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Study The Therapeutic Effect of Melatonin in Treatment of Periventricular Leukomalacia in Rat Model.

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ABSTRACT:
Background:*Periventricular leukomalacia (PVL) is a form of white matter brain injury, which causes motor and cognitive deficits, and is frequently linked to preterm newborns. Inflammation and oxidative stress are important factors in the development of PVL. Brain injury may be minimised by melatonin, a neuroprotective hormone having anti-inflammatory and antioxidant qualities. In order to evaluate melatonin's potential as a treatment alternative, this study examines its therapeutic benefits in a rat model of PVL.*
Method:*Rat pups were split into four groups after approval: test, disease control, treatment control, and negative control. On days 2, 4 and 6, 15 mg/kg of LPS was used to cause premature brain injury. The test group was given melatonin four hours following the last LPS treatment, whereas the negative control group was given saline. The pups' neurodevelopmental reflexes were examined for abnormalities, and on postnatal day 15, they were euthanized so that their brains could be examined using immunohistology and histopathology.*
Results:*The study showed that melatonin treatment significantly improved neurodevelopmental activity and reduced ventricular dilatation in the treatment group compared to the disease control group, indicating its potential neuroprotective effects.*
Conclusion:*This study found that melatonin therapy improved neurodevelopmental outcomes and reduced ventricular dilatation in a rat model of periventricular leukomalacia (PVL), suggesting its potential as a neuroprotective treatment. The results highlight melatonin's antioxidative properties and therapeutic promise for PVL.*
Keywords:*Melatonin, Periventricular leukomalacia (PVL), Therapeutic effect, Rat model, Neuroprotection, Inflammation, Oxidative stress.*

INTRODUCTION

Periventricular leukomalacia (PVL) is characterised by the death of the brain's white matter due to softening of the brain tissue. The disorder is caused by lack of oxygen or blood flow to the periventricular area of the brain. The periventricular area is the area around the ventricles (fluid-filled cavities/spaces in the brain) where nerve fibres carry messages from the brain to the body's muscles(1). Perinatal white matter lesions associated with cerebral palsy appears to involve glutamate excitotoxicity and excess free radical production.(2).

Periventricular leukomalacia (PVL) stands as a formidable challenge in neonatal care, particularly affecting premature infants. Periventricular leukomalacia, characterised by focal necrosis of the white matter adjacent to the cerebral ventricles, poses a significant threat to the neurodevelopmental trajectory of preterm infants. The pathogenesis of PVL involves a complex interplay of ischemia, inflammation, and oxidative stress, ultimately leading to white matter injury(3). Despite advances in understanding the underlying mechanisms, therapeutic options to mitigate the consequences of PVL remain limited. In this exploration, we turn our attention to the hormone melatonin, investigating its neuroprotective properties with a focus on rat studies(4).
Periventricular leukomalacia (PVL) is a condition that primarily affects premature infants, particularly those born before 32 weeks of gestation and most common in very premature and low birth weight babies. PVL can cause damage to the nerve pathways that control motor movements, resulting in muscles that are tight, spastic, or resistant to movement, in addition to being weak(5). Babies with PVL have a higher risk of cerebral palsy and may have motor disorders,delayed cognitive development, coordination problems,vision and hearing impairments . As researchers delve into potential interventions to alleviate the severe consequences of PVL, one intriguing avenue of exploration emerges: the hormone melatonin(6).Melatonin (N-acetyl-5-methoxytryptamine) is an endogenously produced indoleamine secreted by the pineal gland and the secretion is suppressed by light. Melatonin is a highly effective antioxidant(7), free radical scavenger, and has anti-inflammatory effect ,its main physiologic function is

to mediate circadian rhythmicity and seasonality(8). Plenty of evidence supports the utility of melatonin in adults for cancer, neurodegenerative disorders, and ageing(9). In children and neonates, melatonin has been used widely, including for respiratory distress syndrome, bronchopulmonary dysplasia, periventricular leukomalacia (PVL), hypoxic–ischemic encephalopathy and sepsis. In addition, melatonin can be used in childhood sleep and seizure disorders, and in neonates and children receiving surgery(10)). Melatonin has garnered considerable attention for its pleiotropic effects on various physiological processes, including neuroprotection. It readily crosses the blood-brain barrier and exhibits potent antioxidant and anti-inflammatory properties(11), making it an intriguing candidate for neurotherapeutic interventions and for counteracting the damage observed in the white matter surrounding the brain's ventricles, a hallmark of PVL(12). Previous studies have demonstrated the neuroprotective potential of melatonin in diverse neurological disorders(5), prompting investigation into its efficacy in PVL. Melatonin's unique ability to neutralise free radicals positions it as a promising agent for reducing oxidative stress and potentially preserving the delicate white matter surrounding the brain's ventricles(13). Moreover, melatonin has the potential to modulate the inflammatory responses associated with PVL. Inflammation plays a pivotal role in the progression of white matter damage in the periventricular region, and melatonin's capacity to attenuate this response could prove crucial in mitigating the severity of PVL(14).

Preclinical studies, utilising animal models of perinatal brain injury, have provided encouraging insights into melatonin's neuroprotective effects(15). These studies reveal improvements in white matter preservation, reduced inflammation, and enhanced behavioural outcomes in subjects treated with melatonin. However, the translation of these findings from animal models to clinical applications necessitates rigorous evaluation through well-designed clinical trials(16). Rat studies exploring perinatal brain injury have provided encouraging results regarding melatonin's neuroprotective effects(17). Recent animal studies by Lin et al. confirm that melatonin reduces intracerebral cellular inflammatory response and protects neurons against ischemic injury by reducing the oxidative stress, lipid peroxidation, and radical oxygen species generation (18).

While these rat studies provide valuable insights, it's crucial to acknowledge the need for cautious interpretation when translating findings from animal models to human clinical applications. Nevertheless, these preclinical investigations lay the groundwork for further exploration in clinical trials. This study aims to address the current gap in PVL therapeutics by evaluating the impact of melatonin administration in a rat model designed to replicate key aspects of the condition observed in premature infants. By systematically assessing histopathological changes, neurobehavioral outcomes, and molecular markers associated with oxidative stress and inflammation, we seek to unravel the potential therapeutic benefits of melatonin in the context of PVL.

MATERIALS AND METHODS:

Animals and treatment:

The approval for this project is given by Saveetha Dental College. Approval no. BRULAC/SDCH/SIMATS/IAEC/04-2024/05.The animals were driven in an air-conditioned automobile with enough food and water, shielded from harsh sunlight and loud noises, and under constant observation during the journey. For seventy days, these rats were kept in their enclosure.

To generate Preterm brain injury:

Normally delivered rat pups will be injected intraperitoneally with lipopolysaccharide (LPS, 15 mg/kg) on postnatal days 2, 4, and 6 in order to cause premature brain damage. The purpose of this procedure is to simulate neuroinflammation and evaluate its effects on brain development.

Test group:

For the therapeutic test group, the drug will be administered 4 hours after the final dose of LPS injection.This timing is chosen to evaluate the efficacy of melatonin as a treatment rather than a preventative measure and how melatonin can mitigate the established inflammatory response and resultant brain damage caused by LPS-induced preterm brain injury.

Control test group:

Negative Control pups will receive equal amounts of normal saline intraperitoneally on the same schedule. The purpose of this group is to provide a baseline against which to assess the effects of brain injury caused by LPS and the therapuetic medication. Rats in negative control groups do not receive either LPS or minocycline.

GROUP	Details	No.of Animals (pups)

1	Negative control(normal pups)	12
2	Disease control (LPS)	6
3	Treatment control(Melatonin)	6
4	Test group(Melatonin+LPS)	12

Neurodevelopmental reflex testing:
Neurodevelopmental reflex testing will be used to evaluate the puppies in a series of behavioural tests beginning on different days. These tests will include the following: the agility test (PN Day 6), the posture test, the eye opening test(PN Day 12), the cliff avoidance test (PN Day 4), the forelimb grasp test (PN Day 3), the hind limb grasp test (PN Day 3), the righting test (PN Day 3), the hind limb placing test (PN Day 4), and the auditory startle test (PN Day 10).Finding any neurodevelopmental or behavioural problems brought on by the medicines is the goal of these evaluations. Reflex and behavioural testing together offer a thorough assessment of how E. Coli, LPS, and preventative medications affect the pups' neurodevelopment and behaviour. This enables researchers to identify differences between the treated and control groups and assess how effective the therapeutic intervention of melatonin had been.

Post Natal day of starting test	**Test**
3	Forelimb Grasping
3	Hind limb Grasping
3	Righting
4	Hind limb placing
4	Cliff Avoidance
6	Gait
10	Auditory startle
12	Posture
12	Eye Opening

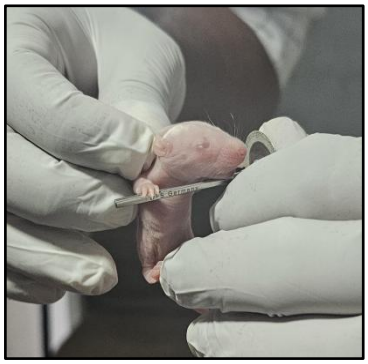

Figure 1:forelimb grasping

Figure 2:hindlimb grasping

Figure 3:hindlimb grasping

Figure 4:Righting reflex



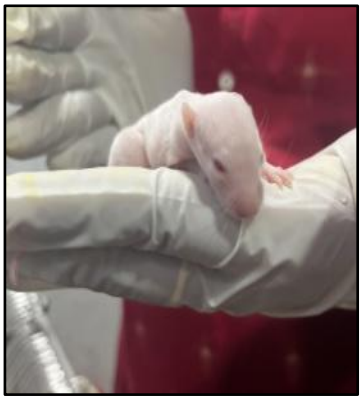


Figure 5:cliff avoidance Figure 6:Gait test Figure 7:Eye opening Figure 8: Posture test

Euthanization and Analysis:
At the age of 15 and 35 days, the pups will be euthanized using carbon monoxide gas inhalation in a CO chamber after sedation with a suitable anaesthetic agent. Brain will be taken for further analysis, such as Western blot, ELISA, qPCR, immunohistochemistry, and other related procedures. These methods will contribute to the evaluation of the cellular and molecular effects of the therapies on different organs, offering a thorough understanding of the biological consequences of LPS, E. coli, and the therapeutic (melatonin) medication. The goal of this strategy is to explain the processes that underlie preterm brain damage and provide possible therapeutic measures.

RESULTS:
TABLE 1 :Group 1-Post Melatonin effects on neurodevelopment in Pups.

Groups	Forelimb grasping	Hindlimb grasping	Righting	Hindlimb placing	Cliff avoidance	Gait	Auditory startle	Posture	Eye opening
Normal control (D1)	5	6	6	6	6	9	10	12	15
Normal control (D2)	5	5	6	7	5	8	11	13	16
Disease control	8	9	4	10	5	6	13	16	15
Treatment control	3	7	3	7	5	9	13	15	16
Test group (D1)	6	7	5	6	5	10	13	17	16
Test group (D2)	6	9	6	8	6	11	14	17	16

TABLE :1
Table 1: Based on the grading of the neurodevelopmental tests for the negative control groups, Disease control group, Treatment control group and Test groups this table shows the maximum developmental activity attained throughout the postnatal days of the pups.

BRAIN HISTOLOGY:

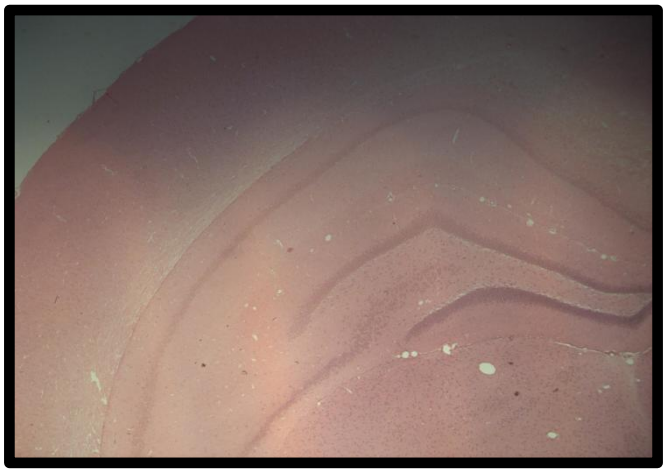

Fig 1. G1 (CONTROL)-Photomicrograph of Brain section
Photomicrograph of Brain section (H&E,X100) showing Normal histoarchitecture of hippocampus and cerebral cortex.

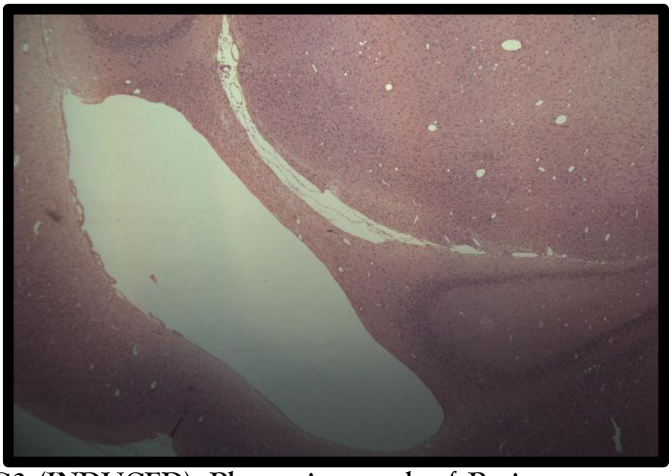

Fig 2. G2 (INDUCED) -Photomicrograph of Brain section (H&E,X100) showing marked Ventricular dilatation.

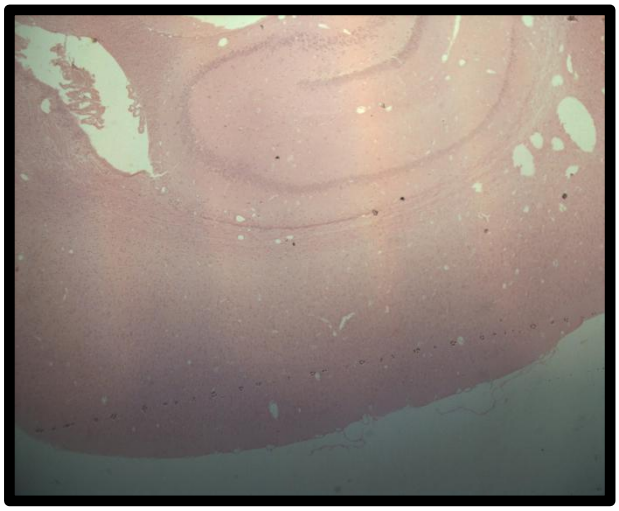

Fig 3. G3(TREATMENT)-Photomicrograph of Brain section (H&E,X100) showing minimal Ventricular dilatation after melatonin treatment .

DISCUSSION:

The data in Table 1 indicates the neurodevelopmental progress of various rat pup groups based on the number of postnatal days (PND) needed to reach particular developmental milestones. The normal control groups (D1 and D2) demonstrated the regular and timely attainment of milestones, including the following: gait (9 and 8 PND), auditory startle (10 and 11 PND), posture (12 and 13 PND), eye opening (15 and 16 PND), righting (6 PND), hindlimb placing (6 and 7 PND), and forelimb grasping (5 PND). These findings imply that these groups' growth and development are normal.

In contrast, the disease control groups representing pups with induced periventricular leukomalacia (PVL) but without any treatment who had PVL and received no treatment showed that there are significant delays in achieving these milestones. For example, forelimb grasping (8 PND), hindlimb grasping (9 PND), righting (4 PND), hindlimb placing (10 PND), gait (6 PND), auditory startle (13 PND), posture (16 PND) and eye opening (15PND) emphasise the destructive effect of PVL on neurodevelopment.

Mixed outcomes were observed in the treatment control group which received an unspecified treatment. However, certain milestones such as hindlimb grasping (7 PND) and gait (9PND) exhibited some

improvement when compared to the disease control group; meanwhile others like forelimb grasping 3 PND and righting 3 PND were even further delayed indicating only partial efficacy of the therapy.
In comparison to the disease control group, the test groups (D1 and D2), who most likely got melatonin treatment, displayed better results. Development milestone like forelimb grabbing (6 PND), hindlimb grasping (7 and 9 PND), righting (5 and 6 PND), hindlimb placement (6 and 8 PND), gait (10 and 11 PND), auditory startle (13 and 14 PND), posture (17 PND), and eye opening (16 PND) were all accomplished more quickly in these groups. The test groups
improved performance as compared to the disease control group implies that melatonin treatment had a beneficial effect on reducing developmental delays brought on by PVL, even though they did not perfectly equal the normal controls.
Melatonin may have a therapeutic effect in reducing the effects of PVL, as evidenced by the improved neurodevelopmental outcomes in the test groups compared to the disease control group. This is evidenced by the earlier attainment of developmental milestones by the test groups.
Even after receiving melatonin therapy, the test groups' developmental progress fell short of that of the normal control groups. This implies that although melatonin has a great deal of neuroprotective effects, normal neurodevelopmental trajectories are not entirely restored by it.

In comparison to the disease control group, the treatment control group's outcomes were inconsistent, with some milestones occurring more slowly. This demonstrates that, although other therapies may have certain advantages, melatonin is the more beneficial option in this situation.The differences in results between the test groups (D1 and D2) highlight the need for additional study to maximise the therapeutic advantages of melatonin by optimising its dosage and administration techniques.Melatonin treatment in this rat model of PVL produced positive effects, indicating that it may be used in human newborn care to prevent or lessen neurodevelopmental deficits linked to PVL. However, before such medicines may be recommended for use in humans, clinical trials and safety evaluations are required.
To summarise, the available data suggests that treating PVL with melatonin presents a viable therapeutic approach for enhancing neurodevelopmental outcomes. However, additional study is necessary to completely realise and comprehend this treatment's potential.

The images of histological sections of rat brain tissue to examine how melatonin administration affects periventricular leukomalacia (PVL). White matter near the lateral ventricles becomes necrotic in PVL in premature infants. The anti-inflammatory, anti-apoptotic, and antioxidant characteristics of melatonin are largely responsible for its reputation as a neuroprotective agent(19). Melatonin targets white matter damage from hypoxia-ischemia or inflammation in PVL. This analysis suggests that melatonin treatment offers some protection to brain tissue in a PVL rat model. The first image shows relatively preserved cortical structure with mild inflammation but no extensive necrosis. The second image reveals disruptions in white matter and signs of past ischemic damage, typical of PVL. The third image indicates that the hippocampal region is largely intact, suggesting melatonin helps preserve critical brain areas. These findings imply that melatonin may reduce oxidative stress and inflammation, minimising white matter damage(20). However, further detailed and quantitative analyses are needed to confirm the extent of its protective effects.

The study by Moretti et al. (2015) demonstrates that melatonin dramatically minimises excitotoxic blood-brain barrier (BBB) breakdown in neonatal rats, which is essential for defending against inflammatory and toxic chemicals that worsen periventricular leukomalacia (PVL)(21). Because of melatonin's anti-inflammatory and antioxidant qualities, which protect against oxidative stress and inflammation, two major causes of PVL disease, neuronal and glial cell function is maintained(22). Furthermore, it appears that melatonin improves the neurodevelopmental trajectories impacted by PVL due to its neuroprotective properties, which include decreased cell death and improved functional results. The findings highlight the potential of melatonin as a treatment agent for PVL and the necessity to maximise its protective effects by optimising dose and timing of delivery(23).
According to a 2010 study by Kaur, Sivakumar, and Ling, melatonin shields the periventricular white matter against damage caused by hypoxia, which is related to PVL (periventricular leukomalacia). By controlling microglial responses and cytokine production, its antioxidant qualities prevent oxidative stress and neuronal death, preserve the integrity of the blood-brain barrier, and reduce inflammation(24). These

results highlight the potential of melatonin to minimise white matter damage and promote neurodevelopmental outcomes in PVL(25).

According to a study by Gressens et al. (2008), agomelatine, a melatonin receptor agonist with 5-HT2C receptor antagonist characteristics, shields mice's developing white matter from excitotoxic damage. This finding is pertinent to the treatment of periventricular leukomalacia (PVL). By lowering excitotoxicity, inflammation, and oxidative stress, the neuroprotective benefits are mediated by melatonin receptor activation and 5-HT2C receptor antagonism(26,27). According to the study by Bouslama et al. (2007), melatonin possesses neuroprotective properties that help avoid learning impairments in newborn mice with brain lesions. White matter damage and developmental delays in periventricular leukomalacia (PVL) may be lessened by melatonin due to its anti-inflammatory and antioxidant characteristics(28), which lower inflammation and oxidative stress(29). Melatonin has the ability to enhance neurodevelopmental and cognitive outcomes in PVL models, which supports its therapeutic promise for brain injuries similar to those caused by PVL. Furthermore, the study's findings on reducing cognitive deficits highlight this potential(30).

According to Jiang et al. (2021), melatonin plays a crucial role in improving myelination and minimising white matter damage by changing A1 to A2 astrocytes through the JAK2/STAT3 pathway, hence improving axonal hypomyelination in septic newborn rats. This result emphasises how melatonin may be able to help with important elements of periventricular leukomalacia (PVL), such as its ability to reduce inflammation associated with PVL. Further investigation into melatonin's clinical application is warranted, since the study's understanding of its mechanism suggests that it may be a useful therapeutic drug for enhancing myelination and neurodevelopmental outcomes in PVL models.(31,32).

FUTURE SCOPE:

The research on melatonin's therapeutic efficacy in the treatment of periventricular leukomalacia in a rat model has a lot of potential for the future of neonatal neuroprotection. The underlying mechanisms of melatonin's neuroprotective qualities may be further investigated, dosage and administration schedules may be optimised to maximise efficacy, potential synergistic effects with other therapeutic agents may be investigated, and results from preclinical studies may be transferred to human neonatal clinical trials. Further investigations may concentrate on the long-term neurodevelopmental consequences, possible adverse reactions, and the viability of integrating melatonin therapy into conventional clinical practice for the avoidance and management of periventricular leukomalacia and other newborn brain damage. In summary, this study could contribute to our knowledge of the pathogenesis of newborn brain injury and enhance treatment approaches for bettering the prognosis of impacted infants.

LIMITATIONS:

Limitations of the study on the therapeutic effect of melatonin in treating periventricular leukomalacia in a rat model include the difficulty of directly translating findings to human clinical practice due to differences in drug metabolism between species, the possibility of differences in disease pathophysiology between rats and humans, and the need for additional research to confirm the efficacy and safety of melatonin therapy in human neonates. Furthermore, periventricular leukomalacia is a complicated disease with a multifaceted etiology and pathophysiology that may not be fully captured in the rat model, which could lead to limitations. Further restrictions might result from the restricted laboratory environment, which might not accurately capture the clinical intricacies and unpredictability found in actual newborn care environments.

CONCLUSION:

Using a rat model, the study investigated the potential benefits of melatonin therapy for treating periventricular leukomalacia (PVL). The treatment groups showed a considerable improvement over the disease control group, as evidenced by the histological analyses and neurodevelopmental assessments. Melatonin therapy specifically improved neurodevelopmental milestones that were significantly compromised in the disease control group, including locomotion, cliff avoidance, righting reflex, forelimb and hindlimb grabbing, and auditory startle. Preventive benefit against brain damage caused by PVL was indicated by the minimal ventricular dilatation seen in the histologically examined rats treated with melatonin. Because melatonin has neuroprotective and antioxidative qualities, these results point to a promising therapeutic potential for melatonin in reducing the adverse effects of PVL.

REFERENCES:

1. National Institute of Neurological Disorders and Stroke [Internet]. [cited 2024 Feb 24]. Periventricular Leukomalacia. Available from: https://www.ninds.nih.gov/health-information/disorders/periventricular-leukomalacia
2. Husson I, Mesplès B, Bac P, Vamecq J, Evrard P, Gressens P. Melatoninergic neuroprotection of the murine periventricular white matter against neonatal excitotoxic challenge. Ann Neurol [Internet]. 2002 Jan [cited 2024 Feb 24];51(1). Available from: https://pubmed.ncbi.nlm.nih.gov/11782987/
3. Google Docs [Internet]. [cited 2024 Aug 3]. 10. Souparnika and Karthik Ganesh - 8044 - 2023 - JPTCP.pdf. Available from: https://drive.google.com/file/d/1oJwrnK7syTeht8NC2PmIdKqXZouHjRNR/view?usp=sharing&usp=embed_facebook
4. Ebenezer JL, Gunapriya R, Ranganathan K, Vijayaraghavan R, Ganesh MK. Determine Cyp17a1 and Ki67 expressions in PCOs induced rat model treated with Sepia pharaonis ink extract proves effective. Indian J Anim Res [Internet]. 2020 Oct 23;(Of). Available from: http://arccjournals.com/journal/indian-journal-of-animal-research/B-4204
5. Microalgae as a potential therapeutic drug candidate for neurodegenerative diseases. J Biotechnol. 2022 Nov 10;358:128–39.
6. Periventricular Leukomalacia [Internet]. [cited 2024 Feb 24]. Available from: https://www.childrenshospital.org/conditions/periventricular-leukomalacia
7. Imtiaz T, Priyadharshini R, Rajeshkumar S, Sinduja P. Green synthesis and Characterization of Silver Nanoparticles Synthesized Using Piper longum and its Antioxidant Activity. J Pharm Res Int. 2021 Nov 24;342–52.
8. Melatonin utility in neonates and children. J Formos Med Assoc. 2012 Feb 1;111(2):57–66.
9. Rajeshkumar S, Ganesh L, Santhoshkumar J. Selenium nanoparticles as therapeutic agents in neurodegenerative diseases. In: Nanobiotechnology in Neurodegenerative Diseases. Cham: Springer International Publishing; 2019. p. 209–24.
10. Website [Internet]. Available from: .https://www.nature.com/articles/pr200733
11. Jayavarsha V, Rajeshkumar S, Lakshmi T, Sulochana G. Green synthesis of selenium nanoparticles study using clove and cumin and its anti- inflammatory activity. J Complement Med Res. 2022;13(5):84.
12. Vishaka S, Sridevi G, Selvaraj J. An analysis on the antioxidant and anti-diabetic properties of rhizome using different solvent systems. J Adv Pharm Technol Res. 2022 Dec;13(Suppl 2):S505–9.
13. Website [Internet]. Available from: https://doi.org/10.1203/01.pdr.0000252546.20451.1a
14. Ramamurthy J, Professor and Head, Department of Periodontics, Saveetha dental College and hospitals, Saveetha institute of medical and technical sciences, Saveetha university. Evaluation of antioxidant and anti inflammatory activity of grape seed oil infused with silver nanoparticles an in vitro study. Int J Dent Oral Sci. 2021 Jul 15;3318–22.
15. [No title] [Internet]. [cited 2025 Jan 5]. Available from: https://www.jrmds.in/articles/effect-of-lupeol-on-proinflammatory-markers-in-adipose-tissue-of-highfat-diet-and-sucrose-induced-type2-diabetic-rats.pdf
16. Garapati B, Malaiappan S, Rajeshkumar S, Murthykumar K. Cytotoxicity of lycopene-mediated silver nanoparticles in the embryonic development of zebrafish—An animal study. Journal of Biochemical and Molecular Toxicology. 2022 Oct 1;36(10):e23173.
17. Rajaraman V, Nallaswamy D, Ganapathy D, Rajeshkumar S, Ariga P, Ganesh K. Effect of hafnium coating on osseointegration of titanium implants: A split mouth animal study. J Nanomater. 2021 Dec 15;2021:1–9.
18. Website [Internet]. Available from: https://www.hindawi.com/journals/ije/2019/9626715/%0A
19. Ponnanikajamideen M, Rajeshkumar S, Vanaja M, Annadurai G. In Vivo Type 2 Diabetes and Wound-Healing Effects of Antioxidant Gold Nanoparticles Synthesized Using the Insulin Plant Chamaecostus cuspidatus in Albino Rats. Can J Diabetes. 2019 Mar;43(2):82–9.e6.
20. Mukundh Chaithanya V, Uma Maheswari TN, Rajesh Kumar S. Anti-inflammatory and cytotoxic effect of lycopene and raspberry in-situ gel. Bioinformation. 2022 Jul 31;18(7):645–50.
21. Gheena S, Ezhilarasan D, Shree Harini K, Rajeshkumar S. Syringic acid and silymarin concurrent administration inhibits sodium valproate-induced liver injury in rats. Environ Toxicol. 2022 Sep;37(9):2143–52.
22. Ezhilarasan D, Rajeshkumar S, Lakshmi T. The role of oxidative stress in chronic liver diseases. In: Role of Oxidative Stress in Pathophysiology of Diseases. Singapore: Springer Singapore; 2020. p. 13–25.
23. Moretti R, Zanin A, Pansiot J, Spiri D, Manganozzi L, Kratzer I, et al. Melatonin reduces excitotoxic blood-brain barrier breakdown in neonatal rats. Neuroscience. 2015 Dec 17;311:382–97.
24. Jaisankar A, Rajeshkumar S. Anti cariogenic and anti inflammatory activity of Achyranthes Aspera mediated silver nanoparticles. J Complement Med Res. 2022;13(3):13.
25. Kaur C, Sivakumar V, Ling EA. Melatonin protects periventricular white matter from damage due to hypoxia. J Pineal Res. 2010 Apr;48(3):185–93.
26. Agomelatine, a melatonin receptor agonist with 5-HT2C receptor antagonist properties, protects the developing murine white matter against excitotoxicity. Eur J Pharmacol. 2008 Jun 24;588(1):58–63.
27. Prabakar J, Senior Lecturer, Department of Public Health Dentistry, Saveetha Dental college, Saveetha Institute of Medical and Technical Sciences, Saveetha University No.162, Poonamallee high road, Chennai 600077, Tamil Nadu, India. In vitro evaluation of cytotoxicity and antioxidant efficacy of bromelain. Int J Dent Oral Sci. 2021 May 20;2516–9.
28. Arthi., MDS Student, Department of Oral and Maxillofacial Pathology, Saveetha Institute of Medical and Technical Science. Anti - oxidant and anti - inflammatory properties of annonamuricata mediated zinc oxide nanoparticles - an in vitro study. Int J Dent Oral Sci. 2021 Aug 16;3761–5.
29. Chaithanya MV, Uma Maheswari TN, Rajeshkumar S. Anti-inflammatory and antioxidant activity of lycopene, raspberry, green tea he
rbal formulation mediated silver nanoparticle. J Indian Acad Oral Med Radiol. 2021 Oct;33(4):397–400.
30. Drury PP, Davidson JO, Bennet L, Booth LC, Tan S, Fraser M, et al. Partial Neural Protection with Prophylactic Low-Dose Melatonin after Asphyxia in Preterm Fetal Sheep. J Cereb Blood Flow Metab [Internet]. 2013 Oct 9 [cited 2024 Jul 14]; Available from: https://journals.sagepub.com/doi/10.1038/jcbfm.2013.174
31. Website [Internet]. Available from: Jiang, S., Wang, H., Zhou, Q., Li, Q., Liu, N., Li, Z., ... Deng, Y. (2021). Melatonin Ameliorates Axonal Hypomyelination of Periventricular White Matter by Transforming A1 to A2 Astrocyte via JAK2/STAT3

Pathway in Septic Neonatal Rats. Journal of Inflammation Research, 14, 5919–5937. https://doi.org/10.2147/JIR.S337499
32. Jiang S, Wang H, Zhou Q, Li Q, Liu N, Li Z, et al. Melatonin Ameliorates Axonal Hypomyelination of Periventricular White Matter by Transforming A1 to A2 Astrocyte via JAK2/STAT3 Pathway in Septic Neonatal Rats. JIR. 2021 Nov 12;14:5919–37.

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