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Original Research Article

Protective Effect of RH Against Benzo[α]pyrene-induced Memory Impairment and Neurodegenerative Changes in Mice

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Abstract

Background: Rohitukine (RH) in Benzo[α]pyrene, an experimental model of cognitive and memory impairment, has not been reported yet. Hence, with the state of the art in repurposing techniques, we redefined the use of RH against CNS disorders, using Benzo[α]pyrene-induced mouse model of cognitive memory impairment. A wide range of components, including plaques of beta-amyloid, tangled neurofibrillary fibres, disappearance of neuronal cells, and anti-inflammatory reactions, collaborate to generate Alzheimer's (AD) disease. Considering that beta-amyloid peptides constitute a crucial part of amyloid-containing plaques, it has been suggested that a significant rise in the production is closely associated with the progression of dementia.
Objectives: The pharmacophore model was employed in a repurposing strategy to identify the characteristics of RH and potential anti-Alzheimer's agents. This approach aimed to assess whether RH can prevent memory impairment and brain damage induced by Benzo[α]pyrene exposure in mice, as well as to investigate the biological mechanisms underlying this protective effect**.**
Materials and Methods: In the present investigation, mice were administered oral RH (20 and 40 mg/kg) starting from the 14th to 28th days, Bαp (5 mg/kg) intraperitoneally every day for 28 days. An open-field test was carried out to evaluate locomotor activity, whereas an elevated plus maze and novel object recognition test were additionally employed to measure non-spatial visualization., ELISA kits were used to analyze animal tissue homogenates for Aβ1-42, anti-inflammatory markers, oxidative damage indicators, and histopathology estimation.
Results: The Repurposing technique with the reported pharmacophore model was used to identify the features of RH and potential anti-Alzheimer agents. The same was followed by docking experiments to rationalize the repurposing, still with receptor interactions. The biological process showed significant results on the 29th day; homogenization of the mice's brain was carried out to perform behavioral, biochemical, and other neuroinflammatory parameters. The Bap-treated mice show decreased activity in EPM and NORT parameters, whereas they show an elevated rise in the level of lipid peroxidation ($p < 0.001$) assay as well as neuroinflammatory parameters; IL6 ($p<0.001$) and Aβ1-42 ($p < 0.001$) simultaneously. The mean±SEM was reported for the results. To analyze the Actophotometer and Object Recognition Task (NORT), two-way ANOVA was conducted, followed by a Bonferroni post hoc test for multiple comparisons. For other molecular, biochemical, and neuroinflammatory results, a one-way ANOVA was performed, followed by Tukey's post hoc test using GraphPad Prism software (Prism 10). However, RH (RH) high doses orally improved behavioral, biochemical, and other neuroinflammatory parameters. In a dose-dependent way, RH improved antioxidant activity as well as interleukin 6 and amyloid beta1-42 parameters.
Conclusion: It has been shown that RH exhibits neuroprotective activity by improving cognitive functions, inhibiting NFKB activity, decreasing oxidative stress parameters, and inhibiting the ERK-MAP kinase activity. Therefore, RH can act as a neuroprotective drug in further models of Alzheimer's.

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Keywords
Repurposing, Alzheimer's disease, benzo[α]pyrene, rohitukine, novel object recognition test, elevated plus maze
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Introduction

Dr. Alois first discovered the neurological disorder in 1906 while conducting research on a patient, Augusta D. He identified histopathological and neurological changes that are considered the diagnostic features of Alzheimer's disease (AD; Khan, 2016). It includes mental disorders caused by dysfunctional neurons in the brain, which can affect cognitive decline (Alzheimer's Association, 2013). AD impacts the neurological system by damaging the neuronal networks, which are required to maintain cognitive behavior ("2023 Alzheimer's disorder facts and Figures," 2023). As per the report, Alzheimer's 2020 stated that a degenerative condition may get worse over time and is more likely to develop symptoms earlier without unreported cognitive changes (Alzheimer's Association, 2020). The epidemiology of AD defines it as the typical form of cognitive impairment that impacts the population, although Australia is known to be the third most common cause of impairment (Eratne et al., 2018). Studies reported that increased production of amyloid precursor protein leads to cell growth and cell division, whereas oxidative phosphorylation inhibits the function of Tau, which causes neurological illness; further, presenilin genes are responsible for the advances in AD (Vellingiri, 2024).

However, the pathophysiology of AD is quite difficult as it includes multiple neurotransmitters. Amyloid beta (Aβ), neurofibrillary tangles, and premature cell death are the primary features that involve Alzheimer's pathophysiology (Imbimbo et al., 2005; Roy et al., 2023; Wang et al., 2023).

Benzo[α]pyrene (Bαp), a polycyclic aromatic hydrocarbon, is commonly found in soil, water, air, grilled foods, and smoke. Human exposure is widespread and poses significant health risks (Bukowska et al., 2022; Liu et al., 2020; Zhang et al., 2016). The neurotransmitter acetylcholinesterase (AChE), responsible for memory, could serve as a diagnostic tool for neurodegeneration associated with environmental pollutants (Niu et al., 2010).

As of now, currently, memantine, rivastigmine, and tacrine are the most common drugs used in the therapy of AD, whereas clinical studies and animal models have supported an extensive list of medicinal products as therapeutic alternatives for the disease, but very few of the medicinal compounds currently exist in drug designing process although substantial advancements have been made for novel pharmaceutical compounds (Aprahamian et al., 2013). Additionally, medicinal plants, such as *Ginkgo biloba*, curcumin, and ginseng, are currently used in clinical and research settings to treat neurological disorders (Ratheesh et al., 2017).

Amoora rohituka and *Dysoxylum binectariferum* (Meliaceae) are medicinal plants known for producing RH ($C_{16}H_{19}O_5N$), an alkaloid with notable anti-cancer activity. Rohitukine (RH), initially extracted from *A. rohituka*, was later found in *D. binectariferum* and serves as a key precursor for flavopiridol (Mohanakumara et al., 2010). RH is found exclusively in four plant species: *A. rohituka* and *D. binectariferum* (Meliaceae) and *Schumanniophyton magnificum* and *Schumanniophyton problematicum* (Rubiaceae). The trunk bark of *D. binectariferum* is the preferred source for RH due to its high yield (Singh et al., 2017). Beyond its anti-cancer effects, RH also exhibits anti-inflammatory (Singh et al., 2019), immunomodulatory (Kumar et al., 2020), anti-fertility (Keshri et al., 2007), and gastroprotective properties (Singh et al., 2012). However, RH's efficacy and mechanism in a Bαp-induced model of cognitive impairment remain unreported. This study aims to evaluate its therapeutic effects in this experimental AD model.

Materials and Methods

Animals

In this study, a total of 58 healthy male Swiss albino mice were employed. Mice weighing 30–35 g were acquired from the National Institute of Pharmaceutical Education and Research (NIPER), Punjab, India. The mice were divided into seven experimental groups and housed in sterilized cages within the animal house. After the procurement of animals, acclimatization was accomplished under preferred conditions recommended by the Committee for Purpose and Supervision of Experiments on Animals (CCSEA). The Institutional Animal Ethics Committee (IAEC) at Lovely Professional University, Phagwara, Punjab, India, authorized the experimental protocol number LPU/IAEC/2023/64, which adhered to the procedure set forth by the CCSEA.

Procurement of Reagents and Chemical Substances

Bαp was procured from Tokyo Chemical Industry (B0085), India. Rohitukine (RH) was extracted as consistent with the protocol. Bαp (5 mg/kg) was dissolved in 1 mL/kg of olive oil and administered intraperitoneal (i.p.). RH was formulated in a normal saline solution and administered orally to the animals at doses of 20 and 40 mg/kg (p.o.) The biochemical kits for measuring interleukin-6 (IL-6), lipid peroxidation (LPO), reduced glutathione (GSH), and amyloid $beta_{1–42}$ ($Aβ_{1–42}$) levels have been purchased from Essence Lifesciences, Chandigarh. All of the other chemicals and reagents were acquired from the local market in Punjab.

Extraction, Purification, and Isolation of Pure Compound RH

The RH was isolated from the natural compound *D. binectariferum* stem bark through air-drying and powdering and extracted with distilled ethanol. The extracts have been filtered, distilled, and dried under a high vacuum to separate solvent traces, resulting in a brown, viscous mass. The extraction and observation identify RH as a known alkaloid with a purity of over 95% (Balaramnavar et al., 2014), as shown in Figure S1.

Experimental Protocol

First, mice were divided into seven groups of eight animals (n = 8). The distribution of the groups and their respective treatments are provided in Table S1. The experimental protocol comprised 28 days, during which groups IV, V, VI, and VII received Bαp induction for 28 days. Bαp (5 mg/kg) was dissolved in 1 mL/kg of olive oil and administered intraperitoneally (Goal et al., 2022). RH, available at low and high doses, was prepared in a normal saline solution and administered orally to animals. From the 14th day onward, groups V, VI, and VII received a low dose of RH (20 mg/kg; Singh et al., 2017), RH high dose (40 mg/kg; Kumar et al., 2020), and donepezil (3 mg/kg; Pattanashetti et al., 2025), all prepared in normal saline and administered orally. Groups I, II, and III received their respective treatments without Bαp induction. Locomotor activity was assessed using the novel object recognition test (NORT) and elevated plus maze (EPM) tests. On the 29th day, the mice were euthanized, and their hippocampal tissue was used for histopathology, biochemical investigation, and molecular target investigation.

Behavioral Parameters

Estimation of Body Weight

The body weight was measured on the following days: 1, 7, 14, 21, and 28 of the experimental schedule.

Estimation of the Open Field Test

The open field maze is one of the most common instruments used to evaluate behavioral activity in animal models. Subsequently, it is the simplest and reliable test that provides behavioral data for assessing the locomotor activity in rodents. The locomotor activity was performed on 0, 21, and 28 days, as per the experimental protocol (Seibenhener & Wooten, 2015).

Estimation of NORT

The NORT is mainly used to assess learning and memory in mice, although the procedure includes habituation day, training day, and testing day. In the NORT procedure, the rodent has the ability to explore two similar objects during its training phase; however, during the testing phase, an entirely novel object is replaced with one identical object. As a result, the mouse will explore the oval object. Then, oval object recognition tests were performed on the 1st and 28th days as per the experimental protocol (Lueptow, 2017). The exploration time was captured by using the software (VJ All Maze Pvt. Ltd., Mumbai).

EPM Task

To assess mouse memory, learning, and apprehension, the EPM was employed. The test evaluated the method, procedure, and results of assessing memory and comprehension. The maze consisted of two open-ended arms and two closed arms, each of which extended from a single middle point. The maze was raised 25 cm above the ground level. At some point in the initial day, every single mouse was relocated to an open arm, far removed from the center position. The transfer latency (TL) was the amount of time it took. In contrast, the movement of the mouse was captured via one of the surrounding arms at the same time, as well as the usage of each of its legs, and was captured by using the software (VJ All Maze Pvt. Ltd., Mumbai). In contrast, on the initial day, the TL latency was evaluated and recorded. If the rodent did not attain one of the limited arms before 90 s had elapsed, the animal was carefully pushed into certainly one of the restrained arms, after which the TL became 90 s. The mouse was subsequently allowed to continue wandering the maze for a further 10 s before being returned to its cage. However, the potential to take into account the newly learnt task was evaluated after 24 h following the initial day of evaluation. The EPM task was performed on 1, 21, and 28 days as per the experimental protocol (Dhingra et al., 2004).

Biochemical Parameters

Homogenization of Mice Brain for Biochemical Parameters

On the 29th day, the mice were euthanized by cervical dislocation. Sooner or later, the brains were removed and handled with isotonic saline at 4°C to separate the hippocampus part of the brain. The tissues have been combined with 0.1 M phosphate-buffered saline (PBS) with a pH of 7.4 to create a 10% weight/volume tissue homogenate. A pH meter was used to adjust the pH to make sure that the hippocampus tissues were blended as they were located in an ice-cooled glass homogenizer (RQ-127, REMI, India) containing a pH of 7.4. After that, the homogenate was then centrifuged at 10,000 *g* for 15 min at −4°C employed centrifuge (CM-12 Plus, REMI, India), and the supernatant was assembled or gathered in sterilized Eppendorf tubes that were stored at −20°C for further biochemical analyses, along with the measurement of oxidative stress markers, inflammatory markers, enzyme-linked immunosorbent assay (ELISA) kits for $A\beta_{1-42}$ peptide, as shown in Figure 5.

Estimation of LPO Levels in Brain

The LPO quantification was accomplished by utilizing the LPO calorimetric assay kit from Elabscience (E-BC-K176-M). Following a 60-min incubation, a stable chromophore with an absorbance was measured at 586 nm. The reagents were organized in steps with the instructions furnished inside the kit.

Estimation of GSH Levels in the Brain

The GSH quantification was executed using the GSH calorimetric assay kit from Elabscience (E-BC-K030-M). The subsequent equation represents the reaction between GSH and DTNB: GSH + DTNB –> GSSH + TNB; GSSH is oxidized glutathione, and TNB is 5-thio-2-nitrobenzoic acid. The reagents were prepared consistent with the instructions provided in the kit.

Determination of Protein

According to Lowry et al. (1951), the methodology was implemented to evaluate the total number of protein content in the hippocampus. The bovine serum albumin, also known as BSA, standard plot was employed for estimating the amount of protein in the samples (Lowry et al., 1951).

Measurement of Neuroinflammatory Biomarkers in Mice Brain Homogenate

Measurement of IL-6 Levels in Mice Brain

The IL-6 levels in mice brains have been employed by using the mouse IL-6 ELISA kit, Elabscience (catalog no: E-EL-M0044). The absorbance of IL-6 in the brain homogenates of mice was expressed in pg/mL.

Estimation of $A\beta_{1-42}$ Level in Mice Brain

$A\beta_{1-42}$ level in mice brains had been quantified by using the $A\beta_{1-42}$ ELISA kit, Elabscience (catalog number: E-EL-M3010). The absorbance of $A\beta_{1-42}$ in the brain homogenates of mice was expressed in pg/mL.

Statistical Analysis

The Mean ± Standard Error of the Mean (SEM) was reported for the results. Actophotometer and NORT were used to conduct a two-way ANOVA, followed by a Bonferroni *post hoc* test for multiple comparisons. Tukey's *post hoc* test was carried out using GraphPad Prism software (Prism 10) following the biochemical, and neuroinflammatory data had been evaluated using one-way ANOVA. The p values < .05 were considered statistically significant.

Histopathological Analysis

Using hematoxylin and eosin staining, hippocampus sections of the mice's brain were evaluated by histopathology. Sections of the hippocampus and cerebral cortex that were 7–9 mm thick were embedded in paraffin wax and fixed in formalin calcium. Following that, it was sliced and dewaxed in xylene, hydrated to lower the alcohol percentage, stained with hematoxylin, dehydrated to raise the alcohol percentage to 70%, and stained with 1% alcoholic eosin. Additionally, it was separated in 90% alcohol and removed in xylene. For histological evaluation, the stained transverse sections were examined and photographed using a Zeiss Primo Star microscope (Kumar et al., 2021).

Results

Computational repurposing: The ligand pharmacophore mapping protocol was employed for mapping of RH on the Caspase-8 inhibitors pharmacophore, which showed good fit values of 2.987, supporting the potential of RH as an anti-Alzheimer's agent, as represented in Figure 1A.

The results further revealed that RH showed good fits for ring aromaticity with coumarin ring, hydrogen bond acceptor lipid at C=O, –OH, and piperidine nitrogen, which showed good fits and strong potential for anti-Alzheimer's activity. Docking of RH with Caspase-8 showed strong hydrogen bond interactions with key active site residues, consistent with earlier findings (Figure 1B).

Behavioral Evaluation

RH Indicates No Locomotor Alteration in Bαp-intoxicated Mice

Locomotor activity was assessed on days 0, 21, and 28 to evaluate RH's effects across all experimental groups. Two-way ANOVA showed no significant difference between BαP-induced AD mice and normal controls. Additionally, donepezil (3 mg/kg), RH low dose (20 mg/kg), and RH high doses showed no significant effects compared to the experimental group. No significant changes in locomotor activity were observed throughout the experiment, as illustrated in Figure 2A.

Effect of RH on Memory Performance in NORT in Bαp-induced AD in Mice

NORT results showed that BαP-treated mice (5 mg/kg; $p <$ .001, 3.25 ± 0.37) had reduced interaction with novel objects compared to normal controls. Additionally, when compared with the experimental control group, standard (donepezil 3 mg/kg; $p <$ .001; 7.875 ± 0.692756), RH low dose (20 mg/kg; $p <$ .05; 6.375 ± 0.754451), and RH high dose ($p <$ .01; 7.25 ± 0.881354) revealed a significant increase in the time spent with the new object. High-dose RH (40 mg/kg) significantly improved cognitive and memory deficits in AD mice, as shown in Figure 2B and 2C.

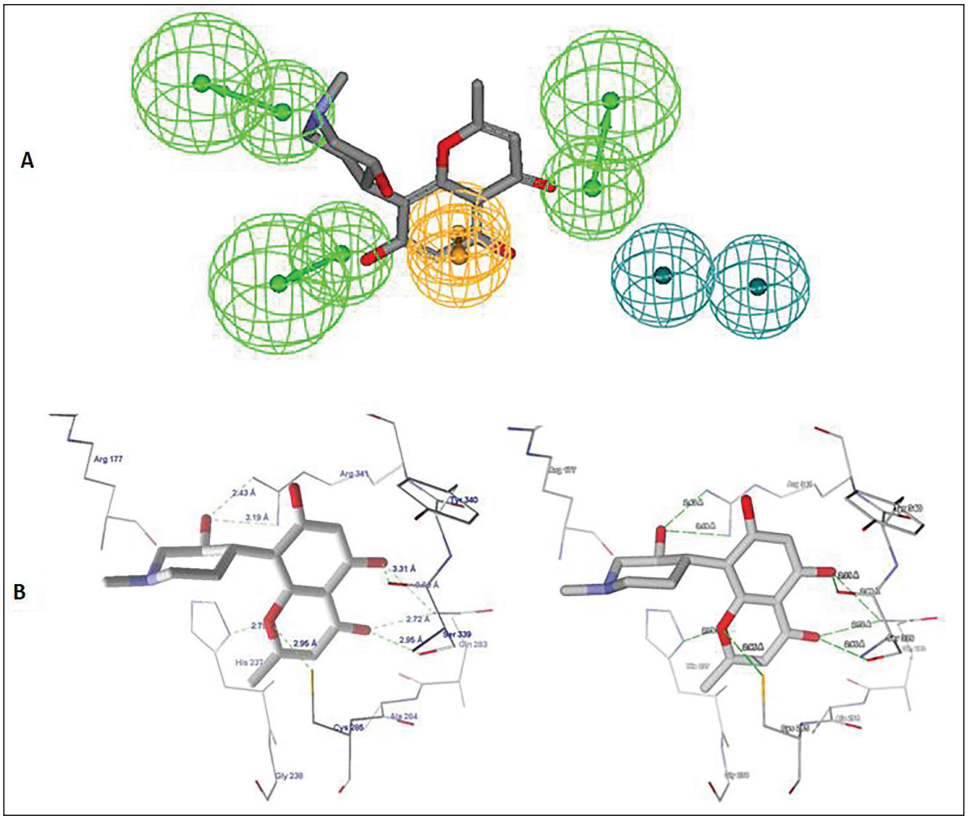


Figure 1. (A) The Ligand Pharmacophore Mapping of the Rohitukine (RH) on Caspase-8 Inhibitors Pharmacophore. (B) The Molecular Docking Interactions of RH with Active Site Residues in Binding Site of Caspase-8.

The Effect of RH in the EPM Test Improved the Learning and Memory in Bαp-induced AD in Mice

EPM tests on days 1, 22, and 28 revealed that BαP-treated mice (5 mg/kg) showed increased TL by day 28, indicating impaired learning and memory. Significant differences were observed compared to the normal group on days 22 and 28, as shown in Figure 2D.

Biochemical Analysis

Effect of RH on LPO Assay in the Hippocampus Region of Bαp Mice

Malondialdehyde (MDA) levels, a marker of LPO, were measured in the hippocampus using an LPO colorimetric assay kit (cat. no. E-BC-K176-M) to assess oxidative stress in BαP-treated mice. One-way ANOVA revealed a significant effect of treatment on hippocampal LPO levels. Compared to the normal control group, the experimental control group (Bαp 5 mg/kg) induced AD and significantly increased hippocampal MDA levels (264.829 ± 18.11006; $p < .001$). Similarly, the standard (donepezil 3 mg/kg), low-dose RH (20 mg/kg), and high-dose RH treatments significantly reduced hippocampal MDA levels in AD mice ($p < .001$) compared to the control group, indicating that high-dose RH attenuated neurodegenerative markers, as shown in Figure 3A.

Effect of RH on GSH in the Hippocampus Region of Bαp-intoxicated Mice

GSH levels in the hippocampus of BαP-treated mice were measured using a GSH colorimetric assay kit (cat. no. E-BC-K030-M) to evaluate oxidative stress. One-way ANOVA revealed a significant effect of treatment on hippocampal GSH levels. Compared with the normal control group, the experimental control group (Bαp 5 mg/kg) induced AD and significantly decreased hippocampal GSH level (77.00593 ± 6.914843; $p < .001$). Similarly, standard (donepezil 3 mg/kg; $p < .001$; 197.816 ± 11.92381), low-dose RH ($p < .01$; 143.392 ± 13.21447), and high-dose RH ($p < .001$; 183.5769 ± 10.37898) treatments significantly increased hippocampal GSH levels in AD mice compared to experimental control group, with high-dose RH showing notable improvement in cognitive deficits, as shown in Figure 3B.

Effect of RH on IL-6 in the Hippocampus Region of Bαp-intoxicated Mice

IL-6 levels in the hippocampus were measured using a mouse IL-6 ELISA kit (cat. no. E-EL-M-K0044) to assess neuroinflammation in BαP-treated mice. One-way ANOVA revealed a significant effect of treatment on hippocampal IL-6 levels. Compared with the normal control group, the

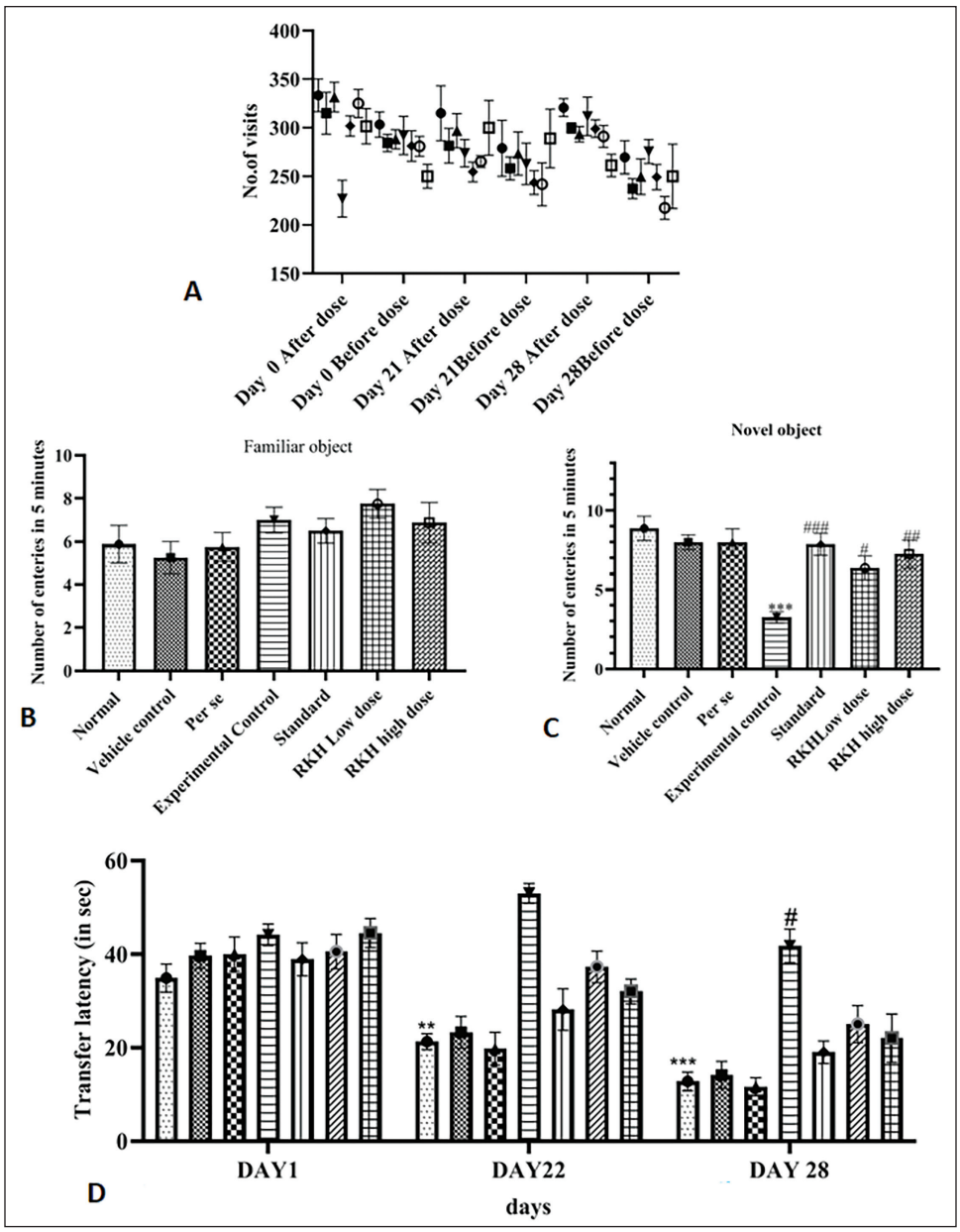


Figure 2. Effect of Rohitukine (RH) on Behavioral Parameters in Benzo[α]pyrene (BαP)-induced Alzheimer's Disease (AD) Mice.

Notes: (A) Locomotor activity was assessed on days 0, 21, and 28 using two-way analysis of variance (ANOVA) with Bonferroni *post hoc* test. (B, C) Novel object recognition test (NORT) and (D) elevated plus maze (EPM) test were analyzed using one-way ANOVA followed by Tukey's *post hoc* test. Statistical significance: *$p < .05$, **$p < .01$, ***$p < .001$ versus normal control; #$p < .05$, ##$p < .01$, ###$p < .001$ versus experimental control (BαP 5 mg/kg). Data are presented as mean ± standard error of the mean (SEM).

experimental control group (Bαp 5 mg/kg) induced AD and significantly increased hippocampal IL-6 levels (1,060.625 ± 25.2211, $p < .001$). Similarly, standard (donepezil, $p < .001$; 333.125 ± 71.3138), low-dose RH (20 mg/kg; $p < .001548$; 125 ± 66.7815), and high-dose RH ($p < .001401$; 25 ± 79.6799) significantly reduced hippocampal IL-6 levels in AD mice compared to control group ($p < .001$), with high-dose RH showing notable improvement in cognitive deficits, as shown in Figure 3C.

Effect of RH on $A\beta_{1-42}$ in the Hippocampus Region of Bαp-intoxicated Mice

$A\beta_{1-42}$ levels in the hippocampus were measured using a mouse $A\beta_{1-42}$ ELISA kit (cat. no. E-EL-M3010) to evaluate treatment efficacy in AD mice. One-way ANOVA revealed a significant effect of treatment on hippocampal $A\beta_{1-42}$ levels. Compared with the normal control group, the experimental control group [23.384 ± 1.5544] (Bαp-induced AD 5 mg/kg) exhibited a significant increase ($p < .001$) in hippocampal Aβ

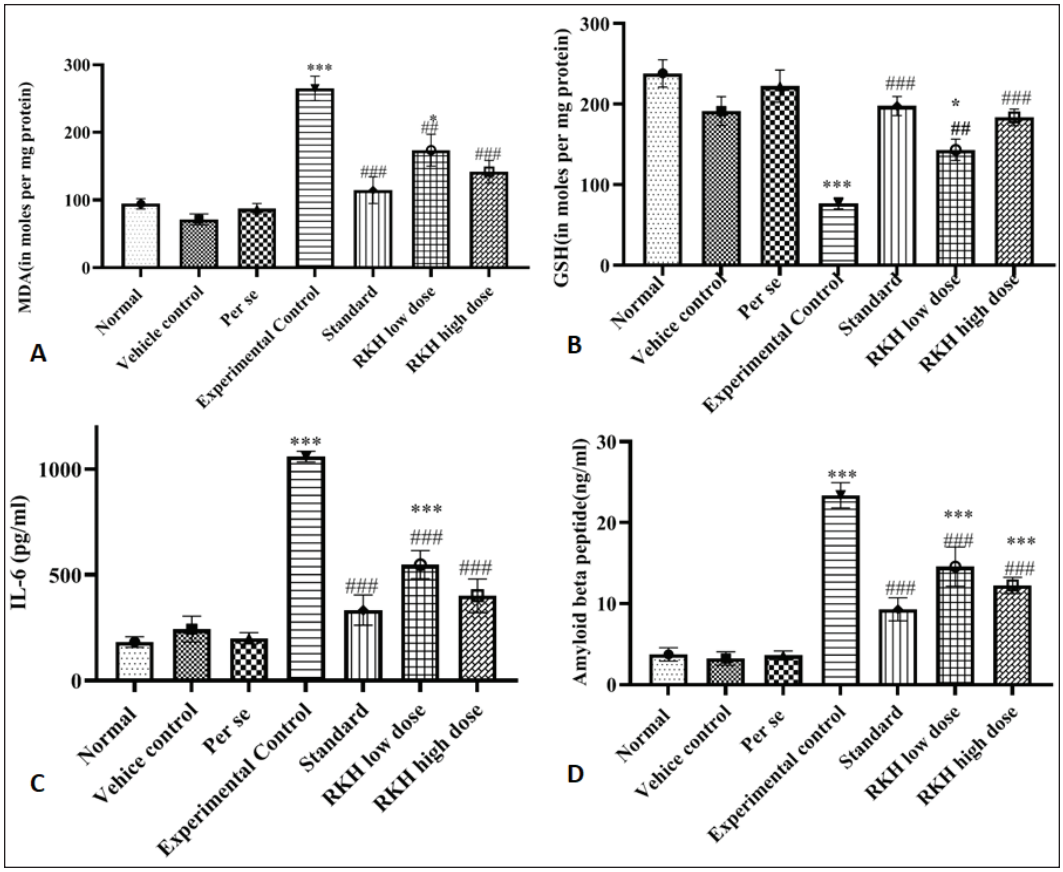


Figure 3. Effect of Rohitukine (RH) on Oxidative Stress and Neuroinflammatory Markers in Benzo[α]pyrene (BαP)-induced Alzheimer's Disease (AD) Mice.

Notes: (A) Lipid peroxidation, (B) reduced glutathione (GSH) levels, (C) interleukin-6 (IL-6), and (D) amyloid beta$_{1-42}$ (Aβ_{1-42}) were measured and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Values are expressed as mean ± standard error of the mean (SEM); *p < .05, **p < .01, ***p < .001 versus normal control; #p < .05, ##p < .01, ###p < .001 versus experimental control (BαP 5 mg/kg).

level. Donepezil (3 mg/kg), RH low dose (20 mg/kg), and RH high doses significantly reduced hippocampal Aβ levels (p < .001) compared to the experimental control, suggesting that high-dose RH may improve cognition, as shown in Figure 3D.

Histopathological Assessment

In histopathology, control, vehicle control, and *per se* showed normal tissue architecture as the tissues were not damaged by the toxin agent (Bαp-induced AD). In these samples, there was no evidence of any damage. Significant damage was reported in a group of mice that received Bαp, that is, (Group 4). Eosinophilic lesions and degeneration of neurons were observed. Damage reversal was most pronounced in the standard treatment group, whereas in the treatment group, the highest damage to neurons was recorded in the high-dose RH-treated group, as this treatment brought regeneration of neurons as well as eosinophilic lesions. Histopathological evidence indicates that treatment doses rejuvenated tissue and mitigated Bαp-induced damage, improving behavioral outcomes. The drug also showed neuroprotective and antioxidant effects, as illustrated in Figure 4.

Discussion

RH, a chromane alkaloid, is gaining attention in pharmacological research due to its diverse properties, including anticancer (Varun et al., 2023), anti-ulcer (Mishra et al., 2014), anti-adipogenic (Balaramnavar et al., 2021), and anti-arthritic (Kumar et al., 2020). Meanwhile, the neurotoxicity of Bαp has been extensively studied (Chepelev et al., 2015; Maciel et al., 2014; Tartaglione et al., 2023; Yang et al., 2017). However, our present study aims to show that the Bαp at a dose of 5 mg/kg i.p. for 28 days induced cognitive and memory impairment in mice (Mawuenyega). However, these animal models are used today as well as tomorrow and remain highly beneficial (Ransohoff, 2018). RH high dose orally dependently improved learning and memory deficits in Bαp-intoxicated mice animal model. Moreover, Bαp administrated resulted in changes in both oxidative and neuroinflammatory

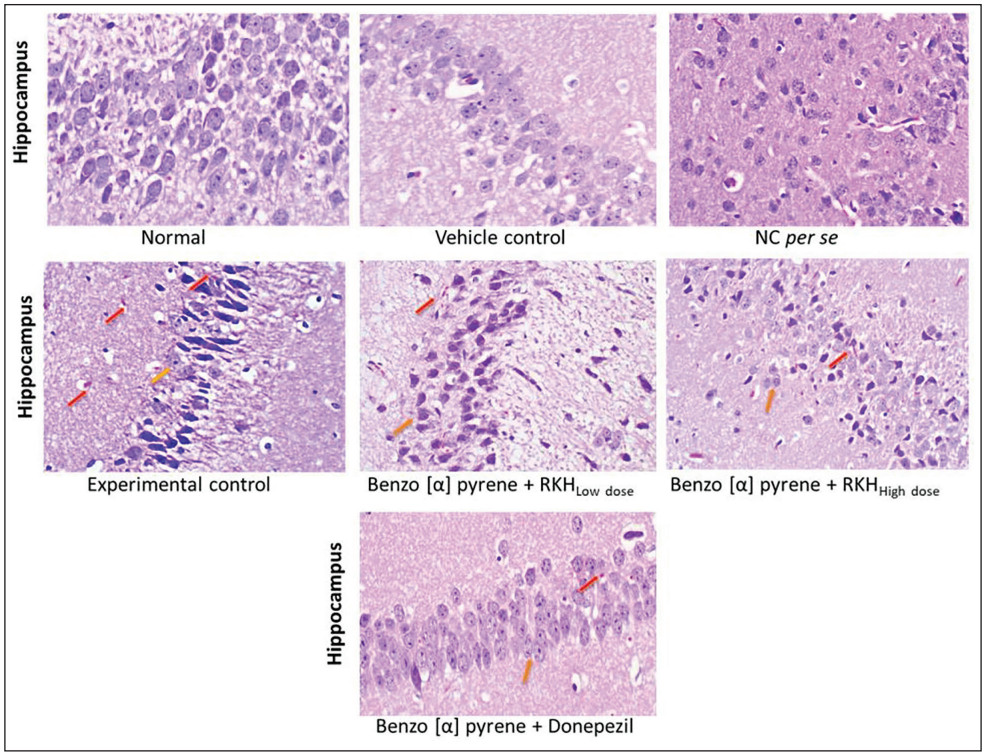


Figure 4. Analysis of Histopathological Data of Transverse Section of Hippocampus Using H&E Staining Where Normal Mice Received Saline Water, Vehicle Control Mice Received Olive Oil, *Per Se* Received High Dose of RH, Experimental Control Received Benzo[α] pyrene (5 mg/kg, i.p.) for 28 Days Whereas Benzo[α]pyrene + RH Low Dose (20 mg/kg, p.o.), Benzo[α]pyrene + RH High Dose (40 mg/kg, p.o.), and Benzo[α]pyrene + Donepezil (3 mg/kg).

Notes: The red arrow shows eosinophilic lesions, and the yellow arrow shows normal neuron. H&E: Hematoxylin and eosin; RH: Rohitukine.

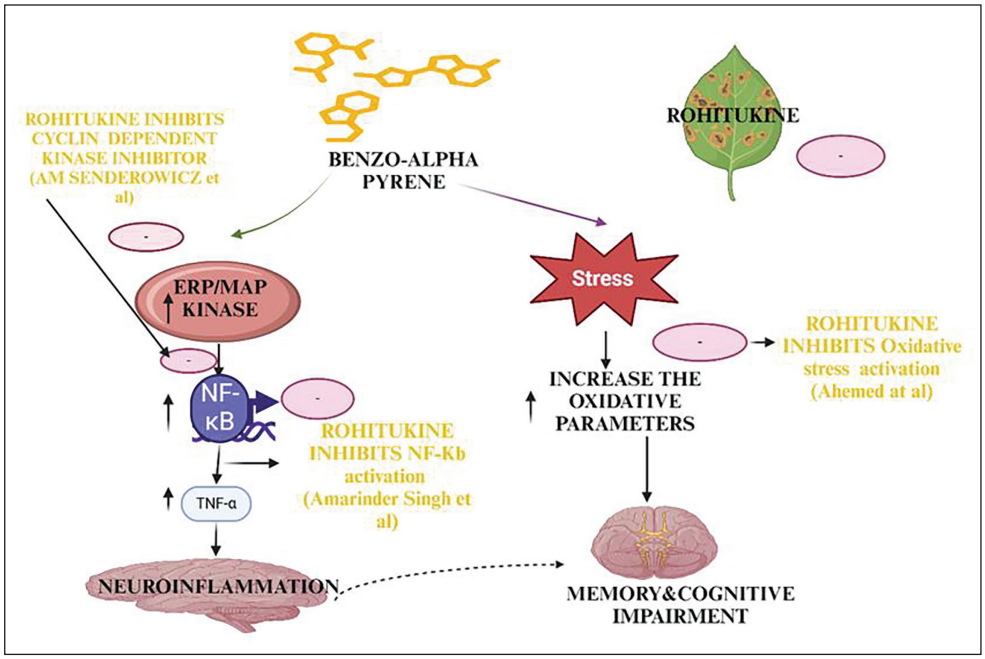


Figure 5. A Hypothesis of Rohitukine (RH) as a Neuroprotective Drug.

Note: NF-κB: Nuclear factor kappa B; TNF-α: Tumor necrosis factor alpha.

markers, which were observed in the brain homogenates of mice after the 28th day. Furthermore, the present study observed that neuroprotective properties in mice by suppressing the progression of cells, cell division and promoting differentiation of cell (Senderowicz & Sausville, 2000) also inhibit the antioxidant activity (Ahmed et al., 2022), decrease the activity of nuclear factor kappa B (NF-κB; Singh et al., 2019), and result in the cognition and memory impairment, and it significantly improved the parameters. RH, as a neuroprotective drug, and its impact on cognitive and memory abilities aid in neurological disorders, as represented in Figure 5.

Conclusion

Our study found that intoxicating mice with Bαp (5 mg/kg) resulted in memory impairment due to increased biochemical and behavioral markers in the hippocampal area. Furthermore, our findings indicate that RH high dose (40 mg/kg) reduced or enhanced memory and learning deficits in mice.

Abbreviations

$A\beta_{1–42}$: Amyloid $Beta_{1–42}$; AChE: Acetylcholinesterase; AD: Alzheimer's disease; ANOVA: Analysis of variance; Bαp: Benzo[α] pyrene; BSA: Bovine serum albumin; CCSEA: Committee for Purpose and Supervision of Experiments on Animals; CNS: Central nervous system; DTNB: 5,5′-Dithiobis(2-nitrobenzoic acid); ELISA: Enzyme-linked immunosorbent assay; EPM: Elevated plus maze; ERK-MAP: Extracellular signal-regulated kinase-mitogen-activated protein; GSH: Reduced glutathione; GSSH: Oxidized glutathione; H&E: Hematoxylin and eosin; IAEC: Institutional Animal Ethics Committee; IL-6: Interleukin-6; LPO: Lipid peroxidation; MDA: Malondialdehyde; NF-κB: Nuclear factor kappa B; NIPER: National Institute of Pharmaceutical Education and Research; NORT: Novel object recognition test; PBS: Phosphate-buffered saline; RH: Rohitukine; SEM: Standard error of the mean; TL: Transfer latency; TNB: 5-Thio-2-nitrobenzoic acid.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

The Institutional Animal Ethics Committee (IAEC) at Lovely Professional University, Phagwara, Punjab, India, allocated the experimental protocol number LPU/IAEC/2023/64, which adhered to the procedure set forth by the Committee for Purpose and Supervision of Experiments on Animals (CCSEA).

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Supplementary Material

Supplementary material for this article is available online.

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