of depression, with higher rates observed in S.D. individuals during more manic episodes of the illness. As a result of these observations, S.D. can cause hypersensitive fluctuations in N.E. levels. An excess of N.E. is thought to cause mania, whereas an insufficient amount of N.E. is believed to cause sorrow [68]. Because altered neurotransmission is a hallmark of anxiety and tremor, our findings indicate that decreased N.E. levels were seen in S.D. fishes which may be involved in the pathophysiology of altered behaviour. At the same time, the groups under treatment showed improvement and consistency in behaviour.

To summarise, this zebrafish research is vital because it supports previously published rodent and clinical findings on pro-cognitive and anxiolytic effects of GABA analogues and the link between these effects and circadian rhythms [7-10,21]. Our findings suggest that P-GABA positively impacts zebrafish cognitive and affective processes so that zebrafish models can be utilized to study how cognitive and behavioural processes interact with circadian rhythms in humans and other animals. Finally, zebrafish may prove to be a helpful tool for developing novel cognitive and affective disorders therapies that target the central GABA system due to their remarkable high-throughput drug screening capacity [68, 69].

5. CONCLUSION

In summary, the present work indicates that P-GABA has favourable effects on enhancing learning and memory activities in the context of sleep deprivation in a zebrafish model. The findings of histological examinations corroborate the behavioural observations. Several studies have suggested that these effects are related to P- GABA's effects on the neuro-immune and dopaminergic systems. According to the findings, this study demonstrated that P-GABA could reverse the decline in cognitive function and rise in anxiety associated with sleep deprivation through reducing brain oxidative stress and the modulation of AChE activity. There is a need for additional research to demonstrate the molecular

process in higher evolutionary orders, which will aid in the extrapolation of results to clinical trials and repositioning P-GABA as a potential therapeutic option for sleep deprivation and associated cognitive dysfunctions.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Colten HR, Altevogt BM. Sleep disorders and sleep deprivation: An unmet public health problem. National Academies Press; 2006.
2. Bishir M, Bhat A, Essa MM, Ekpo O, Ihunwo AO, Veeraraghavan VP, et al. Sleep deprivation and neurological disorders. BioMed Research International. 2020;2020:5764017.
3. Mahalakshmi AM, Ray B, Tuladhar S, Bhat A, Bishir M, Bolla SR, et al. Sleep, brain vascular health and ageing. GeroScience. 2020;42:1257–1283.
4. Potter GDM, Skene DJ, Arendt J, Cade JE, Grant PJ, Hardie LJ. Circadian rhythm and sleep disruption: Causes, metabolic consequences, and countermeasures. Endocrine Reviews. 2016;37:584–608.
5. Gorgoni M, D'Atri A, Lauri G, Rossini PM, Ferlazzo F, De Gennaro L. Is sleep essential for neural plasticity in humans, and how does it affect motor and cognitive recovery? Neural Plasticity. 2013;2013: 103949.
6. Bhat A, Pires AS, Tan V, Babu Chidambaram S, Guillemin GJ. Effects of sleep deprivation on the tryptophan metabolism. International Journal of Tryptophan Research. 2020;13: 117864692097090.
7. Shivalingaiah SB, Tuladhar S, Mahalakshmi AM, Muthukumar P, Yannam SK, Rashan L, et al. Sleep and gonadotrophin hormones. International Journal of Nutrition, Pharmacology, Neurological Diseases. 2021;11:17.
8. Irwin MR, Wang M, Ribeiro D, Cho HJ, Olmstead R, Breen EC, et al. Sleep loss activates cellular inflammatory signaling. Biological Psychiatry. 2008;64:538–540.
9. Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. Journal of Lipid Research. 2009;50:S29–S34.
10. Takemiya T, Suzuki K, Sugiura H, Yasuda S, Yamagata K, Kawakami Y, et al. Inducible brain COX-2 facilitates the recurrence of hippocampal seizures in mouse rapid kindling. Prostaglandins & Other Lipid Mediators. 2004;71:205–216.
11. Reppert SM, Weaver DR. Molecular analysis of mammalian circadian rhythms. Annu. Rev. Physiol 2001;63:647-76.
12. Sleep-related disorders among a healthy population in South India. Panda S, Taly AB, Sinha S, Gururaj G, Girish N, Nagaraja D. Neurol India. 2012;60:68–74.
13. Schibler U, Ripperger J, Brown SA. Peripheral circadian oscillators in mammals: Time and food. J Biol Rhythms 2003;18:250-60.
14. Tischkau SA, Mitchel JW, Tyan SH, Buchanan GF, Gillette MU. Ca^{2+}/CAMP response element-binding protein (CREB)-dependent activation of per1 is required for light induced signaling in the suprachaismatic nucleus circadian clock. J Biol Chem. 2003;278:718-23.
15. Morin LP. The circadian visual system. Brain Res Rev 1994;19:102-27.
16. Sheeba V, Sharma VK, Shubha K, Chandrashekaran MK, Joshi A. The effect of different light regimes on adult life span in drosophila melanogaster is partly mediated through reproduction output. J Biol Rhyth. 2000;15:380-92.
17. Tynes T, Hannerik M, Andersen A, Vistnes A, Haldorsen T. Incidence of breast cancer in norwegian female radio and telegraph operators. Cancer Causes Control. 1996;7: 197-204.
18. Rafnssion V, Tulinius H, Jonasson JG, Hrafnkelsson J. Risk of breast cancer in female flight attendants: A population –

based study (Iceland). Cancer Causes Control. 2001;12:95-101.

19. Kerengi NA, Pandula E, Feuer. GM. Oncostatic effects of the pineal gland. Drug Metabol Drug Interact. 1990;8:313-9.

20. Rosenthal NE, Joseph-Vanderpool JR, Levendosky AA, Johnston SH, Allen R, Kelly KA, Souetre E, Schultz PM, Starz KE. Phase-shifting effect of bright morning light as treatment for delayed sleep phase syndrome. Sleep. 1990;13:354-61.

21. Lewy AJ, Sack RL, Singer CM, White DM, Hoban TM. Winter depression and the phase-shift hypothesis for bright light's therapeutic effects: History, theory and experimental evidence. J Biol Rhythms. 1988;3:121-34.

22. Eastman CI. Squashing versus nudging circadian rhythms with artificial bright light: Solutions for shift work? Perspect. Biol Med. 1991;34:181-95.

23. Moore RY, Speh JC. GABA is principal neurotransmitters of the circadian system. Neurosci. Lett. 1993;150:112-6.

24. Wagner S, Castel M, Gainer H, Yarom Y. GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity. Nature. 1997;387:598-603.

25. Devaraj U, Shanmugasundaram T, Balamurugan E. Glutamate elicits therapeutic response in light-induced sleep-deprived Zebrafish, Danio rerio. Biosc. Biotech. Res. Comm. 2021;14(4): 1577-83.
Available:http://dx.doi.org/10.21786/bbrc/14.4.32

26. Mirunalani S, Subrmanian P. Temporal oscillations of thyroid hormones in long term melatonin treated rats. Pharmazie 2004;60:52-6.

27. Subramanian P, Sundaresan S, Balamurugan E. Temporal oscillations of phosphatases in N-Phthaloyl gamma-aminobutyric acid treated rats. Indian J Exp Biol. 1998;36:1141-3.

28. Subramanian P, Balamurugan E. Temporal oscillations of serum electrolytes in N-Phthaloyl GABA- treated rats. Pharmacol Biochem Behav. 1999;62:511-4.

29. Batista FLA, Lima LMG, Abrante IA, de Araújo JIF, Batista FLA, Abrante IA, Magalhães EA, de Lima DR, Lima MdCL, do Prado BS, et al. Antinociceptive activity of ethanolic extract of *Azadirachta indica* A. Juss (Neem, Meliaceae) fruit through opioid, glutamatergic and acid-sensitive ion pathways in adult zebrafish (*Danio rerio*).

30. Hurtado-Alvarado G, Domínguez-Salazar E, Pavon L, Velázquez-Moctezuma J, Gómez-González B. Blood-brain barrier disruption induced by chronic sleep loss: low-grade inflammation may be the link. Journal of Immunology Research. 2017; 2016:4576012.

31. Dumitru G, El-Nashar HAS, Mostafa NM, Eldahshan OA, Boiangiu RS, Todirascu-Ciornea E, Hritcu L, Singab ANB. Agathisflavone isolated from *Schinus polygamus* (Cav.) Cabrera leaves prevents scopolamine-induced memory impairment and brain oxidative stress in zebrafish (Danio rerio). Phytomedicine. 2019;58: 152889.

32. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. Measurement of protein using bicinchoninic acid. Anal. Biochem. 1985; 150:76–85.

33. Zanandrea R, Abreu MS, Piato A, Barcellos LJG, Giacomini ACVV. Lithium prevents scopolamine-induced memory impairment in zebrafish. Neurosci. Lett. 2018;664:34–37.

34. Alderman SL, Bernier NJ. Ontogeny of the corticotropin-releasing factor system in zebrafish, Gen Comp Endocrinol; 2009.

35. Egan RJ, Bergner CL, Hart PC, Cachat JM, Canavello PR, Elegante MF, Elkhayat SI, Bartels BK, Tien AT, Tien DH, Mohnot S, Beeson E, Glasgow E, Amri H, Zukowska Z, Kalueff AV. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish, Brain Behavioual Research; 2009.

36. Li Y, Du X, Liu C, Wen Z, Du J. Reciprocal regulation between resting microglial dynamics and neuronal activity in vivo. Developmental Cell. 2012;23:1189–1202.

37. Wu Y, Dissing-Olesen L, MacVicar BA, Stevens B. Microglia: dynamic mediators of synapse development and plasticity. Trends in Immunology. 2015;36:605–613.

38. Kang JE, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, et al. Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. Science. 2009;326: 1005–1007.

39. Alves G, Brønnick K, Aarsland D, Blennow K, Zetterberg H, Ballard C, et al. CSF amyloid-beta and tau proteins, and

cognitive performance, in early and untreated Parkinson's disease: the Norwegian ParkWest study. Journal of Neurology, Neurosurgery, and Psychiatry. 2010;81:1080–1086.

40. Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, et al. Sleep drives metabolite clearance from the adult brain. Science. 2013;342:373–377.

41. Patel NS, Paris D, Mathura V, Quadros AN, Crawford FC, Mullan MJ. Inflammatory cytokine levels correlate with amyloid load in transgenic mouse models of Alzheimer's disease. Journal of Neuroinflammation. 2019;2:9.

42. Nadjar A, Wigren HKM, Tremblay ME. Roles of microglial phagocytosis and inflammatory mediators in the pathophysiology of sleep disorders. Frontiers in Cellular Neuroscience. 2017; 11:250.

43. Cope EC, LaMarca EA, Monari PK, Olson LB, Martinez S, Zych AD, et al. Microglia play an active role in obesity-associated cognitive decline. The Journal of Neuroscience. 2019;38:8889–8904.

44. Ornelas A, Zacharias-Millward N, Menter DG, Davis JS, Lichtenberger L, Hawke D, et al. Beyond COX-1: The effects of aspirin on platelet biology and potential mechanisms of chemoprevention. Cancer Metastasis Reviews. 2018;36:289–303.

45. Moro MA, De Alba J, Cárdenas A, De Cristóbal J, Leza JC, Lizasoain I, et al. Mechanisms of the neuroprotective effect of aspirin after oxygen and glucose deprivation in rat forebrain slices. Neuropharmacology. 2000;39:1309–1318.

46. Everson CA. Clinical assessment of blood leukocytes, serum cytokines, and serum immunoglobulins as responses to sleep deprivation in laboratory rats. American Journal of Physiology- Regulatory, Integrative and Comparative Physiology. 2005;289:R1054–R1063.

47. Mullington JM, Simpson NS, Meier-Ewert HK, Haack M. Sleep loss and inflammation. Best Practice & Research. Clinical Endocrinology & Metabolism. 2010;24:775–784.

48. He J, Hsuchou H, He Y, Kastin AJ, Wang Y, Pan W. Sleep restriction impairs blood-brain barrier function. The Journal of Neuroscience. 2014;34:14697–14706.

49. Kata D, Földesi I, Feher LZ, Hackler L, Puskas LG, Gulya K. A novel pleiotropic effect of aspirin: beneficial regulation of

proand anti-inflammatory mechanisms in microglial cells. Brain Research Bulletin. 2017;132: 61–74.

50. Wang Y, Wu Y, Li L, Zheng J, Liu R, Zhou J, et al. Aspirintriggered lipoxin a4 attenuates LPS-induced pro-inflammatory responses by inhibiting activation of NF-κB and MAPKs in BV-2 microglial cells. Journal of Neuroinflammation. 2011;8:95.

51. Craig LA, Hong NS, Kopp J, McDonald RJ. Reduced cholinergic status in hippocampus produces spatial memory deficits when combined with kainic acid induced seizure. Hippocampus. 2008;18: 1112–1121.

52. Masters CL, Bateman R, Blennow K, Rowe CC, Sperling RA, Cummings JL. Alzheimer's disease. Nat. Rev. Dis. Prim. 2015;1:15056.

53. Liu PP, Xie Y, Meng XY, Kang JS. History and progress of hypotheses and clinical trials for Alzheimer's disease. Signal Transduct. Target. Ther. 2019;4:1–22.

54. Doody RS, Dunn JK, Clark CM, Farlow M, Foster NL, Liao T, Gonzales N, Lai E, Massman P. Chronic donepezil treatment is associated with slowed cognitive decline in Alzheimer's disease. Dement. Geriatr. Cogn. Disord. 2001;12: 295–300.

55. Bullock R, Touchon J, Bergman H, Gambina G, He Y, Rapatz G, Nagel J, Lane R. Rivastigmine and donepezil treatment in moderate to moderately-severe Alzheimer's disease over a 2-year period. Curr. Med. Res. Opin. 2005;21: 1317–1327.

56. Lyketsos CG, Carrillo MC, Ryan JM, Khachaturian AS, Trzepacz P, Amatniek J, Cedarbaum J, Brashear R, Miller DS. Neuropsychiatric symptoms in Alzheimer's disease. Alzheimer's Dement. 2011;7:532–539.

57. Tay TL, Savage JC, Hui CW, Bisht K, Tremblay M. Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. The Journal of Physiology. 2017;595:1929–1945.

58. Tönnies E, Trushina E. Oxidative Stress, Synaptic Dysfunction, and Alzheimer's Disease. J. Alzheimer's Dis. 2017;57: 1105–1121.

59. Jafarian S, Ling KH, Hassan Z, Perimal-Lewis L, Sulaiman MR, Perimal EK. Effect of zerumbone on scopolamine-induced

memory impairment and anxiety-like behaviours in rats. Alzheimer's Dement. 2019;5:637–643.

60. Naderali E, Nikbakht F, Ofogh S, Rasoolijazi H. The role of rosemary extract (40% carnosic acid) in degeneration of hippocampal neurons induced by kainic acid in the rat: The behavioral and histo-chemical approach. J. Integr. Neurosci. 2018;17:31–43.

61. Karim N, Khan I, Abdelhalim A, Abdel-Halim H, Hanrahan JR. Molecular docking and antiamnesic effects of nepitrin isolated from *Rosmarinus offcinalis* on scopola-mine-induced memory impairment in mice Biomed. Pharmacother. 2017;96:700–709.

62. Song H, Xu L, Zhang R, Cao Z, Zhang H, Yang L, Guo Z, Qu Y, Yu J. Rosemary extract improves cognitive deficits in a rats model of repetitive mild traumatic brain injury associated with reduction of astrocytosis and neuronal degeneration in hippocampus. Neurosci. Lett. 2016;622: 95–101.

63. Gerlai R. Zebrafish and relational memory: Could a simple fish be useful for the analysis of biological mechanisms of complex vertebrate learning? Behav. Process. 2017;141:242–250.

64. Meshalkina DA, Kizlyk MN, Kysil EV, Collier AD, Echevarria DJ, Abreu MS, Barcellos LJG, Song C, Kalue AV. Understanding zebrafish cognition. Behav. Process. 2017;141:229–241.

65. Xu X, Scott-Scheiern T, Kempker L, Simons K. Active avoidance conditioning in zebrafish (*Danio rerio*). Neurobiol. Learn. Mem. 2007;87:72–77.

66. Aoki R, Tsuboi T, Okamoto H. Y-maze avoidance: An automated and rapid associative learning paradigm in zebrafish. Neurosci. Res. 2015;91:69–72.

67. Braida D, Ponzoni L, Martucci R, Sparatore F, Gotti C, Sala M. Role of neuronal nicotinic acetylcholine receptors (nAChRs) on learning and memory in zebrafish. Psychopharmacology. 2014; 231:1975–1985.

68. Hamilton TJ, Morrill A, Lucas K, Gallup J, Harris M, Healey M, Pitman T, Schalomon M, Digweed S, Tresguerres M. Establishing zebrafish as a model to study the anxiolytic effects of scopolamine. Sci. Rep. 2017;7:15081.

69. Gerstner JR, Yin JCP. Circadian rhythms and memory formation, Nature Reviews Neuroscience. 2010;11:577.

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