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RESEARCH ARTICLE

Evaluation of Hordenine's Therapeutic Potential in Alzheimer's Disease-induced Cognitive and Oxidative Impairments

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Abstract: ***Introduction*:** This research aimed to investigate the potential of Hordenine (HR) against Alzheimer's Disease (AD) induced by Streptozotocin (STZ) in Wistar rats by evaluating its impact on cognitive function, oxidative stress, inflammatory cytokines, and neuroprotective biomarkers in comparison to donepezil.

***Methods*:** The study involved five groups of Wistar rats: a control group, a group with STZ-induced AD, and three treatment groups receiving varying doses of HR (50 mg/kg and 75 mg/kg) and donepezil (5 mg/kg). Over 28 days, the animals underwent various behavioural tests to assess cognitive function, along with biochemical analyses to measure A+cetylcholinesterase (AChE) activity, oxidative stress markers, inflammatory cytokines (IL-1β, TNF-α), and nuclear factor kappa B (NF-κB) levels, and histological examination. Additionally, molecular docking studies were performed to assess the interaction of HR with AChE.

***Results*:** STZ administration caused significant cognitive decline, oxidative stress, and elevated inflammatory markers. HR supplementation, particularly at 75 mg/kg, significantly improved cognition, reduced oxidative stress, and decreased pro-inflammatory cytokines (IL-1β, TNF-α), as well as NF-κB levels, while increasing Brain-Derived Neurotrophic Factor (BDNF) expression. Molecular docking studies revealed strong binding of HR to AChE, suggesting potential inhibitory effects.

***Discussion*:** Hordenine demonstrated promising neuroprotective effects against STZ-induced neurotoxicity by improving cognition and reducing oxidative stress and inflammation, suggesting HR's potential as an adjunct therapy for Alzheimer's disease, offering a protective mechanism that may complement existing treatments like donepezil.

***Conclusion*:** The research shows that the medicinal plant HR exhibits neuroprotective potential against AD induced by STZ. Further research involving clinical trials is warranted to fully establish the efficacy and safety of HR in the treatment of AD.

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1. INTRODUCTION

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder that mainly affects the elderly and is the leading cause of dementia worldwide. [1]. It is characterized by cognitive regression, memory loss, and behavioural modifications [2]. The pathology of AD is strongly linked with the accumulation of amyloid plaques and tau proteins in the brain, leading to neurological dysfunction and cell death [3]. Despite widespread research, the mechanisms causing the disease remain unknown, and the currently available treatments have been limited to symptomatic relief rather than curative interventions. The complexity of AD is predisposed by various factors, including genetic, environmental, and lifestyle. This underscores the necessity for a multilayered approach to research and treatment [4, 5].

The progression of AD is linked to Oxidative Stress (OS), apoptosis, and inflammation [6, 7]. OS results from an imbalance between the production of Reactive Oxygen Species (ROS) and the potential to neutralize them, causing cellular damage leading to neurodegeneration [8]. Apoptosis is considered a major factor leading to brain shrinkage and cognitive decline [9, 10]. The activation of microglia and astrocytes due to β-amyloid plaques and neurofibrillary tangles leads to pro-inflammatory cytokine release, increasing

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neuroinflammation and contributing to AD progression [11-13].

ROS plays a key role in activating signaling pathways, including MAPKs, specifically p38 MAPK, which are being intensely studied. Stressful conditions, including ROS and inflammatory signals, trigger activation of p38MAPK. Previous studies have established the role of p38MAPKs in AD development, suggesting that reducing p38MAPK activity may serve as a potential treatment [14]. Brain-Derived Neurotrophic Factor (BDNF) assists in controlling neurogenesis and retaining synaptic plasticity [15]. Due to its neuroprotective properties, BDNF offers potential to reverse memory and cerebral deficits linked with AD [16].

AD worsens cognitive impairments *via* neuronal damage and synapse depletion. It is characterized by the accumulation of amyloid-β peptides (Aβ) and neurofibrillary masses in brain cells [17]. Neuroinflammation, OS, and amyloid formation are considered primary pathogenic processes [18]. Research has shown that AD patients with high Aβ accumulation have elevated nuclear factor-κB (NF-κB) levels. Excessive Aβ also accelerates acetylcholine degradation [19].

Streptozotocin (STZ) injection *via* the Intracerebroventricular (ICV) route is a widely used animal model that effectively mimics AD features, including memory loss, OS, neuroinflammation, degeneration, cholinergic dysfunction, and impaired glucose metabolism. The STZ model provides a clinically related platform for studying the mechanism and potential treatments [20].

Acetylcholinesterase (AChE), the key neurotransmitter involved in cholinergic neurotransmission, contributes to AD development [21]. It affects OS, inflammation, apoptosis, and protein aggregation [22]. Studies show a relationship between dioxin exposure and AChE-related dysfunction. The AhR-dependent pathway is crucial in understanding dioxin-induced AChE dysfunction. Studies have enhanced our understanding of binding agents to AChE for neuroprotection and AD management [23]. To check the neuroprotective activity of HR, we conducted molecular docking between AChE and HR.

The main aim of AD medication research is to prevent and delay detrimental processes. Alkaloid hormone (4-(2-Dimethylaminoethyl) phenol), found in cacti, certain algae, fungi, and cereal seedlings such as barley and sorghum, shows anti-tumour, anti-inflammatory, antioxidant, and antibacterial properties [24].

Hordenine (HR) can alleviate nerve pain, reduce neuroinflammation [25], decrease inflammation in diabetic nephropathy [26], and protect against lung damage induced by lipopolysaccharides [27, 28]. HR exhibits antibacterial and antiviral properties, reducing inflammation from SARS-CoV-2 and herpes viruses [29, 30]. The mechanisms underlying HR's protective effects against experimentally induced AD are not fully understood. Research on HR therapeutic potential against STZ-induced AD is limited.

Hordeum vulgare (barley) has been used in Chinese agriculture since 2000 BCE. Its derivative, Hordeum vulgare malt, is created by sprouting and drying barley fruits and is used in traditional Chinese medicine [31]. It is believed to promote Qi circulation and is used to treat conditions like indigestion, food retention, and hyperprolactinemia [32]. *Hordeum vulgare* is a versatile crop used in food, medicine, and ornaments. It is resilient to cold, drought, and salt, and is cultivated year-round in Yunnan province, China [33].

Hordeum vulgare has demonstrated anti-inflammatory, antibacterial, antioxidant, and antitumor properties [34]. Hordenine, a key compound in barley, is structurally similar to stimulants in bitter orange and is included in performance supplements [35]. It shows protective effects against nerve damage and inflammation [36]. However, its mechanism in AD remains unclear, and limited research exists on its effects against STZ-induced AD. This study aimed to investigate whether hordenine could reduce oxidative stress and neuroinflammation in STZ-induced AD in rats.

2. MATERIALS AND METHODS

2.1. Ethics Statement

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of K.R. Mangalam University, Haryana, India, under approval number KRMU/CCSEA/RES/IAEC-2023-8. Animal care adhered to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA). Wistar rats weighing between 180-200 g were sourced from an institutional animal facility. They were housed in hygienic cages within a controlled environment maintained at 22 ± 3°C and a 12-hour light/dark cycle. Throughout the experiment, all rats had unrestricted access to food and water.

2.2. Experimental Drugs and Other Chemicals

STZ, Donepezil (DZ), and HR were sourced from Merck Specialties Pvt. Ltd., Mumbai, India. Biochemical assay kits were sequentially procured from eBioscience, Biocompare, Elabscience, Mybiosource, and ThermoFisher Scientific, all based in the USA. Additional reagents and chemicals were supplied by Thermo Fisher Scientific, Mumbai, India.

2.3. Experimental Design

The study aimed to explore the impact of HR on animals exposed to STZ. A total of 30 Wistar rats of either sex, aged 10-12 weeks, were divided into 5 groups with 6 rats in each group. The study was conducted over a period of 28 days, with the following experimental groups (Table **1**).

Each rat was assigned to one of five groups (Groups I to V). Group I received vehicle control with standard saline treatment. Group II, III, IV, and V rats were administered with STZ at a dose of 3 mg/kg/10µl (i.c.v, rate of 1µl per min) infused into each bilateral ventricle once to induce AD as reported in earlier studies [37]. Group III animals received oral supplementation of 50 mg/kg (p.o.) HR daily for 28 days. Group IV animals received 75 mg/kg (p.o.) HR daily for 28 days. Group V animals received a standard drug, DZ,

Table 1. Experimental groups (n=6).

S. No.	Group Name	Groups
1	I	Vehicle Control
2	II	STZ 3mg/kg (i.c.v) once (disease control)
3	III	STZ 3mg/kg (i.c.v) once + HR low dose 50 mg/kg (p.o.) for 28 days (treatment control)
4	IV	STZ 3mg/kg (i.c.v) once +HR high dose 75 mg/kg (p.o.) for 28 days (treatment control)
5	V	STZ 3mg/kg (i.c.v) once +Donepezil 5 mg/kg (p.o.) for 28 days (standard control)

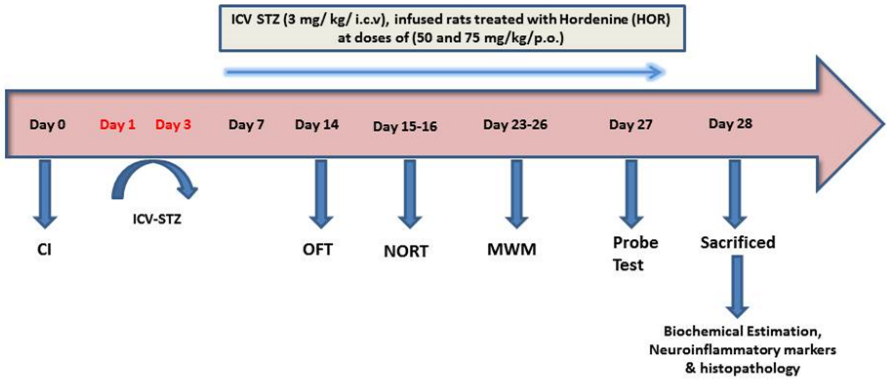

Fig. (1). The layout of the medication plan plus the timeline for evaluating several experiment components. Day 0 represents the time of the surgical procedure, specifically, I.C.V. STZ refers to intracerebroventricular administration of streptozotocin. CI stands for cannula implantation, MWM denotes the Morris water maze, OFT represents the open field test, and NORT signifies the novel object recognition test. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

at a dose of 5 mg/kg daily for the same duration. Fig. (**1**) illustrates the study timeline.

2.4. Surgical Procedure

The surgical procedure was designed to induce AD in rats using i.c.v. administration of STZ. Rats were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) to provide proper sedation, analgesia, and muscle relaxation. The skull area was shaved, and the rats were cleaned with povidone-iodine. They were then positioned on a stereotaxic apparatus for the precise delivery of STZ into the lateral ventricles.

The depth of anesthesia was evaluated by the absence of reflex responses, and a heating pad was used to maintain body temperature throughout the surgery. A midline incision was made in the scalp, and small burr holes were drilled at specific stereotaxic coordinates to insert a 30-gauge stainless steel cannula. STZ (3 mg/kg in 10 µl) was infused slowly (1 µl/min) bilaterally into the ventricles using a microinjection syringe.

After surgery, the incision site was closed with sutures, and povidone-iodine was applied to prevent infection. Postoperative analgesia was provided to minimize discomfort. Rats were placed in individual cages and closely monitored until they had fully recovered from anesthesia. They were observed for any signs of abnormalities, which were promptly addressed. Throughout the 28-day experimental period, the rats were regularly monitored for health status, body weight, and behavioural changes. Upon completion of the study, animals were sacrificed for tissue collection. Brain tissues were immediately harvested, rinsed with ice-cold isotonic saline, and then further processed for biochemical and histopathological analysis.

2.5. Body Weight Measurement and Percentage Change Calculation

The animals' body weights were recorded on the first day of the trial (before STZ exposure) and on the final day (28th day). The percentage change in body weight was calculated using the following formula.

Percentage Change= Initial Body Weight/Final Body Weight−Initial Body Weight ×100

2.6. Open-Field Test (OFT)

Mild stress can often be identified through an OFT by observing alterations in emotionality and exploratory behaviours [38]. The study was conducted in a square wooden box measuring 80 x 80 x 40 cm, with 16 identical 4 x 4 cm squares, red walls, and a black-lined white floor. Each animal was individually placed in the center of the box and observed for 3 minutes. Changes in exploratory capacity were assessed by measuring the frequency of ambulation and total exploratory rearing. Emotionality was evaluated by recording the frequency of grooming and the amount of voided feces.

2.7. The Novel Object Recognition Test (NORT)

The NORT, a widely used behavioural assay for studying memory recognition in rats, is a simple test that can be completed in just 3 days. This test has been performed in an open wooden box measuring (70×70×25 cm), containing similar items of various shapes and sizes that make it difficult for the animals to move. The test is divided into three phases. The first phase is habituation, during which rats are allowed 10 minutes to acclimate to the open field, followed by the second phase, the sample phase, where rats spend 10 minutes exploring two similar objects, A1 and A2.The final phase is the examination stage, which occurs 24 hours after the sample phase.

During the examination stage, the rats investigate a novel object alongside the familiar object from the sample phase. The exploration time is limited to 5 minutes. The arena and objects are sanitized with 5% ethanol. Object exploration is defined as the rat smelling, touching, and licking objects with its front legs or snout. The rat's preference for the novel object is determined by calculating the discrimination index, which involves dividing the total time spent exploring both objects [39].

2.8. Morris Water Maze (MWM) Test

To evaluate the animals' learning and memory abilities, a black pool with a depth of 50 cm and a circumference of 180 cm was used in the MWM test. The pool was partitioned into four quadrants: the northeastern quadrant was the first, and the southeastern quadrant was the second. The southwestern quadrant was designated as the third, while the northwestern quadrant was the fourth [40]. An inconspicuous, circular platform with a diameter of 10 cm was positioned in the third quadrant, with the water level maintained at 40 cm. Skimmed milk was added to the water to make it opaque, concealing the platform located below the surface (1 cm). Visual hints were attached to the walls surrounding the pool to help the animals navigate towards the platform. An automated tracking system and video camera monitored the rats, recording their route, speed, and duration. Accurate measurements were recorded and analyzed for the duration spent in the quadrant targeted, the opposite quadrant, and the total number of crossings.

2.9. Tissue Homogenate Preparation

Following the exploration phase, the rats were anesthetized using diethyl ether. Physiological saline (ice-cold and isotonic) was gently washed over the carefully dissected brains, which were placed on an ice-cold plate. The brains were then separated into left and right hemispheres with a very sharp blade to isolate the hippocampal tissue. The hippocampi were weighed and then homogenized in ice-cold PBS (pH 7.4) using a Teflon-glass homogenizer. Homogenates were then subjected to centrifugation at 10,000 rotations per min for 15 mins. The supernatants collected from this process were then preserved at a temperature of -80°C for future biochemical parameter analysis [41].

2.10. Measurement of AChE Content

The AChE concentrations in the brain tissue of both treated and control animals were analyzed [42]. Hippocampal tissues from the treated animals were suspended in a 0.25 M sucrose solution and incubated for 30 mins. Following incubation, the tissues were preserved. Subsequently, the samples were centrifuged at 10,000 rpm to obtain a supernatant, which was then analyzed using a spectrophotometric method. The AChE content was measured by reading the absorbance at 412 nm, and the results were expressed as nanograms per gram of tissue (ng/g tissue).

2.11. Oxidative Stress and Antioxidant Markers Detection

For evaluation of the levels of these elements in the brain tissues of both control as well as treated rats, the markers for OS, including LPO and nitrite, along with antioxidants such as GSH, catalase, and SOD, were quantified *via* the assessment test kits and methods specified by the producer (Biocompare, USA).

2.12. Quantification of Inflammatory Markers

The levels of IL-1β and TNF-α in the brains of both groups of animals, including controls and treated animals, were determined using specific examination test kits, in accordance with the guidelines provided by the manufacturers.

2.13. Determination of BDNF Levels in Brain Tissue

BDNF levels in brain homogenates were measured using a commercially available ELISA kit, as per the manufacturer's instructions. Absorbance was recorded at 450 nm using a microplate reader to quantify BDNF concentration. All samples were analyzed in duplicate.

2.14. Histopathological Studies of Hippocampal and Cortical Regions

Biochemical analysis of the cortical as well as hippocampal regions was excised from all groups, including control and treatment, and cut into small pieces, followed by embedding in paraffin and sectioning to 4-6 µm of thickness. Hematoxylin and eosin were utilized for staining slices. Finally, slides were photographed using a light microscope, which was equipped with a camera.

2.15. Molecular Docking

Based on the resolution and extensive sequence coverage, a 3D X-ray crystallographic structure of AChE was obtained from the RCSB Protein Data Bank (PDB ID: 4bdt) [43]. Removal of water molecules, ions, and covalent ligands was performed by using the Dock Prep procedure available in the UCSF Chimera program [44]. The Kolmann charges were calculated, and subsequently, polar hydrogen atoms were introduced. The active site and list of key amino acid residues interacting with ligands of such macro molecules were taken from the literature. The 3D structures of HR and

DZ were downloaded from the PubChem database. This structure was checked for any errors. Then, by using Open Babel, hydrogen atoms were added, and the structures were converted into PDBQT format for Docking studies [45]. Based on the Active site's information on AchE, the center of the Grid Box was set at center x = -2.299405, center y = -35.609262, and center z = -52.048214, and the optimal box size was calculated using the eBoxSize Script developed by Matthias and Mark [46]. For Auto Dock 4.2, Lamarckian Genetic Algorithm was used with 1000 runs in Auto Dock 4.2 [47]. The best-docked pose was selected based on its binding energy score and the presence of significant interactions in the active site.

2.16. Statistical Analysis

No animals, experimental units, or data points were excluded from the analysis. All collected data were included to ensure the accuracy and reliability of the study findings. Biochemical analysis results were presented as mean ± SEM for the 3 replicates. Group differences were evaluated using one-way ANOVA followed by Tukey's post hoc test. Data were considered significant if $p < 0.05$.

3. RESULTS

3.1. Effect of HR on the STZ-induced Weight Changes in the Rats

Rats treated with STZ showed a gradual decrease in body weight over the course of the treatment period, with a significant reduction observed by the end of the fifth week compared to the control group. The decrease in body weight was effectively prevented by DZ (5 mg/kg). Similarly, HR (50 and 75 mg/kg) strongly inhibited the decrease in body weight (Fig. **2A**).

3.2. Effect of HR on Behavioral Changes Induced by STZ in Alzheimer's Animals as Evaluated by the Object Recognition Test

Behavioral changes in treated and control animals were measured as displayed in Fig. (**2B** and **C**). AD animals induced with STZ displayed reduced exploration compared to control animals. Nevertheless, in AD rats exposed to STZ, supplementation with 50 and 75 mg/kg of HR resulted in a substantial increase in exploration and discrimination indices. The STZ-induced behavioral changes were also significantly reversed by the conventional drug DZ.

3.3. Influence of HR on STZ-induced Behavioral Alterations in Alzheimer's Rats as Measured by the OFT

Behavior changes in both controls, as well as treated rats, were monitored *via* OFT, with details provided in Fig. (**3**). Rats with STZ-induced AD showed reduced ambulation occurrence, higher rearing regularity, increased grooming occurrence, and more fecal output (greater number of fecal pellets) compared to the animals of the control group. Moreover, supplementation with HR at doses of 50 and 75 mg/kg reversed the STZ-induced increases in ambulation and rearing rates and decreases in grooming frequency and fecal output. Importantly, these behavioral changes induced by STZ were also reversed by the standard drug DZ.

3.4. Influence of HR on Behavioral Modifications in Alzheimer's Rats Induced by STZ as Assessed by the Morris Water Maze (MWM) Test

The MWM test findings revealed a significant decline in memory and learning in STZ-induced AD rats (Fig. **4**). An increased escape latency signifies a significant decline in the learning capability of AD animals. The reduced time spent in

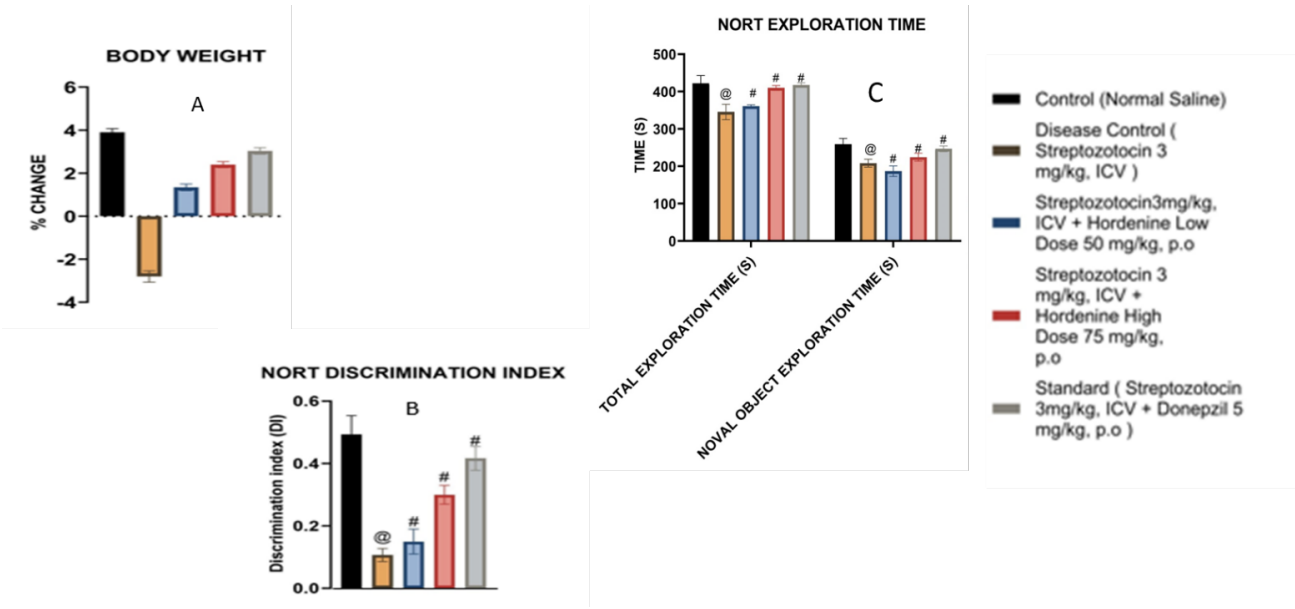

Fig. (2). Impact of HR on body weight, overall exploration, novel exploration, and discrimination in rats. Values are shown as mean ± SEM, with @ $p < 0.001$ compared to the vehicle group, and $^{\#}p < 0.001$ compared to the STZ group (n=6). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

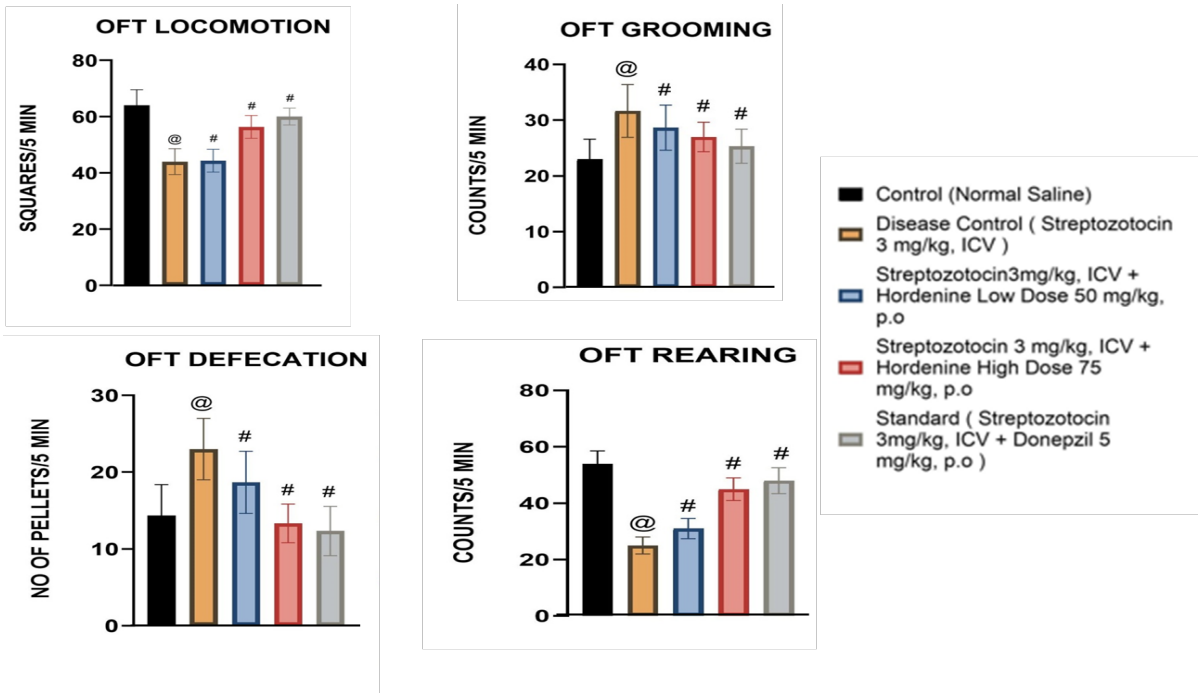

Fig. (3). Effect of HR on grooming, locomotion, rearing, defecation, in rats. Values are depicted as mean ± SEM, with *@p* < 0.001 *vs*. vehicle, and *#p* <0.001 *vs*. STZ (n=6). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

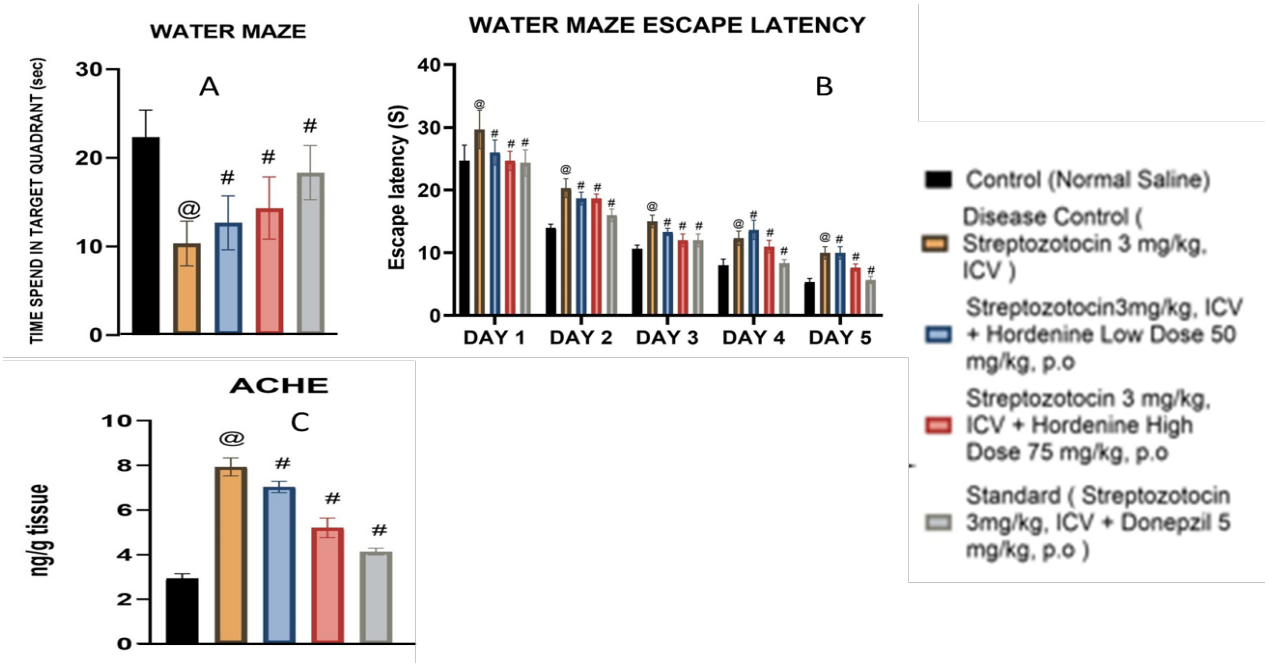

Fig. (4). Influence of HR on time devoted to target quadrants, escape latency, and AChE levels in rats. Data are shown as mean ± SEM with *@p* < 0.001 compared to the vehicle group, and *#p* < 0.001 compared to the STZ group (n=6). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

the targeted quadrant signifies that the STZ effectively prolonged escape latency in addition to impaired memory. Treatment with HR substantially alleviated these parameters. STZ-induced AD rats displayed a considerable enhancement in the time spent in the targeted quadrant as well as a reduction in escape latency in treatment with 50 and 75 mg/kg of HR (Fig. **4A** as well as **4B**). Furthermore, the standard drug treatment DZ notably improved the behavioral transformations caused by STZ in AD rats.

3.5. Influence of HR on AChE Levels in the Tissues of the Brain of AD Animals Induced by STZ-induced

In AD animals, brain AChE levels were notably higher compared to control rats. However, treatment with 50 and 75

mg/kg HR substantially lowered AChE concentrations in the brain tissues of rats, as depicted in Fig. (**4C**). Treatment with the standard medication DZ also significantly lowered AChE concentrations in the tissues of the brain of AD rats. Both HR and DZ treatments exhibited similar effects.

3.6. Influence of HR on OS as Well as Antioxidant Markers in AD Rats Induced by STZ

In rats administered STZ, lipid peroxidation significantly increased, as evidenced by a significant rise in MDA concentrations in both the cortex and hippocampus ($p < 0.001$), indicating Oxidative Stress (OS). The 50 mg/kg dose of HR did not show significant effects, whereas the 75 mg/kg dose significantly reduced LPO concentrations associated with the lower dose. The antioxidant potential of HR is illustrated in Fig. (**5A**) ($p < 0.0001$).

The AD rats displayed a substantial reduction in GSH levels in both the hippocampus and cortex. Administration of HR affected the degree of GSH depletion induced by STZ, with the 75 mg/kg dosage notably increasing levels of GSH as compared to STZ-administered rats (Fig. **5B**). Conversely, the low HR dose (50 mg/kg) produced no significant change in GSH levels relative to the control rats.

Catalase activity in the brain was reduced in rats administered with STZ ($p < 0.001$). Treatment with oral HR at 75 mg/kg markedly increased catalase activity as compared to STZ-treated rats. DZ (5 mg/kg) significantly enhanced catalase action in the hippocampus as well as frontal cortical areas of STZ-treated rats, as illustrated in Fig. (**5C**). Conversely, the lower dose of HR (50 mg/kg) does not mitigate the reduced catalase action observed in STZ-administered rats.

Significantly lower SOD concentrations were observed in the cortex as well as the hippocampus of animals administered with STZ. Treatment with HR at 75 mg/kg significantly restored SOD levels in STZ-treated rats, as shown in Fig. (**5D**), whereas the low dose of HR, *i.e.*, 50 mg/kg, did not exhibit significant effects on SOD levels.

Nitrite amounts in the cortex as well as hippocampus of STZ-treated rats were notably elevated. Treatment with varying HR doses at 75 mg/kg in these rats resulted in a substantial and dose-dependent reduction in nitrite quantities, as shown in Fig. (**5E**). In contrast, the lowest HR dose (50 mg/kg) did not significantly alter nitrite levels in rats administered STZ.

3.7. Impact of HR on Inflammatory Markers in the Brain Tissues of AD Animals Induced by STZ

Fig. (**6**) depicts the concentrations of inflammatory indicators, including IL-1β and TNF-α, in the brains of both control and treated rats. In Figs. (**6A** and **6B**), higher concentrations of TNF-α plus IL-1β are evident in the brains of AD rats administered with STZ compared to the control group. Importantly, AD rats treated with 75 mg/kg of HR showed a

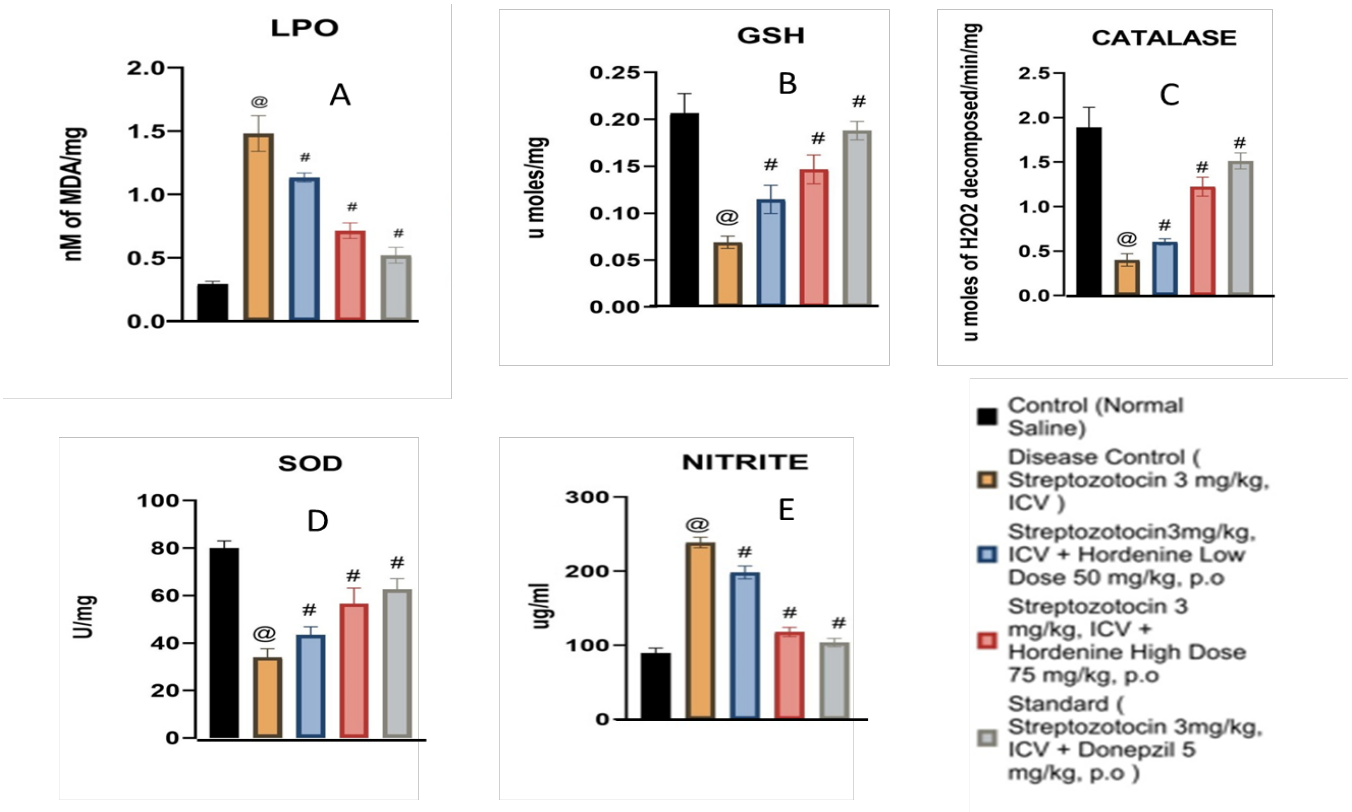

Fig. (5). Effect of HR on LPO (**A**), GSH (**B**), Catalase (**C**), SOD (**D**), and Nitrite (**E**) in rats. Data are presented as mean ± SEM with @$p <$ 0.001 compared to the vehicle group, and $^{\#}p < 0.001$ compared to the STZ group (n=6). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

marked decrease in IL-1β and TNF-α levels. HR treatment efficiently controlled IL-1β and TNF-α concentrations, which reversed to nearly standard levels, comparable to the effects seen in the standard DZ group.

3.8. Impact of HR Upon NF-kB plus BDNF Concentrations in the Brain Tissue of AD Animals Induced by STZ

Fig. (**6**) also shows the levels of NF-κB/p65 and BDNF in the tissues of the brains of both treated and control rats. In AD rats induced by STZ, NF-kB/p65 concentrations were significantly increased, while BDNF levels were reduced. Treatment with HR effectively regulated these markers. HR administration increased BDNF expression and decreased NF-κB/p65 concentrations in the brain tissue of STZ-induced AD rats (Fig. **6C** and **6D**). Comparable effects were perceived in AD rats treated with DZ.

3.9. Influence of HR on Brain Tissue Histopathology in AD Rats Induced by STZ

The hippocampal and cortical areas of the group I showed anatomical features (Fig. **7 CO (a)** and **HP (a)**). In contrast, hippocampal and cortex slides of STZ-induced AD rats showed the disrupted architecture and regions with reduced cellular density (Fig. (**7**) **CO (b)** and **HP (b)**). Furthermore, Fig. (**7**) **(CO (c)** and **HP (c)**) demonstrates that rats treated with the standard, *i.e.*, DZ, retained a normal histological architecture. Similarly, rats administered HR (75 mg/kg) exhibited a neuroprotective effect, as indicated by reduced histological alterations and preservation of characteristic cellular structures (Fig. **7**: **CO (d)** and **HP (d)**), compared to the changes observed with standard therapy (Group V; Fig. (**7**): **CO (e)** and **HP (e)**).

3.10. Molecular Docking

The binding energy between AChE and HR has been determined to be -6.56 kcal/mol. Fig. (**8**) depicts the 3D and 2D interaction—HR binding interactions with the active region of AChE. Figure 8 depicts the tertiary structure of AChE, whereby the active site is occupied by HR, as indicated by the blue dashed box. The magnified perspective (on the right) provides a comprehensive depiction of the molecular interactions occurring between HR and the adjacent amino acid residues. The primary interactions seen in this study encompass conventional hydrogen bonding with HIS447 and TYR341, a carbon-hydrogen bonding interaction with TYR83, and Pi-Pi stacking interactions with TYR337 and TRP86. The HIS447 protein serves as a vital anchoring site by forming hydrogen bonds. At the same time, the aromatic ring participates in Pi-Pi stacking with TRP86, indicating a robust interaction between the ligand and receptor that may hinder the enzymatic function of AChE.

The binding energy between AChE and DZ was found as -8.1 kcal/mol. depicts the 3D and 2D interaction. The active site of AChE is occupied by DZ, as indicated by the highlighted dashed box. The magnified perspective comprehensively depicts the molecular interactions between DZ and the adjacent amino acid residues. The primary interactions seen in this study encompass conventional hydrogen bonding with TYR124, PHE295, and ARG296, and Pi-Pi stacking interactions with TRP286. At the same time, the aromatic moiety of DZ participates in pi-pi stacking with TRP286, indicating a robust interaction between the ligand and receptor that may effectively inhibit the enzymatic function of AChE.

The molecular docking study elucidated the mechanism of action of HR on AChE, where HR exhibited a binding

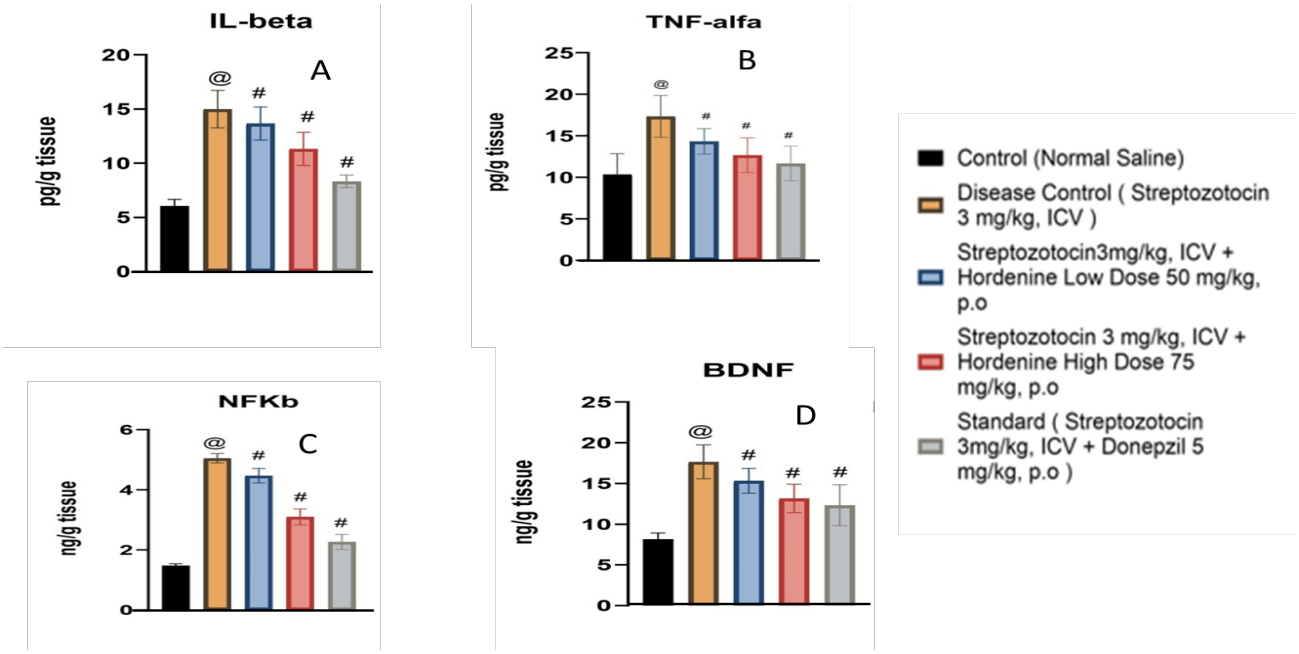

Fig. (6). Effect of HR on IL-β (**A**) and TNF-α (**B**), NF-κB (**C**), and BDNF (**D**) levels in rats. Data are presented as mean ± SEM with @$p <$ 0.001 compared to the vehicle group, and $^{\#}p < 0.001$ compared to the STZ (n=6). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

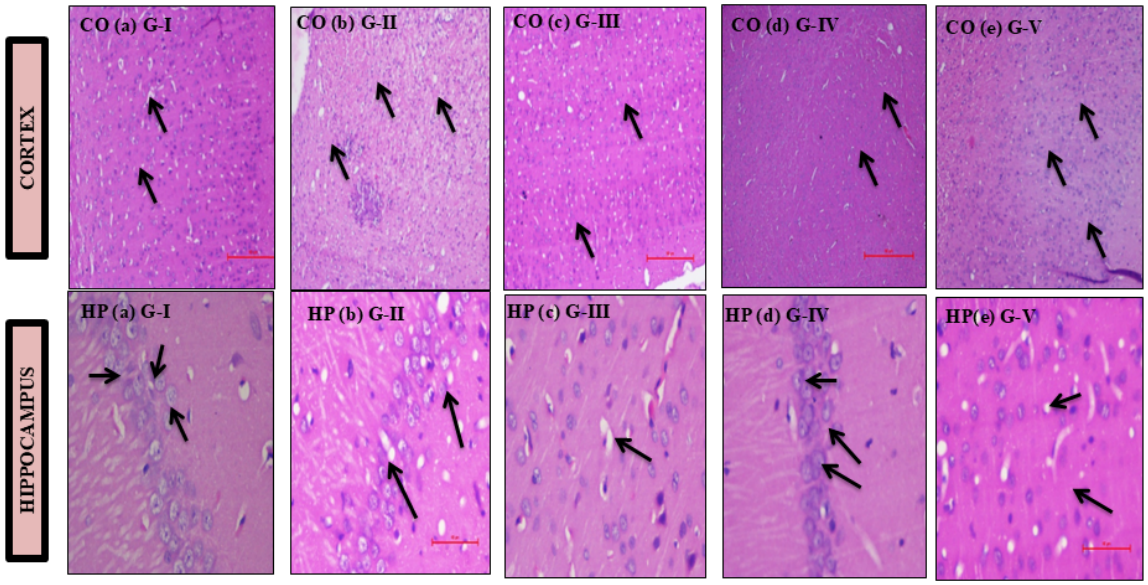

Fig. (7). The cortical and hippocampus regions of the brain microphotographs from the group I *i.e.*, vehicle control, group-II (disease control), group-III, IV (HR treated groups), and group V (standard control). In group I (CO (a) and HP (a)), slides showed well-defined neural cell morphology with no signs of inflammation or neural degeneration. The STZ-administered group (group II) (CO (b) and HP (b)) exhibited significant inflammation, including pyknosis, neural degeneration, and multiple vacuoles (indicated by arrows), indicating severe neuroinflammation. In group III (CO (c) and HP (c)), and IV (CO (d) and HP (d)), treatment with HR (50 mg/kg) results in a slight reduction of neuroinflammation, whereas HR (75 mg/kg) treatment showed marked suppression in neuroinflammation. The standard control group (5 mg/kg) (CO (e) and HP (e)) showed disrupted architecture and regions with reduced cellular density, indicating a suppression of the neuroinflammatory process (n=6). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

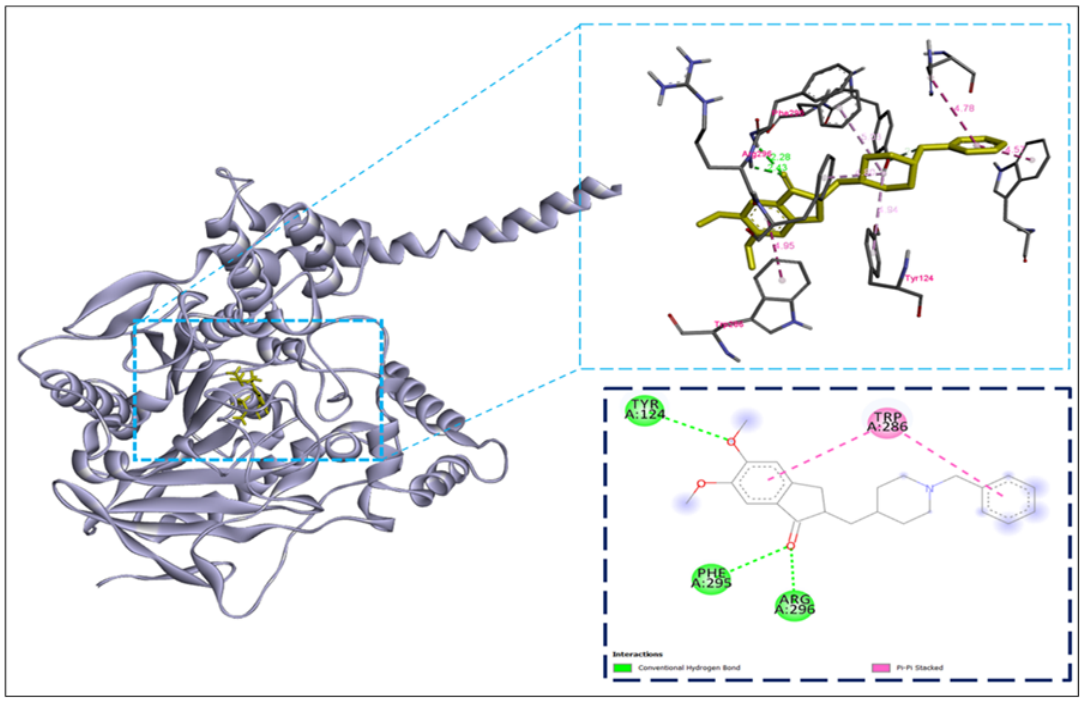

Fig. (8). Molecular interactions between Hordenine (HR) and surrounding amino acid residues of Acetylcholinesterase (AChE) at the active site. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

affinity of –6.56 kcal/mol, compared to the standard drug DZ (–8.1 kcal/mol). This suggests the potential efficacy of HR in AChE inhibition and highlights its relevance for therapeutic interventions in neurodegenerative disorders.

4. DISCUSSION

This study offers evidence that HR has significant neuroprotective effects in a STZ-induced model of AD in rats. The data suggest that HR may mitigate the key pathological fea-

tures of AD, including cognitive decline, OS, and neuroinflammation, highlighting its potential as a therapeutic agent. One of the standout findings is HR's ability to improve cognitive function and behavior in STZ-induced AD rats, particularly at the 75 mg/kg dosage.

Behavioral assessments, such as the OFT, NORT, and MWM, demonstrated that HR reversed memory deficits, reduced anxiety-like behaviors, and improved exploration. These results are comparable to standard treatment i.e., DZ, indicating the potential of HR in cognitive enhancement [48]. The neuroprotection activity of HR may be due to increased levels of BDNF in the treatment group, leading to an influence on synaptic function and neurogenesis. Furthermore, AChE, an enzyme involved in neurotransmission, levels were significantly reduced by HR [49].

Additionally, AChE, a key enzyme involved in cholinergic neurotransmission, was found to have lower levels in the brain of the treatment group [50], as it causes acetylcholine to break down quickly, thus reducing cholinergic action. HR improves cognitive performance by enhancing cholinergic transmission, as indicated by the reduction in AChE activity. Molecular docking experiments further corroborate this mechanism by confirming that HR binds to AChE efficiently and potentially blocks its activity. OS is a major factor in the development of AD [51].

The results of the study also indicate that HR enhances protective antioxidant defenses by increasing the levels of GSH, catalase, and SOD, while significantly reducing oxidative stress markers such as MDA [52]. These antioxidant attributes play an essential role in minimising the oxidative damage caused by STZ, which is the main cause of the neurodegenerative processes related to AD. Our results align with previous research, in which HR reduces Parkinson's-like motor deficits by activating DRD2 and lowering oxidative stress, supporting its potential as a neuroprotective supplement [53].

HR significantly decreases neuroinflammation, an additional significant component in the pathogenesis of AD. According to the study, pro-inflammatory cytokines, including TNF-α and IL-1β, were found to be considerably reduced in the brain tissues of rats administered STZ-induced AD [51]. Additionally, HR reduced the activity of NF-κB, an important regulator of inflammatory reactions in the brain. The effects are also supported by earlier research showing that HR protects dopaminergic neurons by inhibiting the NF-κB and MAPK pathways, offering potential for the treatment of Parkinson's disease [25]. The combination of decreased NF-κB activity and elevated BDNF expression implies that HR reduces inflammation and supports neuroprotection and synaptic plasticity, both of which have significance for preserving cognitive functions in AD [54].

The neuroprotective advantages associated with HR are further demonstrated by histological evaluations, which showed decreased neuroinflammation and preservation of neuronal architecture in the cortical and hippocampus areas of rats receiving HR. This suggests that HR minimises the histological alterations, like neuronal degeneration and microglial activation, that are commonly indicative of AD [55]. The molecular docking examinations, which reveal that HR has a substantial binding affinity to AChE comparable to DZ, offer additional information on the mechanism of action of the drug. This implies that cholinergic modulation, an important process in the treatment of AD, could be the mechanism by which HR exerts its therapeutic benefits, aligning with recent in-silico studies focused on AChE-targeted drug discovery [56, 57].

This study has several limitations. One key limitation is the use of hordenine, which has shown neuroprotective effects against STZ-induced Alzheimer's disease in Wistar rats. However, the exact mechanisms through which hordenine exerts its neuroprotective action remain unclear. While various parameters such as oxidative stress, inflammation, behavioral assessments, and histopathological analysis were utilized to validate Alzheimer's disease in this study, other important markers, including apoptotic and fibrotic indicators, were not evaluated. These factors represent potential areas for further investigation. Possible study limitations include the use of animal models that may not fully replicate human AD pathology, the relatively small sample size, as well as the short duration of treatment. These factors may limit the results to clinical settings.

The results of this research demonstrate HR as an achievable prospect for the development of novel AD treatment approaches, possibly in addition to currently recommended therapies like DZ. Although these preclinical findings are promising, further investigation is needed to determine the therapeutic significance of HR in AD patients. Further investigation initiatives should focus on verifying the safety, effectiveness, and optimal dosage of HR in clinical trials, as well as evaluating its long-term influence on AD development.

CONCLUSION

This research shows that the Chinese medicinal plant HR exhibits neuroprotective potential against AD induced by STZ. Decreased MDA and elevated SOD in brain tissues demonstrated that HR treatment significantly suppressed neuroinflammation and OS efficiently reversed cognitive deterioration. Moreover, HR significantly enhanced cholinergic neurotransmission by reducing AChE activity, thereby improving cognitive functions such as memory. The decrease in pro-inflammatory cytokines (IL-1β, TNF-α) and NF-κB levels further highlights the anti-inflammatory properties of HR.

The observed therapeutic effect of HR may be due to its potential to regulate oxidative pathways, suppress inflammation, and support neuronal integrity. Additionally, its impact on BDNF expression suggests a role in enhancing synaptic plasticity and neurogenesis, which are essential for memory formation. HR demonstrated optimistic effects comparable to the standard, making it a promising neuroprotective alternative. These complex actions position HR as a convincing candidate for multi-targeted therapy in AD.

Additionally, HR's antioxidant and anti-inflammatory effects may provide extensive benefits in reducing the progression of disease. Additionally, its natural origin offers low toxicity, which encourages further research as a complementary or alternative treatment. Further, exploring the precise molecular mechanisms of HR will be significant in heightening its neuroprotective potential.

Histopathological evaluations showed that HR treatment preserved neural cell morphology and reduced neuroinflammation in the hippocampal and cortical regions. Moreover, molecular docking studies indicated a significant binding affinity of HR to AChE, suggesting that HR can effectively inhibit AChE activity, akin to the standard drug DZ, although with slightly less binding energy. These findings suggest that HR, particularly at higher doses, holds promise as a therapeutic agent for alleviating the cognitive and neurochemical disturbances associated with AD. Further research, including clinical trials, is warranted to fully establish the efficacy and safety of HR in the treatment of AD.

AUTHORS' CONTRIBUTIONS

The authors confirm their contribution to the paper as follows: M.A.: writing original draft, editing, validation & data curation; M.S. & S.K.: supervision, methodology and editing; P.B.: visualization, data curation; N.T.: formal analysis and visualization; S.S.: data curation and editing.

LIST OF ABBREVIATIONS

AChE	=	A+cetylcholinesterase
AD	=	Alzheimer's Disease
Aβ	=	Amyloid-β Peptides
BDNF	=	Brain-Derived Neurotrophic Factor
HR	=	Hordenine
ICV	=	Intracerebroventricular
NF-κB	=	Nuclear Factor Kappa B
OS	=	Oxidative Stress
ROS	=	Reactive Oxygen Species
STZ	=	Induced by Streptozotocin

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of K.R. Mangalam University, Haryana, India, under approval number KRMU/CCSEA/RES/IAEC-2023-8.

HUMAN AND ANIMAL RIGHTS

Animal care adhered to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA).

This study adheres to internationally accepted standards for animal research, following the 3Rs principle. The ARRIVE guidelines were employed for reporting experiments involving live animals, promoting ethical research practices.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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