ORIGINAL ARTICLE

Protective Effect of Mirtazapine on Brain Tissue in Bleomycin-induced Neurotoxicity in Rats Model

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Abstract

Background: Neurotoxicity refers to the detrimental impact of chemical or biological agents on the structure and function of the nervous system. It can result from various interrelated mechanisms, including oxidative stress, neuroinflammation, mitochondrial dysfunction, and neuronal apoptosis. Bleomycin (BLM), a commonly used chemotherapeutic agent, has been associated with neurotoxic effects, potentially mediated by oxidative damage and mitochondrial impairment. However, the neuroprotective potential of mirtazapine against BLM-induced neurotoxicity in central nervous system tissues remains inadequately explored. **Aim:** This study aimed to investigate the protective effects of mirtazapine on brain tissue in a rat model of BLM-induced neurotoxicity, with a particular focus on histopathological alterations. **Methods:** Thirty-five adult male Wistar rats were randomly assigned to five groups: Control, BLM, BLM plus mirtazapine (15 or 30 mg/kg), and mirtazapine-only groups. Neurotoxicity was induced through a single intratracheal dose of BLM (5 mg/kg), followed by daily oral administration of mirtazapine for 14 days. On day 15, animals were euthanized, and brain tissues were harvested for histological examination. **Results:** Histological assessment revealed that BLM caused significant neuronal degeneration in the cerebral cortex and hippocampus. Mirtazapine at 15 mg/kg provided moderate neuroprotection, while the 30 mg/kg dose markedly preserved cortical and hippocampal architecture, indicating substantial histological improvement. **Conclusion:** Mirtazapine exhibited a dose-dependent neuroprotective effect against BLM-induced brain toxicity, likely through the preservation of neuronal integrity. These findings highlight its potential as a therapeutic candidate for mitigating chemotherapy-related neurotoxicity.

Key words: Bleomycin, brain, histopathology, mirtazapine, neurotoxicity

INTRODUCTION

Neurotoxicity refers to the detrimental effects of chemical or biological agents on the structure and function of the nervous system. It can arise through various mechanisms, including oxidative stress, neuroinflammation, mitochondrial dysfunction, and neuronal apoptosis.[1] These events are frequently triggered by environmental toxins, chemotherapeutic agents, and certain pharmaceuticals, often leading to cognitive impairment and neurodegeneration.[2] Among these mechanisms, oxidative stress plays a pivotal role in disrupting redox homeostasis and promoting the overproduction of reactive oxygen species, which, in turn, causes mitochondrial damage, lipid peroxidation, and ultimately, neuronal death.[3,4] Systemic oxidative stress induced by various agents may influence brain tissue either directly or indirectly through inflammatory mediators or the diffusion of free radicals. Bleomycin (BLM), a glycopeptide antibiotic, is widely utilized as an antineoplastic agent.[5,6] It belongs to a class characterized by glycosylated peptide cores derived from bacterial species, which enables the disruption

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of cellular processes.[7] BLM is commonly incorporated into combination chemotherapy regimens, including adriamycin, BLM, vinblastine, dacarbazine and BLM, etoposide, adriamycin, cyclophosphamide, oncovin, procarbazine, prednisone, for the treatment of various malignancies.[8] Since its Food and Drug Administration approval in 1975, it has remained a cornerstone in the treatment of squamous cell carcinomas, testicular cancers, and malignant lymphomas.[9] Consequently, BLM induces deoxyribonucleic acid strand breaks, particularly at the 3′–4′ phosphodiester bonds in deoxyribose, leading to G2-phase cell cycle arrest and impaired cellular regeneration.[10,11] Under normal physiological conditions, BLM does not penetrate the blood–brain barrier (BBB) effectively. However, modifications, such as osmotic BBB disruption can significantly enhance its central nervous system (CNS) entry. Rodent and canine studies have shown that this increased CNS exposure leads to overt neurotoxicity, unlike other chemotherapeutics, such as cyclophosphamide, which cause minimal neural damage under similar conditions.[12] Pulmonary fibrosis is the most recognized adverse effect of BLM.[13] Given the potential for central neurotoxic effects when BLM reaches CNS tissues, there is increasing interest in identifying protective agents that can mitigate its neural impact.[12] One such agent is mirtazapine, an atypical antidepressant from the noradrenergic and specific serotonergic antidepressant class. Mirtazapine acts by antagonizing central presynaptic α2-adrenergic autoreceptors and heteroreceptors, thereby enhancing the release of norepinephrine and serotonin. It also selectively blocks 5-HT2 and 5-HT3 receptors, facilitating 5-HT1A-mediated neurotransmission and contributing to its anxiolytic and antidepressant effects.[14] Beyond its psychiatric indications, mirtazapine has shown promising results in mitigating organ toxicity from chemotherapeutic agents, such as BLM. Abdelhady *et al*. (2023) demonstrated that mirtazapine alleviated BLM-induced pulmonary fibrosis in rats by downregulating key fibrogenic and inflammatory markers, including TGF-β, PDGF-BB, TIMP-1, and α-SMA. It also reduced hydroxyproline levels and the expression of inflammatory molecules, such as ICAM-1, MCP-1, and CXCL4, suggesting a dual anti-inflammatory and anti-fibrotic effect mediated through antioxidant mechanisms and NLRP3 inflammasome modulation.[15] Furthermore, mirtazapine has demonstrated neuroprotective potential in models of chemically induced neurotoxicity. Alharthy *et al*. (2023) reported that in a cadmium-induced neurotoxicity model, mirtazapine attenuated hippocampal and cortical damage through Nrf2 upregulation and inhibition of the TLR4/NF-κB pathway, with corresponding reductions in tumor necrosis factor-alpha, Interleukin (IL)-1β, and IL-6 expression.[16] A recent review further supports its therapeutic promise across neurological conditions, such as epilepsy, Alzheimer's disease, Parkinson's disease, and chemotherapy-induced cognitive dysfunction, highlighting its capacity to modulate oxidative stress, reduce inflammation, and inhibit neuronal apoptosis.[17] Despite increasing evidence supporting the biochemical and molecular neuroprotective actions of mirtazapine, its histological impact on brain tissue following chemotherapeutic insult remains largely underexplored. Histopathological evaluation plays a critical role in visualizing structural alterations, including neuronal degeneration, gliosis, and inflammatory cell infiltration, which are essential for confirming tissue-level protection in neurotoxicity models.[18] Therefore, integrating histological analysis can provide a comprehensive understanding of mirtazapine's protective effects and substantiate molecular findings through direct morphological evidence. For instance, Gulec *et al.* (2013) reported that mirtazapine preserved neuronal integrity and reduced histological signs of degeneration and inflammation in the brains of cisplatin-treated rats, highlighting its potential in mitigating chemotherapy-induced neurotoxicity.[19] However, the neuroprotective potential of mirtazapine against BLM-induced neurotoxicity in CNS tissues remains inadequately explored. Therefore, this study aims to investigate the protective effect of mirtazapine on brain tissue in a BLM-induced neurotoxicity rat model, focusing on histological changes and underlying molecular mechanisms.

MATERIALS AND METHODS

Animal selection

35 Adult male Albino Wister rats weighing between 200 and 220 g were obtained from the Clinical Pharmacy College, King Faisal University. They were housed under standard environmental conditions and a 12-h light-dark cycle with free access to food and water.

Ethical approval

All experiments were performed by the "Ethical Conduct for Use of Animals in Research" Guidelines of King Faisal University, Kingdom of Saudi Arabia. All animal care and experimental procedures were approved by the Animal Research Ethics Committee at King Faisal University under ethical approval No. (KFU-REC-2023- NOV-ETHICS1562).

Drugs and chemicals

The chemicals for the experiments, including BLM 15 mg (Bleocip 15IU, Cipla Pharmaceutical Company, Mumbai, India), Mirtazapine 15 mg and 30 mg, were purchased from local Riyadh Pharma, and sodium thiopental 500 mg was purchased from Sigma-Aldrich (St.Louis, MO, USA).

Induction of neurotoxicity in rats

After anesthetizing the rats with sodium thiopental 20 mg/kg IP, they were placed on their back, and they were injected with BLM 5 mg/kg, dissolving it in Normal saline to

a final concentration of 1 mg/mL/200 g and administered as a single dose through intratracheal instillation.

Treatment intervention

Thirty-five male Wistar albino adult rats were randomly allocated into five groups, as follows:

I. Normal control (Ctrl) group ($n = 6$). Rats received normal saline through intratracheal instillation as a single dose for 14 days.
II. Diseased group: BLM ($n = 6$). Rats received a single dose of BLM (5 mg/kg) through intratracheal instillation.
III. Treated group: BLM+Mirtazapine 15 mg (BLM+M15) ($n = 6$). Rats received a single dose of BLM (5 mg/kg) through intratracheal instillation. After 1 h, start mirtazapine (15 mg/kg/day, orally) for 14 days.
IV. Treated group: BLM+Mirtazapine 30 mg (BLM+M30) ($n = 6$). Rats received a single dose of BLM (5 mg/kg) through intratracheal instillation. After 1 h, start mirtazapine (30 mg/kg/day, orally) for 14 days.
V. Ctrl group: Mirtazapine only (Mirt) ($n = 6$). Rats received mirtazapine (15 mg/kg/day, orally) only for 14 days.

The drug treatment extends until day 14, with the rats euthanized under anesthesia on day 15. The brain tissues were dislocated. Washed well in 10 % formalin for histochemical investigation.

Histological examination of tissue

After incubating the sample of brain, formalin solution for tissue fixation for a full day, each group was individually allocated. The Leica TP1020 device, a German-manufactured model, was utilized for tissue processing. Tissues underwent a series of alcohol dehydration steps, ranging from 70% to 100% concentration, using Scharlau alcohol solution to remove water and replace it with alcohol. Subsequently, Sigma Aldrich. Xylene, a chemical compound, was used to remove alcohol and facilitate wax infiltration within the tissues at a temperature of 70°C. In the tissue embedding stage in wax, the Leica1160 device was used at a temperature of 70°C. Subsequently, it was cooled down to 4°C and left to cool. The tissue was sliced to a thickness of 5 micro using the Leica RM 2255 microtome and placed on a slide, then left on a hot surface at 40°C to dry. After drying, the slides were stained with hematoxylin and eosine stain from Sigma. Finally, the slides were photographed using an Olympus BX51 microscope and a Dp70 Camera.

RESULTS

Histological features of the normal Ctrl group

The Ctrl group exhibited normal histological features of both the cerebral cortex and hippocampus. Neuronal cells appeared well-organized with intact morphology, prominent nuclei, and no evidence of degeneration, gliosis, or inflammatory infiltration [Figures 1-4].

The examined brain sections from the Ctrl Group [Figures 1-4] revealed apparently normal cerebral cortex and hippocampus.

Diseased group, BLM

In contrast, the BLM Group demonstrated severe histopathological alterations. The cerebral cortex showed extensive neuronal degeneration, cytoplasmic vacuolation, and areas of gliosis. The hippocampus revealed disrupted pyramidal cell layers, shrunken hyperchromatic neurons, and increased pericellular spaces, indicating significant neurotoxicity [Figures 5-8].

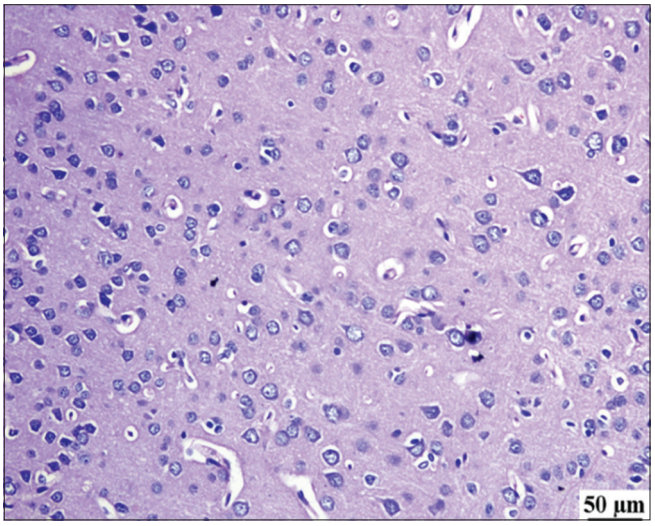

Figure 1: Photomicrograph of brain, cerebral cortex control group showing apparently normal cerebral cortex hematoxylin and eosin

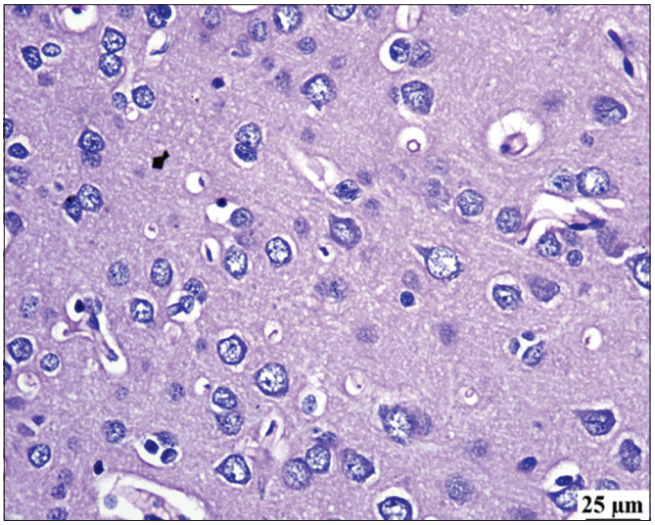

Figure 2: Photomicrograph of brain, cerebral cortex control group higher magnification showing apparently normal cerebral cortex hematoxylin and eosin

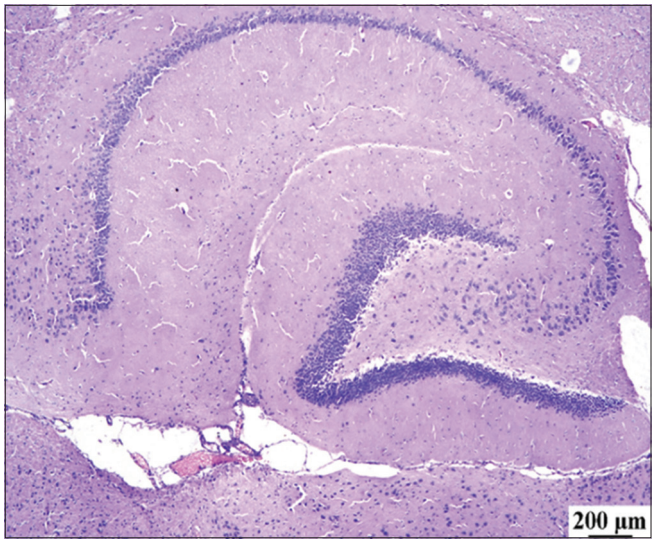

Figure 3: Photomicrograph of brain, hippocampus control group showing apparently normal hippocampus hematoxylin and eosin

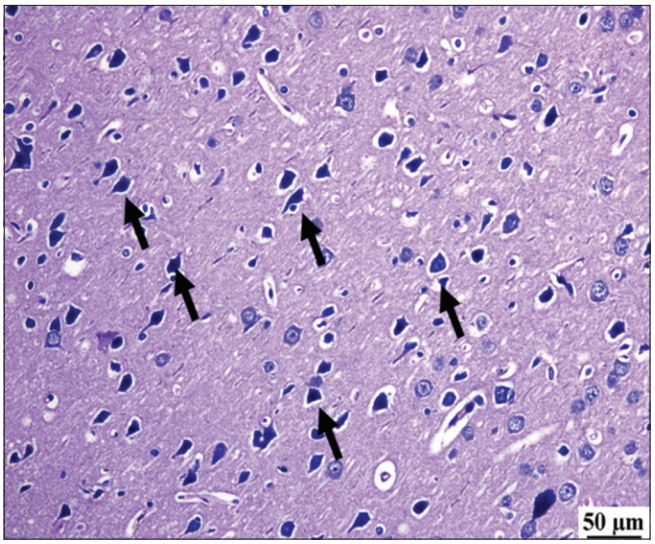

Figure 5: Photomicrograph of brain, cerebral cortex bleomycin group showing numerous dark degenerating neurons (arrows) hematoxylin and eosin

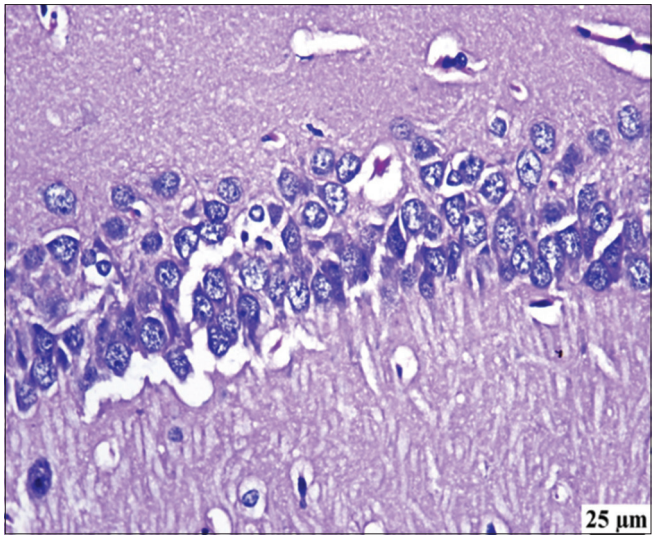

Figure 4: Photomicrograph of brain, hippocampus control group higher magnification showing apparently normal hippocampus hematoxylin and eosin

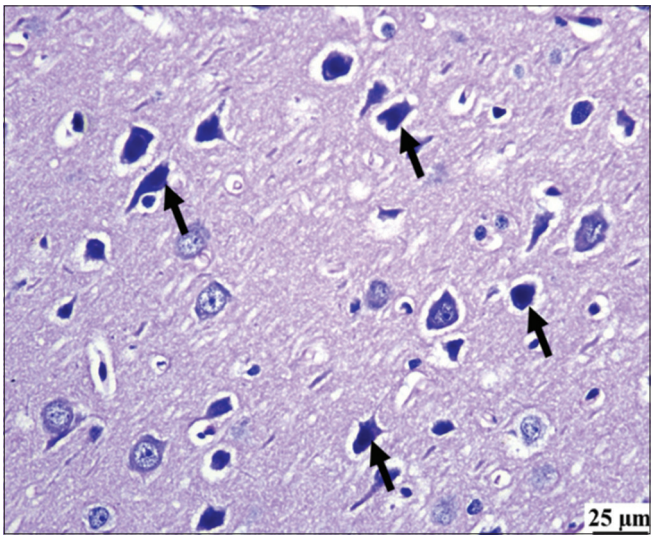

Figure 6: Photomicrograph of brain, cerebral cortex bleomycin group higher magnification showing numerous dark degenerating neurons (arrows) hematoxylin and eosin

BLM Group [Figures 5-8] showed numerous dark degenerating neurons within the cerebral cortex and hippocampus.

Treated group: BLM+Mirtazapine 15 mg (BLM+M15)

BLM+M15 Group exhibited mild improvement in histological structure compared to the BLM-only group. The cerebral cortex showed partial restoration of neuronal arrangement with reduced vacuolation and less prominent gliosis. The hippocampus was relatively preserved but still showed scattered degenerative neurons and mild disorganization [Figures 9-12].

BLM+M15 Group [Figures 9-12] showed moderate improvement as the cerebral cortex contained numerous dark degenerating neurons meanwhile, numerous dark degenerating cells were detected in the hippocampus.

Treated group: BLM+Mirtazapine 30 mg (BLM+M30)

BLM+M30 Group showed marked histological improvement. The cerebral cortex appeared nearly normal, with well-arranged neurons and minimal pathological alterations. The hippocampus demonstrated improved cytoarchitecture [Figures 13-16].

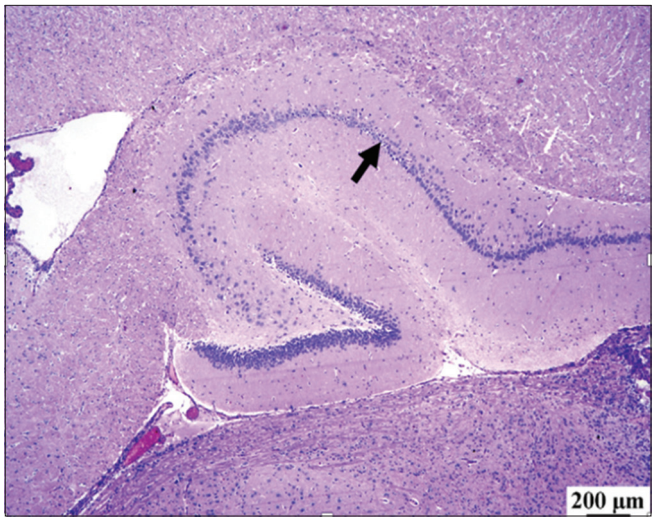

Figure 7: Photomicrograph of brain, hippocampus bleomycin group showing numerous dark degenerating neurons (arrows) hematoxylin and eosin

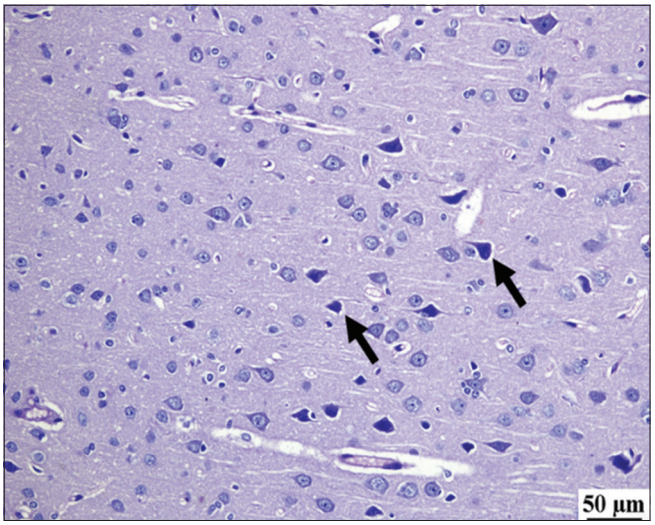

Figure 9: Photomicrograph of brain, cerebral cortex bleomycin+mirtazapine 15 group showing few dark neurons (arrow) hematoxylin and eosin

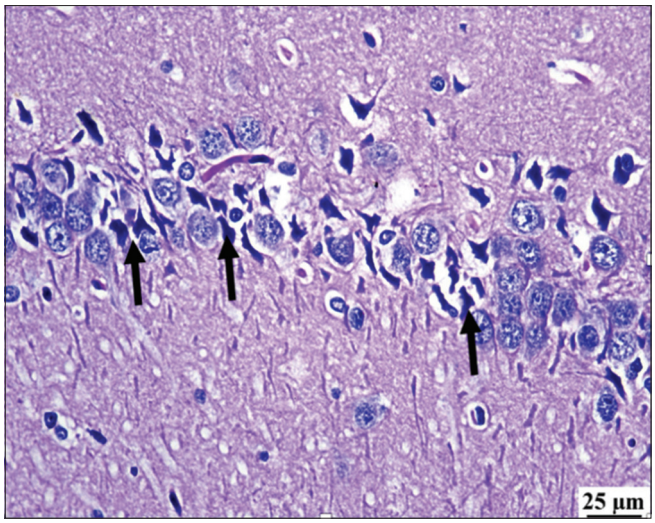

Figure 8: Photomicrograph of brain, hippocampus bleomycin group higher magnification showing numerous dark degenerating neurons (arrows) hematoxylin and eosin

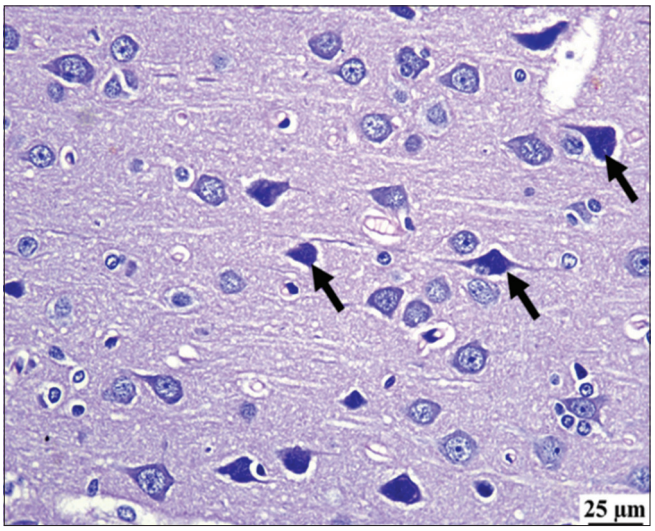

Figure 10: Photomicrograph of brain, cerebral cortex bleomycin+mirtazapine 15 group higher magnification showing few dark neurons (arrow) hematoxylin and eosin

BLM+M30Group [Figures 13-16] exhibited marked improvement as the examined sections revealed the existence of few degenerating neurons within the cerebral cortex and hippocamps.

Ctrl: Mirtazapine only (Mirt)

Mirt Group showed brain histology comparable to the normal Ctrl group, with preserved neuronal architecture and no apparent pathological changes in either the cerebral cortex or hippocampus [Figures 17-20].

Mirt Group [Figures 17-20] showed apparently normal brain sections.

DISCUSSION

The present histological findings underscore the pronounced neurotoxic effects of BLM, as evidenced by neuronal degeneration, cytoplasmic vacuolation, gliosis, and disruption of hippocampal pyramidal layers, consistent with chemotherapy-induced oxidative stress and neuroinflammation. In contrast, the group treated with mirtazapine alone preserved normal brain architecture, indicating its safety at the administered dose, in line with evidence describing its brain antioxidant effects. Co-administration of mirtazapine with BLM demonstrated a clear dose-dependent neuroprotective effect: Group M15 exhibited mild structural improvement, whereas Group M30 showed substantial preservation of neuronal integrity and

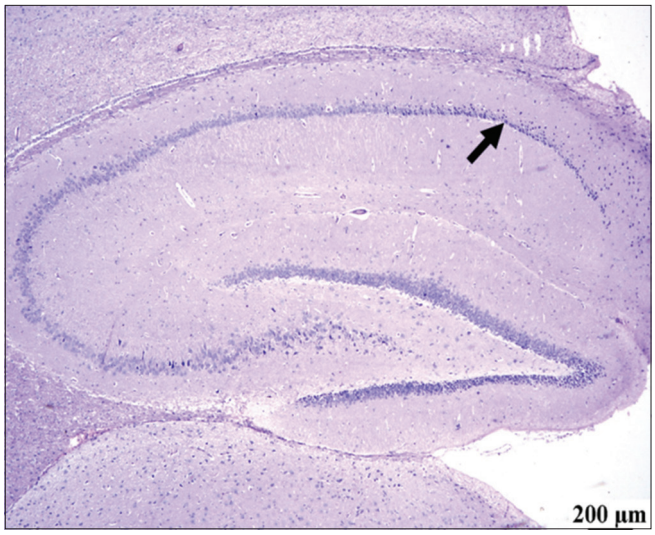

Figure 11: Photomicrograph of brain, hippocampus bleomycin+mirtazapine 15 group showing numerous dark neurons (arrow) hematoxylin and eosin

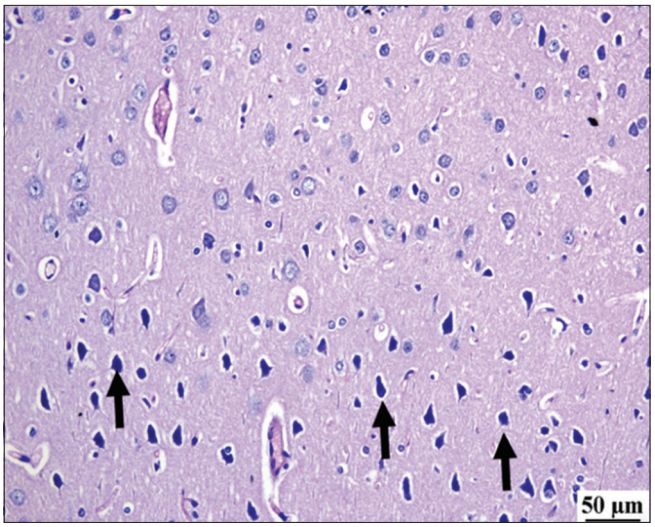

Figure 13: Photomicrograph of brain, cerebral cortex bleomycin+mirtazapine 30 group showing few dark neurons (arrow) hematoxylin and eosin

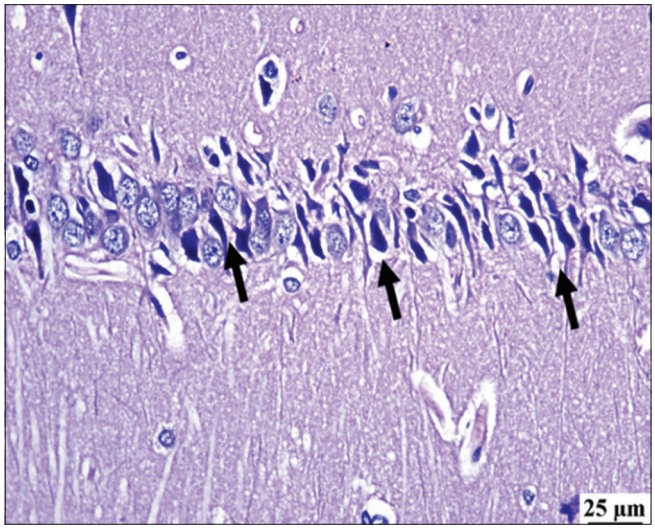

Figure 12: Photomicrograph of brain, hippocampus bleomycin+mirtazapine 15 group, higher magnification showing numerous dark neurons (arrow) hematoxylin and eosin

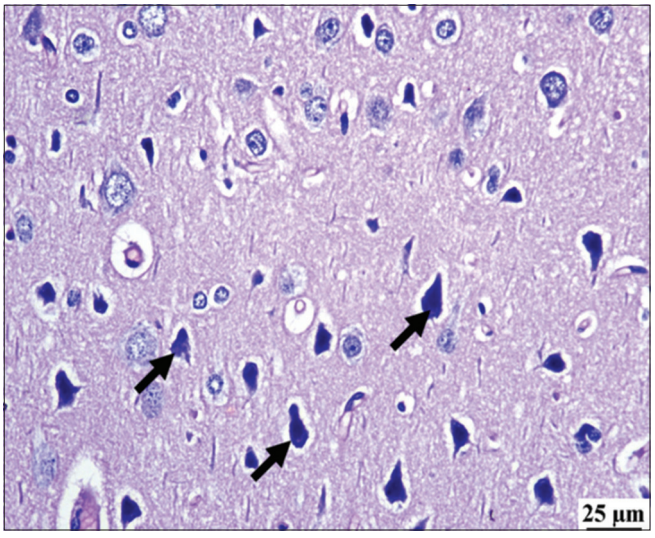

Figure 14: Photomicrograph of brain, cerebral cortex bleomycin+mirtazapine 30 group higher magnification showing few dark neurons (arrow) hematoxylin and eosin

marked reduction in inflammatory and degenerative changes. Although BLM is predominantly known to cause pulmonary fibrosis, evidence for its neurotoxicity remains limited. Most pre-clinical and clinical studies have not focused on its impact on the CNS, possibly due to its poor penetration across the BBB under systemic administration. However, some experimental studies suggest that BLM can exert neurotoxic effects under specific conditions. For instance, direct intracerebral injection in rodents has been shown to induce significant brain tissue damage, including necrosis, edema, and inflammation.[20] In addition, Neuwelt *et al*. (1983) demonstrated that systemic administration of BLM in animals with osmotically disrupted BBB resulted in hemorrhagic necrosis, gliosis, and marked neurotoxicity.[12] These findings, though derived from non-standard delivery routes, suggest that BLM may possess latent neurotoxic potential that could be unmasked in pathological conditions involving BBB disruption, possibly mediated by mechanisms, such as oxidative stress and neuroinflammation. These outcomes align with prior studies reporting mirtazapine's neuroprotective potential through antioxidant and anti-inflammatory mechanisms, likely mediated by mitochondrial stabilization and modulation of oxidative stress pathways.[21] A key strength of this study lies in its well-designed experimental framework, which included both safety (mirtazapine-only) and dose-response arms, enabling a clear distinction of neuroprotective efficacy. In addition, focusing on well-characterized brain regions (cerebral cortex and hippocampus) enhanced the histopathological clarity and relevance. Despite these promising findings,

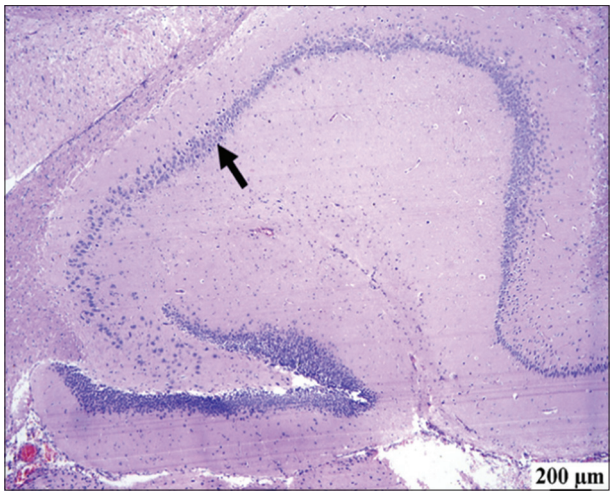

Figure 15: Photomicrograph of brain, hippocampus bleomycin+mirtazapine 30 group showing few dark neurons (arrow) hematoxylin and eosin

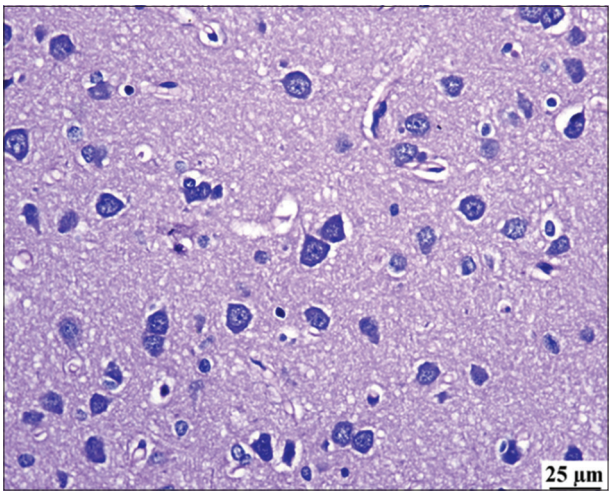

Figure 18: Photomicrograph of brain, cerebral cortex mirtazapine group higher magnification showing apparently normal cerebral cortex hematoxylin and eosin

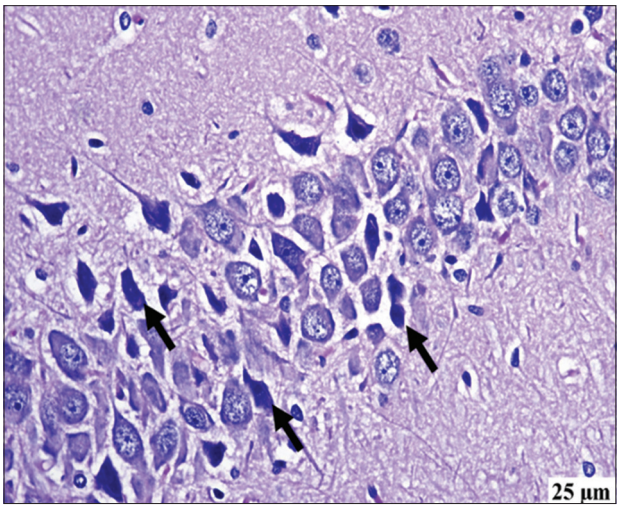

Figure 16: Photomicrograph of brain, hippocampus bleomycin+mirtazapine 30 group higher magnification showing few dark neurons (arrow) hematoxylin and eosin

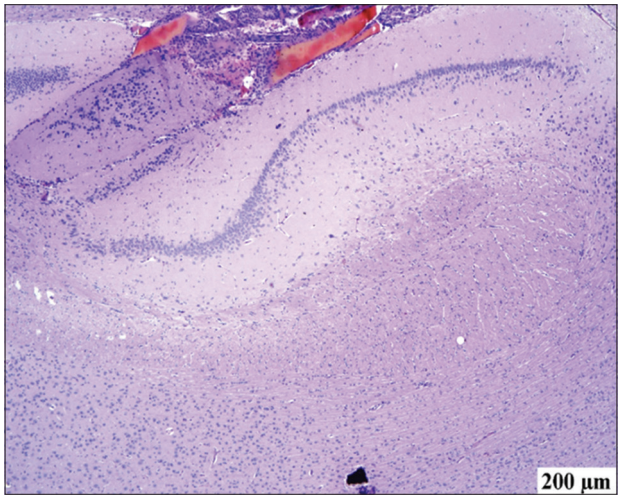

Figure 19: Photomicrograph of brain, hippocampus mirtazapine group showing apparently normal hippocampus hematoxylin and eosin

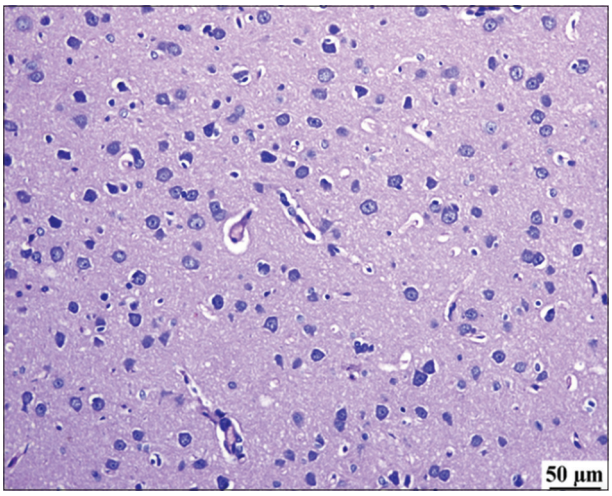

Figure 17: Photomicrograph of brain, cerebral cortex mirtazapine group showing apparently normal hippocampus hematoxylin and eosin

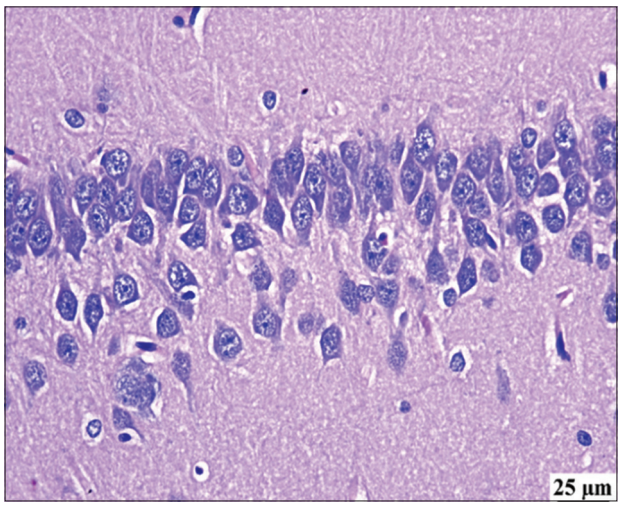

Figure 20: Photomicrograph of brain, hippocampus mirtazapine group, higher magnification showing apparently normal hippocampus hematoxylin and eosin

further investigation is warranted to enhance mechanistic understanding and translational value. Future studies should explore molecular biomarkers, such as oxidative stress indicators and mitochondrial regulators (e.g., SIRT1, PARKIN, PGC-1α, NRF1, and MFN2). Behavioral and cognitive assessments would help link histological protection to actual neurological function. Comparing mirtazapine with other neuroprotective agents and employing diverse neurotoxicity models would expand the generalizability. Finally, establishing the long-term safety profile and therapeutic window of high-dose mirtazapine is essential.

CONCLUSION

In conclusion, this study provides robust pre-clinical evidence that mirtazapine mitigates BLM-induced neurotoxicity in a dose-dependent manner, supporting further investigation into its potential as a neuroprotective agent.

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