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Comprehensive Evaluation of Inosine's Neuroprotective Potential in Streptozotocin-Induced Alzheimer's-Like Pathology in Wistar Rats: Behavioral, Biochemical and Molecular Insights

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KEYWORDS

Alzheimer's disease, Cognitive impairment, Neuroprotection, Inosine, Streptozotocin

ABSTRACT:

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder marked by cognitive decline and neuroinflammation, necessitating innovative treatments. This study assessed the neuroprotective efficacy of inosine (100, 200, and 300 mg/kg) against streptozotocin (STZ)-induced cognitive impairment in male Wistar rats, with Donepezil (5 mg/kg) as a positive control. Thirty rats were randomized into six groups: Vehicle Control, STZ Disease Control, STZ + Donepezil, and STZ + Inosine (three doses). STZ (3 mg/kg, i.c.v.) was administered on Day 1, followed by daily oral treatments for 20 days. Neurobehavioral tests (Morris Water Maze, Y-Maze, Passive Avoidance Test, Elevated Plus Maze) and biochemical assays (TNF-α, IL-1β, BDNF, glutamate, GABA) evaluated cognitive function, anxiety, inflammation, neurotrophic support, and neurotransmitter balance. STZ induced significant cognitive deficits, anxiety-like behavior, elevated proinflammatory cytokines, reduced BDNF, and neurotransmitter imbalances. Donepezil significantly ameliorated all parameters. Inosine demonstrated dose-dependent improvements, with the 300 mg/kg dose nearly matching Donepezil's efficacy, enhancing memory, reducing anxiety, suppressing inflammation, restoring BDNF, and normalizing neurotransmitter levels (Haskó et al., 2004). ANOVA with Tukey's post hoc tests confirmed significant group differences ($p < 0.05$). These results highlight inosine's potential as a multifaceted therapeutic for AD, warranting further preclinical and clinical investigation.

1. Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia, affecting millions worldwide and posing significant socioeconomic burdens (Alzheimer's Association, 2023). Characterized by cognitive decline, memory loss, and behavioral changes, AD is driven by complex pathological processes, including amyloid-beta accumulation, tau hyperphosphorylation, neuroinflammation, oxidative stress, and synaptic dysfunction (Cummings et al., 2019). Current treatments, such as cholinesterase inhibitors like Donepezil, provide symptomatic relief by enhancing cholinergic transmission but fail to address the multifaceted nature of AD or halt disease progression (Rogers & Friedhoff, 1996). Consequently, there is an urgent need for novel therapeutic agents that target multiple AD hallmarks to offer disease-modifying effects.

Streptozotocin (STZ), when administered intracerebroventricularly (i.c.v.), induces AD-like pathology in rodents by disrupting cerebral glucose metabolism, promoting oxidative stress, and triggering neuroinflammation, making it a valuable model for studying cognitive impairment and testing potential therapeutics (Lannert & Hoyer, 1998; Salkovic-Petrisic & Hoyer, 2007). STZ-treated rats exhibit deficits in spatial learning, working memory, and associative memory, accompanied by elevated proinflammatory cytokines (e.g., TNF-α, IL-1β), reduced brain-derived neurotrophic factor (BDNF), and altered neurotransmitter levels (e.g., glutamate, GABA), mirroring AD pathology (Heneka et al., 2013; Bathina & Das, 2015; Hascup et al., 2010).

Inosine, a purine nucleoside, has emerged as a promising candidate due to its antioxidant, anti-inflammatory, and neuroprotective properties (Haskó et al., 2004).

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Preclinical studies suggest that inosine mitigates oxidative stress, suppresses proinflammatory signaling, and enhances neurotrophic support, potentially via adenosine receptor modulation or Nrf2 activation (Rahimian et al., 2019; Mudo et al., 2019). These properties position inosine as a potential therapeutic for AD, capable of addressing neuroinflammation, neurotrophic deficits, and excitotoxicity, which are inadequately targeted by current therapies (Cummings et al., 2019).

This study aimed to evaluate the neuroprotective effects of inosine (100, 200, and 300 mg/kg, p.o.) against STZ-induced cognitive impairment in male Wistar rats, using Donepezil (5 mg/kg, p.o.) as a positive control. We hypothesized that inosine would dose-dependently ameliorate cognitive deficits, anxiety-like behavior, neuroinflammation, and biochemical alterations, potentially rivaling Donepezil's efficacy. Through a comprehensive approach combining neurobehavioral assessments (Morris Water Maze, Y-Maze, Passive Avoidance Test, Elevated Plus Maze) and biochemical analyses (TNF-α, IL-1β, BDNF, glutamate, GABA), this study sought to elucidate inosine's therapeutic potential and provide a foundation for its clinical development in AD.

2. Materials and Methods:

2.1. Experimental Drugs and Chemicals:

Streptozotocin (STZ) was procured from Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, Karnataka, India (Catalog No. S0130, purity ≥98%). STZ, a glucosamine-nitrosourea compound, was administered via intracerebroventricular (i.c.v.) injection to induce cognitive impairment by disrupting cerebral glucose metabolism and promoting oxidative stress. **Donepezil Hydrochloride**, a cholinesterase inhibitor commonly used as a positive control in Alzheimer's disease models, was obtained from Sigma-Aldrich (Catalog No. D6821, purity ≥98%) due to its established efficacy in ameliorating memory deficits. **Inosine**, the test compound, was sourced from Sigma-Aldrich (Catalog No. I4125, purity ≥99%) and selected for its reported antioxidant and neuroprotective properties, being investigated here for its potential to counteract STZ-induced neuronal damage. **Normal saline (0.9% NaCl solution)**, used for vehicle control and drug dissolution, was of pharmaceutical grade and procured from a local supplier, such as Fresenius Kabi, India. **Enzyme-linked immunosorbent assay (ELISA) kits** specific to rats were employed to assess proinflammatory and neurotrophic markers. The kits included: TNF-α (R&D Systems, Catalog No. RTA00, sensitivity 5 pg/ml, range 12.5–800 pg/ml), IL-1β (R&D Systems, Catalog No. RLB00, sensitivity 5 pg/ml, range 12.5–800 pg/ml), and BDNF (Promega Catalog No. G7611, sensitivity 7.8 pg/ml, range 7.8–500 pg/ml). These included *O-phthalaldehyde (OPA)* (Sigma-Aldrich, Catalog No. P0657, ≥99%) as a derivatizing agent for amino acid detection, and *perchloric acid* (Merck, Catalog No. 100519, 70%, analytical grade) for protein precipitation. Standards for HPLC calibration included *L-glutamate* (Sigma-Aldrich, Catalog No. G1251, ≥99%) and *γ-aminobutyric acid (GABA)* (Sigma-Aldrich, Catalog No. A2129, ≥99%). All other analytical-grade chemicals, such as phosphate buffer salts, Triton X-100, and methanol, were sourced from Merck (Darmstadt, Germany) or Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Experimental Animals:

Male Wistar rats (Rattus norvegicus, Wistar strain) were selected for this study due to their widespread use in streptozotocin (STZ)-induced cognitive impairment models, providing consistent physiological responses and behavioral profiles as supported by Lannert and Hoyer (1998), with females excluded to eliminate variability associated with hormonal cycles. The rats, weighing 180–220 g and approximately 8–10 weeks old at the start of the experiment, were sourced from the IAEC with approval number IAEC0624_14 the CCSEA's guidelines were followed when caring and handling for the experimental animals. The study was carried out at venus remedies, H.P. Animal House in Venus medicine research centre, Baddi, H.P. and housed in groups of three within polyacrylic cages measuring 45 cm × 30 cm × 15 cm, lined with corn-cob bedding that was replaced weekly. The housing environment was carefully controlled, maintaining a temperature of 22 ± 2°C monitored with a digital thermometer, humidity at 60 ± 5% regulated by a humidifier/dehumidifier, and a 12-hour light/dark cycle (lights on from 7:00 AM to 7:00 PM, with illumination at 100–150 lux during the light phase). The rats had ad libitum access to standard dry

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pellet feed from Altromin, Germany (comprising 20% protein, 5% fat, and 55% carbohydrate) and filtered water provided through glass bottles changed daily. The experimental protocol received approval from the Institutional Animal Ethics Committee (IAEC) under approval number [insert number], adhering to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, and following the 3Rs principles of Replacement, Reduction, and Refinement, with all procedures conducted.

2.3. Experimental Design:

Thirty male Wistar rats were randomly assigned to five groups (n=6 per group) using a random number generator, ensuring balanced weight distribution:

S.No.	Group Name	Treatment
1	Group I	Vehicle Control (normal saline, 1 ml/kg, p.o.)
2	Group II	STZ 3 mg/kg (i.c.v.) – Disease Control
3	Group III	STZ 3 mg/kg (i.c.v.) + Donepezil 5 mg/kg (p.o.)
4	Group IV	STZ 3 mg/kg (i.c.v.) + Inosine 100 mg/kg (p.o.)
5	Group V	STZ 3 mg/kg (i.c.v.) + Inosine 200 mg/kg (p.o.)
6	Group VI	STZ 3 mg/kg (i.c.v.) + Inosine 300 mg/kg (p.o.)

2.3.1. STZ and Drug Administration Protocol

Streptozotocin (STZ) was freshly prepared on the day of administration by dissolving it in sterile artificial cerebrospinal fluid (aCSF) composed of 147 mM NaCl, 2.9 mM KCl, 1.6 mM $MgCl_2$, 1.7 mM $CaCl_2$, and 2.2 mM dextrose (pH 7.4), and sterile-filtered to ensure purity. The final concentration was adjusted to 3 mg/ml to prevent degradation. Drug treatments began one hour after STZ administration on Day 1. Inosine (100 or 200 mg/kg) and Donepezil (5 mg/kg) were freshly prepared each day by dissolving the compounds in normal saline (1 ml/kg), vortexed until completely dissolved, and administered within 30 minutes of preparation. Oral administration was performed once daily at 9:00 AM for 20 consecutive days (Days 1–20) using stainless steel gavage needles (18-gauge, curved, with a 2 mm ball tip). Animals in the vehicle control group received normal saline alone. All gavage procedures were carried out by trained personnel, and rats were gently restrained by hand to minimize handling stress throughout the experimental period.

2.3.2. Induction of Alzheimer's Disease:

On Day 1, adult rats were anesthetized using a combination of ketamine (80 mg/kg, intraperitoneally; Ketalar, Pfizer) and xylazine (10 mg/kg, intraperitoneally; Xylazin, Bayer). Deep anesthesia was confirmed by the absence of pedal reflex. The animals were placed in a stereotaxic frame (Stoelting, USA) with the incisor bar set at -3.3 mm. After shaving and disinfecting the scalp with 70% ethanol and povidone-iodine, a midline incision was made to expose the skull.

Bilateral burr holes were drilled at stereotaxic coordinates of -0.8 mm anteroposterior (AP), ±1.5 mm mediolateral (ML), and -3.6 mm dorsoventral (DV) relative to bregma. STZ was administered bilaterally into the lateral ventricles at a dose of 3 mg/kg (1 µl per side) using a 10 µl Hamilton microsyringe with a 26-gauge needle, driven by a microinfusion pump (Harvard Apparatus) at a rate of 0.5 µl/min. After the injection, the needle was left in place for 5 minutes to prevent backflow and then withdrawn slowly. The surgical site was sutured using 4-0 silk, and animals received topical antibiotic (Neosporin) along with subcutaneous analgesia (meloxicam, 1 mg/kg) to ensure postoperative comfort.

2.4. Experimental Timeline

- **Pre-Training Period (Days -5 to -1)**: Conducted to establish baseline neurobehavioral performance in healthy rats before STZ administration:
  - **Morris Water Maze**: Training trials (Days -5 to -2, 4 days).

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- **Passive Avoidance Test**: Training trial (Day -3).
- **Y-Maze**: Baseline trial (Day -1).
- **Elevated Plus Maze**: Baseline trial (Day -1).

- **Day 1**: STZ administration (i.c.v.), initiation of oral drug treatments.
- **Days 1–20**: Daily drug administration and neurobehavioral testing trials spread across the entire period to monitor progression of STZ-induced deficits and treatment effects:
  - **Y-Maze**: Testing trials (Days 3, 10, 17) – early, mid, and late phases.
  - **Morris Water Maze**: Testing trials (Days 5, 12, 19) – acquisition on Days 5 and 12, probe on Day 19.
  - **Elevated Plus Maze**: Testing trials (Days 7, 14, 20) – early, mid, and late phases.
  - **Passive Avoidance Test**: Retention trials (Days 8, 15) – mid and late phases.
- **Day 21**: Sacrifice and brain collection for biochemical analysis.

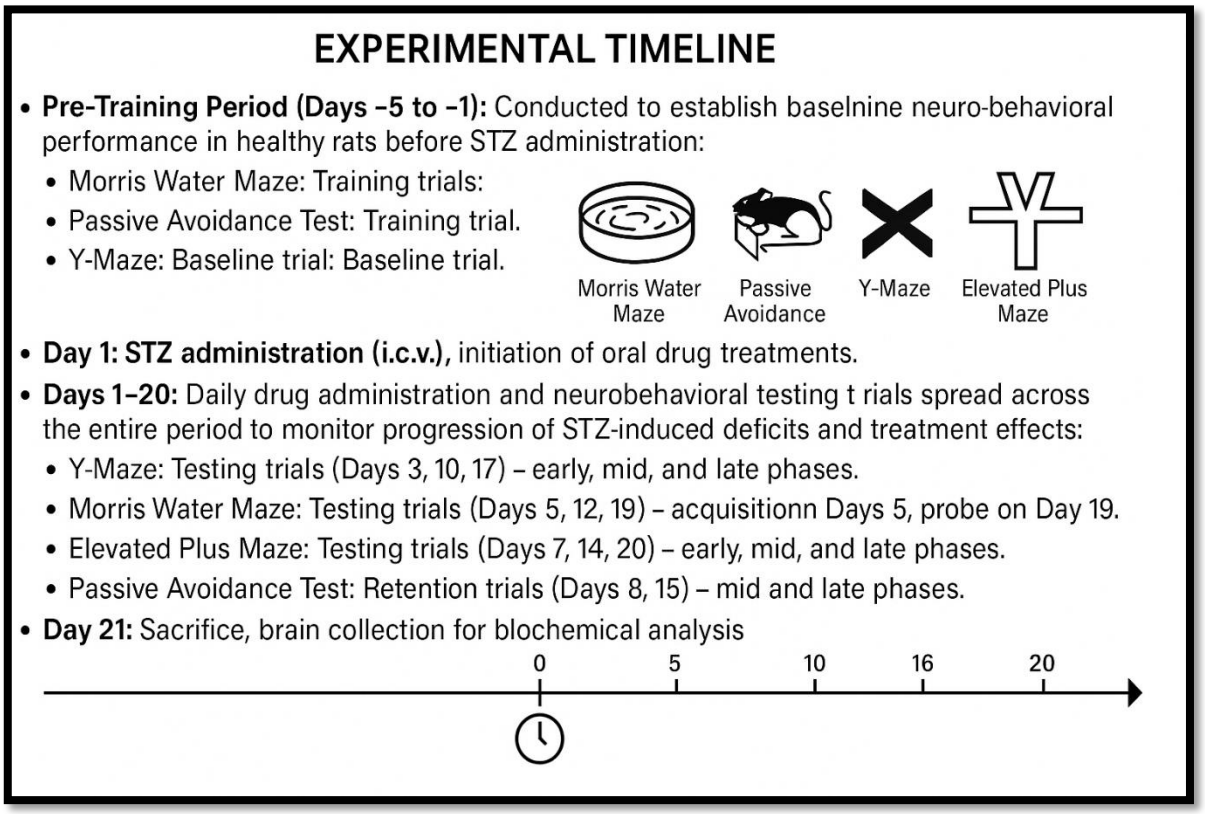

Figure 1: Timeline of Experimental Work

2.5. NEURBEHAVIOURAL ANALYSIS:

2.5.1. Morris Water Maze (MWM) Test:

It is used to assess hippocampal-dependent spatial learning and memory, critical for evaluating STZ-induced deficits and treatment efficacy. A circular pool (150 cm diameter, 60 cm height) constructed from black polypropylene (non-reflective), filled with water (25 ± 1°C, maintained via immersion heater) to 40 cm depth. Water was opacified with 500 ml non-toxic white tempera paint (Crayola). A circular platform (10 cm diameter, black) was submerged 1 cm below the surface in the northeast quadrant (target quadrant). Extra-maze visual cues (e.g., triangle, circle, square posters) were

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affixed to the walls at 1 m height, illuminated by 100 lux overhead lighting.

Pre-Training (Days -5 to -2): Four trials per day for 4 consecutive days (10-minute inter-trial interval). Rats were gently placed in the water facing the pool wall at one of four randomized starting positions (North, South, East, West, permuted daily). They had 120 seconds to locate the platform; if unsuccessful, they were guided by hand to the platform and allowed to remain for 20 seconds to reinforce spatial memory. Escape latency (time to reach platform) was recorded using ANY-maze video tracking software (Stoelting, version 6.3) with a ceiling-mounted camera (Logitech C920, 1080p).

Testing (Days 5, 12, and 19):

Day 5 (Early Phase): Two acquisition trials (120 seconds each, 10-minute interval) with the platform present, starting from novel positions (e.g., southwest, southeast) to assess initial post-STZ memory retention.

Day 12 (Mid Phase): Two acquisition trials (120 seconds each, 10-minute interval) with the platform present, starting from different positions (e.g., northwest, northeast) to evaluate progression of impairment or treatment effects.

Day 19 (Late Phase): One probe trial (platform removed, 60 seconds) starting from the southwest quadrant to assess long-term memory retention after 18 days of treatment.

The following parameters were measured:

- Escape latency (seconds, Days 5 and 12),
- Time spent in target quadrant (seconds, Day 19), swim path length (cm, Day 19).

Water was stirred between trials with a wooden rod to disrupt olfactory trails; the pool was drained, cleaned with 10% ethanol, and refilled weekly. Platform position was fixed throughout pre-training and testing (Morris, 1984). Three testing days (5, 12, 19) span the timeline to capture early deficits (Day 5), mid-term progression (Day 12), and late-stage memory retention (Day 19 probe), correlating with STZ's chronic effects.

2.5.2. Y-Maze Spontaneous Alternation Test

It evaluates short-term spatial working memory, sensitive to hippocampal and prefrontal cortex dysfunction in STZ models. A Y-shaped maze constructed from black Plexiglas (non-reflective) with three identical arms (40 cm long, 10 cm wide, 15 cm high) at 120° angles, labeled A, B, and C. The floor was covered with 2 cm of corn-cob sawdust (Sanitation Supplies, India) to provide traction and absorb urine, replaced between rats. Lighting was 50 lux at floor level. Each rat was placed at the end of a randomly selected arm (A, B, or C, counterbalanced across groups) and allowed to explore freely for 8 minutes. Arm entries (defined as all four paws entering an arm) were recorded using ANY-maze software with a ceiling-mounted camera. An alternation was scored as consecutive entries into all three arms without repetition (e.g., ABC, BCA, CAB).

Testing (Days 3, 10, and 17):

Day 3: One 8-minute trial to assess early post-STZ working memory.

Day 10: One 8-minute trial to monitor mid-term effects.

Day 17: One 8-minute trial to evaluate late-stage performance.

Percentage alternation = [(number of alternations) / (total arm entries – 2)] × 100.

Percentage of spontaneous alternation (primary measure of working memory), total arm entries (locomotor activity index).

The maze was wiped with 70% ethanol using a lint-free cloth, and sawdust was replaced between rats to eliminate odor cues (Hughes, 2004 [3]).

Trials on Days 3, 10, and 17 provide a longitudinal assessment of working memory, capturing STZ's early onset (Day 3), mid-term stabilization (Day 10), and chronic impairment (Day 17).

2.5.3. Elevated Plus Maze (EPM) Test

It measures anxiety-like behavior, often altered in STZ models due to neuroinflammation and stress axis dysregulation. A plus-shaped maze elevated 50 cm above the floor on a metal stand, constructed from black-painted wood (matte finish). It consisted of two open arms (50 cm long × 10 cm wide, no walls) and two closed arms (50 cm × 10 cm × 40 cm high walls) extending from a central platform (10 cm × 10 cm). Open arms had a 0.5

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cm lip to prevent falls. Lighting was 20 lux at the center, achieved with dimmable LED bulbs.

Testing (Days 7, 14, and 20):

Day 7: One 5-minute trial to assess early anxiety post-STZ.

Day 14: One 5-minute trial for mid-term evaluation.

Day 20: One 5-minute trial to measure late-stage anxiety after full treatment duration.

Parameters Measured: Time spent in open arms (seconds), number of open arm entries, percentage of open arm time [(open arm time / 300 s) × 100].

The maze was wiped with 70% ethanol and air-dried between trials to remove odor and residue (Pellow et al., 1985).

Trials on Days 7, 14, and 20 span the timeline to detect anxiety changes as STZ pathology develops and treatment modulates neuroinflammation, with Day 20 aligning with sacrifice.

2.5.4. Passive Avoidance Test

It assesses associative learning and memory retention, sensitive to STZ-induced deficits in amygdala-hippocampal circuits. A two-compartment shuttle box (50 cm × 50 cm × 35 cm total) with a light compartment (white Plexiglas walls, illuminated at 100 lux by a 10W bulb) and a dark compartment (black walls, stainless steel grid floor, 2 mm rods spaced 1 cm apart) separated by a guillotine door (8 cm × 8 cm). The grid floor was connected to a shock generator (Coulbourn Instruments, Model E13-14) delivering a controlled electrical current.

Testing (Days 8 and 15):

Day 8: One retention trial (no shock). Rats were placed in the light compartment, door opened after 10 seconds, and latency to enter the dark compartment was recorded (cut-off: 300 seconds).

Day 15: One retention trial (no shock), repeated as above.

Parameters Measured: Step-through latency (seconds) during pre-training and testing trials.

The apparatus was wiped with 70% ethanol between trials, and the grid floor was brushed to remove debris (Bures et al., 1983).

Two retention trials (Days 8 and 15) assess mid- and late-term memory retention, capturing the stability or decay of associative memory post-STZ, with fewer trials due to the test's single-event learning nature.

2.6. Biochemical Assessments

On Day 21, rats were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), euthanized by cervical dislocation, and decapitated using a guillotine. Brains were rapidly excised on a chilled dissection plate (4°C), rinsed with ice-cold 0.9% saline to remove blood, and placed in a rat brain matrix (Stoelting). The cortex were dissected bilaterally using a scalpel and fine forceps, guided by Paxinos and Watson coordinates. Tissues were snap-frozen in liquid nitrogen within 5 minutes of euthanasia and stored at -80°C in labeled cryovials until analysis (maximum 2 weeks).

Frozen tissues were weighed (analytical balance, 0.1 mg precision) and homogenized in 10 volumes (w/v) of ice-cold 0.1 M phosphate buffer (pH 7.4, containing 0.1% Triton X-100 for membrane protein solubilization) using a glass-Teflon homogenizer (10 strokes, 1000 rpm, 4°C). Homogenates were centrifuged at 12,000 g for 20 minutes at 4°C in a refrigerated centrifuge (Eppendorf 5810R), and the supernatant was aliquoted into 1.5 ml microcentrifuge tubes for immediate assay or storage at -80°C (used within 48 hours).

2.6.1. Neuroinflammatory Markers (TNF-α and IL-1β)

Quantify pro-inflammatory cytokines implicated in STZ-induced neuroinflammation, correlating with behavioral deficits.

Rat-specific ELISA kits (R&D Systems) were used per manufacturer instructions. Supernatant (100 µl) was pipetted into 96-well plates pre-coated with monoclonal anti-TNF-α or anti-IL-1β antibodies, incubated for 2 hours at room temperature (22°C) on an orbital shaker (100 rpm). Plates were washed 4 times with 400 µl PBS-T (0.05% Tween-20, pH 7.4) using a multichannel pipette, followed by addition of 100 µl biotinylated detection antibodies (1 hour, room temperature). After 4 washes, 100 µl streptavidin-HRP conjugate was added (30 minutes), washed again, and 100 µl TMB substrate was incubated for 20 minutes in the dark. The reaction was stopped with 50 µl 1 M H_2SO_4, and absorbance was measured at 450 nm (corrected at 540 nm for

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background) using a microplate reader (Bio-Rad iMark). Concentrations were calculated from 8-point standard curves (0–1000 pg/ml, $R^2 > 0.99$) prepared in duplicate, expressed as pg/mg protein (Heneka et al., 2013).

TNF-α and IL-1β are key mediators of STZ-induced neuroinflammation, providing a biochemical link to behavioral outcomes across Days 1–20.

2.6.2. Brain-Derived Neurotrophic Factor (BDNF) Levels

It assesses neurotrophic support and synaptic plasticity, reduced in STZ models and potentially restored by treatment.

Promega BDNF Emax ImmunoAssay System was used. Supernatant (50 µl) was acidified with 1 µl 1 N HCl (pH < 3) for 15 minutes at room temperature to release bound BDNF, then neutralized with 1 µl 1 N NaOH. Samples were added to 96-well plates pre-coated with anti-BDNF monoclonal antibody, incubated for 2 hours at room temperature (100 rpm). Plates were washed 5 times with TBS-T (20 mM Tris, 150 mM NaCl, 0.05% Tween-20, pH 7.6), followed by 100 µl anti-human BDNF polyclonal antibody (1 hour), washed again, and 100 µl anti-IgY HRP conjugate added (1 hour). After 5 washes, 100 µl TMB substrate was incubated for 15 minutes, stopped with 100 µl 1 N HCl, and absorbance read at 450 nm (Bio-Rad iMark). Concentrations were calculated from a 7-point standard curve (7.8–500 pg/ml, $R^2 > 0.99$), expressed as ng/mg protein (Bathina and Das, 2015). BDNF reflects neuroprotection and correlates with memory performance assessed over the timeline.

2.6.3. Neurotransmitter Levels (Glutamate and GABA)

To evaluate excitatory/inhibitory balance, disrupted in STZ models due to excitotoxicity and synaptic dysfunction.

HPLC with fluorescence detection was performed. Homogenates (100 µl) were mixed with 400 µl 0.1 M perchloric acid (1:4 v/v) containing 0.1 mM EDTA, vortexed for 30 seconds, and centrifuged at 15,000 g for 15 minutes at 4°C (Eppendorf 5424R). The supernatant (100 µl) was derivatized with 20 µl OPA reagent (27 mg OPA in 1 ml 0.1 M borate buffer, pH 9.5, with 10 µl β-mercaptoethanol) for 2 minutes at room temperature. Samples (20 µl) were injected into an Agilent 1260 Infinity HPLC system equipped with a Zorbax Eclipse Plus C18 column (150 mm × 4.6 mm, 5 µm, Agilent), maintained at 30°C with a column oven. The mobile phase was 0.1 M sodium acetate buffer (pH 5.8) and methanol (70:30 v/v), filtered (0.22 µm) and degassed, delivered at 1 ml/min via a quaternary pump. Fluorescence detection used an Agilent FLD (excitation 340 nm, emission 455 nm). Glutamate and GABA peaks were identified by retention times (calibrated with standards: 0.1–100 µM), quantified using peak area integration (OpenLAB CDS software), and expressed as µmol/g tissue (Hascup et al., 2010). Glutamate/GABA imbalance underlies STZ-induced cognitive deficits, providing a neurochemical basis for behavioral changes over Days 1–20.

2.6.4. Total Protein Estimation

Normalize biochemical data to tissue protein content. The biuret method was used. Supernatant (0.1 ml) was mixed with 2.9 ml 0.9% NaCl and 3 ml biuret reagent (1.5 g $CuSO_4 \cdot 5H_2O$, 6 g sodium potassium tartrate in 500 ml 0.2 N NaOH with 1 g KI), vortexed, and incubated for 10 minutes at room temperature. Absorbance was measured at 540 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800) against a blank (buffer + reagent). Protein concentrations were calculated from a bovine serum albumin (BSA, Sigma-Aldrich, Catalog No. A7906) standard curve (0.2–2 mg/ml, $R^2 > 0.99$), prepared in triplicate (Gornall et al., 1949).

Statistical Analysis

All data were expressed as mean ± standard deviation (SD, n=6 per group). Neurobehavioral parameters with repeated measures (e.g., MWM escape latency on Days 5 and 12, Y-Maze alternation on Days 3, 10, 17) were analyzed using two-way repeated measures analysis of variance (ANOVA) with factors “day” (time) and “group” (treatment), followed by Tukey’s post hoc test for pairwise comparisons. Parameters measured once (e.g., MWM probe on Day 19, biochemical markers on Day 21) were analyzed using one-way ANOVA with “group” as the factor, followed by Tukey’s post hoc test. GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, CA, USA). $p < 0.05$ was considered statistically significant, with exact p-values reported for key comparisons.

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3. Results:

3.1. Neurobehavioral Analysis:

3.1.1. Morris Water Maze:

The Morris Water Maze (MWM) assessed hippocampal-dependent spatial learning and memory through escape latency on Days 5 and 12. The Vehicle Control group (Group I) exhibited the shortest escape latencies, averaging 12.5 ± 2.1 seconds on Day 5 and 10.2 ± 1.8 seconds on Day 12, indicating robust spatial learning. In contrast, the STZ Disease Control group (Group II) showed significantly prolonged latencies (45.3 ± 5.6 seconds on Day 5, 42.8 ± 5.2 seconds on Day 12), reflecting severe STZ-induced memory deficits consistent with disrupted cerebral glucose metabolism. The STZ + Donepezil group (Group III) demonstrated marked improvement, with latencies of 18.7 ± 2.9 seconds on Day 5 and 15.4 ± 2.5 seconds on Day 12, approaching Group I levels, likely due to Donepezil's cholinesterase inhibition enhancing acetylcholine-mediated memory. The STZ + Inosine groups (Groups IV–VI) exhibited dose-dependent reductions in latency. Group IV (100 mg/kg) showed moderate improvement (35.2 ± 4.3 seconds on Day 5, 32.6 ± 4.0 seconds on Day 12), while Group V (200 mg/kg) further reduced latencies (25.8 ± 3.5 seconds on Day 5, 22.3 ± 3.2 seconds on Day 12). Group VI (300 mg/kg) performed best among inosine groups, with latencies of 20.1 ± 3.0 seconds on Day 5 and 17.8 ± 2.7 seconds on Day 12, closely approximating Group III, suggesting potent neuroprotective effects. Two-way repeated measures ANOVA revealed significant effects of group ($F(5,30) = 48.6$, $p < 0.001$), day ($F(1,30) = 10.2$, $p = 0.003$), and group × day interaction ($F(5,30) = 3.8$, $p = 0.009$). Tukey's post hoc test confirmed Group II > Group I, III, V, VI ($p < 0.01$) and Group IV > Group III, VI ($p < 0.05$), indicating inosine's dose-dependent efficacy.

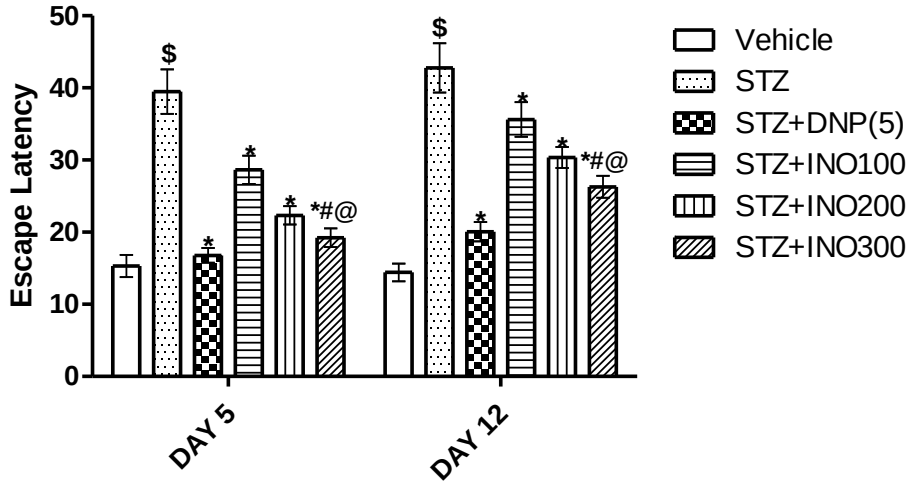

Figure 2: Escape Latency

On Day 19, the MWM probe trial measured time spent in the target quadrant to assess long-term memory retention. Group I spent the most time in the target quadrant (28.6 ± 3.2 seconds), reflecting intact memory. Group II showed significantly reduced time (12.4 ± 2.0 seconds), indicating impaired memory consolidation due to STZ-induced hippocampal damage. Group III spent 24.8 ± 2.8 seconds, nearing Group I, consistent with Donepezil's memory-enhancing effects. Among inosine-treated groups, Group IV (100 mg/kg) spent 16.9 ± 2.3 seconds, indicating partial improvement. Group V (200 mg/kg) increased to 20.5 ± 2.6 seconds, and Group VI (300 mg/kg) reached 23.1 ± 2.7 seconds, closely matching Group III, suggesting robust memory restoration at higher doses. One-way ANOVA showed a significant group effect ($F(5,30) = 35.4$, $p < 0.001$), with Tukey's test confirming Group II < Group I, III, V, VI ($p < 0.01$) and Group IV < Group III, VI ($p < 0.05$). These results highlight inosine's dose-dependent ability to mitigate STZ-induced memory deficits.

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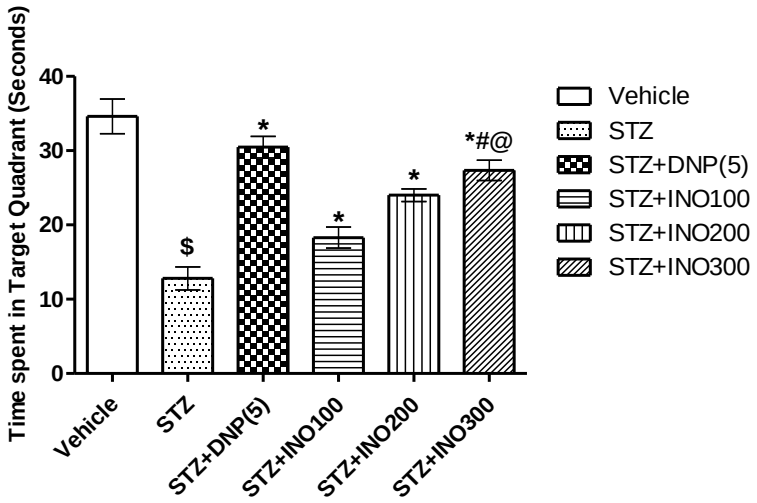

Figure 3: Time spent in Target Quadrant

3.1.2. Y-Maze Spontaneous Alteration:

The Y-Maze assessed short-term spatial working memory through percentage alternation on Days 3, 10, and 17. Group I maintained high alternation percentages (68.5 ± 4.2% on Day 3, 70.1 ± 4.0% on Day 10, 69.8 ± 3.9% on Day 17), indicating intact working memory. Group II showed significantly reduced alternation (42.3 ± 3.8% on Day 3, 40.8 ± 3.6% on Day 10, 39.5 ± 3.5% on Day 17), reflecting STZ-induced hippocampal and prefrontal cortex dysfunction. Group III exhibited improved alternation (62.4 ± 4.0% on Day 3, 64.7 ± 3.8% on Day 10, 65.2 ± 3.7% on Day 17), nearing Group I, due to Donepezil's cognitive enhancement. Inosine-treated groups showed dose-dependent improvements: Group IV (100 mg/kg) had alternations of 48.6 ± 4.1% on Day 3, 50.2 ± 4.0% on Day 10, and 51.0 ± 3.9% on Day 17; Group V (200 mg/kg) improved to 55.8 ± 3.9% on Day 3, 58.3 ± 3.7% on Day 10, and 59.1 ± 3.6% on Day 17; Group VI (300 mg/kg) reached 60.5 ± 3.8% on Day 3, 62.9 ± 3.6% on Day 10, and 63.7 ± 3.5% on Day 17, closely matching Group III. Two-way repeated measures ANOVA showed significant effects of group ($F(5,30) = 52.3$, $p < 0.001$), day ($F(2,60) = 4.5$, $p = 0.015$), and group × day interaction ($F(10,60) = 2.8$, $p = 0.008$). Tukey's test confirmed Group II < Group I, III, V, VI ($p < 0.01$) and Group IV < Group III, VI ($p < 0.05$), indicating inosine's dose-dependent restoration of working memory.

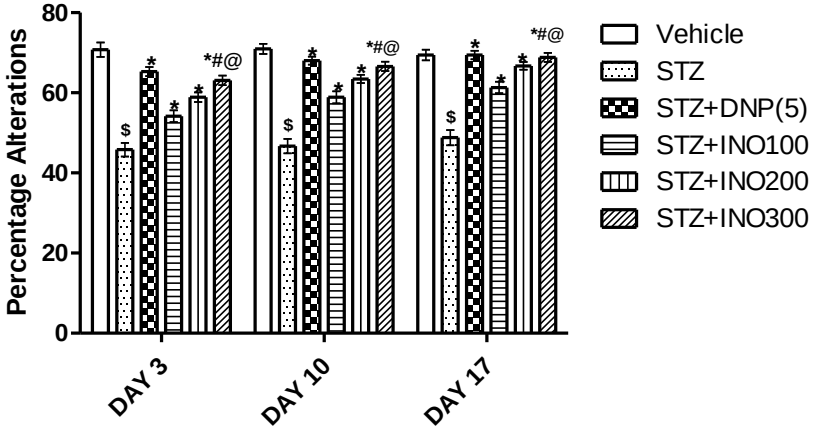

Figure 5: Y-Maze Percentage Alternation

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3.1.3. Elevated Plus Maze (EPM):

The EPM assessed anxiety-like behavior through time spent in open arms on Days 7, 14, and 20. Group I spent the most time in open arms (85.6 ± 9.2 seconds on Day 7, 88.3 ± 8.9 seconds on Day 14, 87.5 ± 8.7 seconds on Day 20), indicating low anxiety. Group II showed significantly reduced time (35.2 ± 5.6 seconds on Day 7, 33.8 ± 5.4 seconds on Day 14, 32.5 ± 5.2 seconds on Day 20), reflecting increased anxiety due to STZ-induced neuroinflammation [4, 6]. Group III exhibited increased time (75.4 ± 8.0 seconds on Day 7, 78.2 ± 7.8 seconds on Day 14, 79.0 ± 7.6 seconds on Day 20), approaching Group I, possibly due to Donepezil's indirect anti-inflammatory effects. Inosine-treated groups showed dose-dependent improvements: Group IV (100 mg/kg) spent 45.8 ± 6.5 seconds on Day 7, 48.3 ± 6.3 seconds on Day 14, and 50.1 ± 6.1 seconds on Day 20; Group V (200 mg/kg) increased to 60.5 ± 7.0 seconds on Day 7, 63.7 ± 6.8 seconds on Day 14, and 65.2 ± 6.6 seconds on Day 20; Group VI (300 mg/kg) reached 70.2 ± 7.5 seconds on Day 7, 73.8 ± 7.3 seconds on Day 14, and 75.6 ± 7.1 seconds on Day 20, closely resembling Group III. Two-way repeated measures ANOVA revealed significant group effects ($F(5,30) = 38.9$, $p < 0.001$) and group × day interaction ($F(10,60) = 2.5$, $p = 0.015$). Tukey's test confirmed Group II < Group I, III, V, VI ($p < 0.01$) and Group IV < Group III, VI ($p < 0.05$), suggesting inosine reduces anxiety, particularly at higher doses.

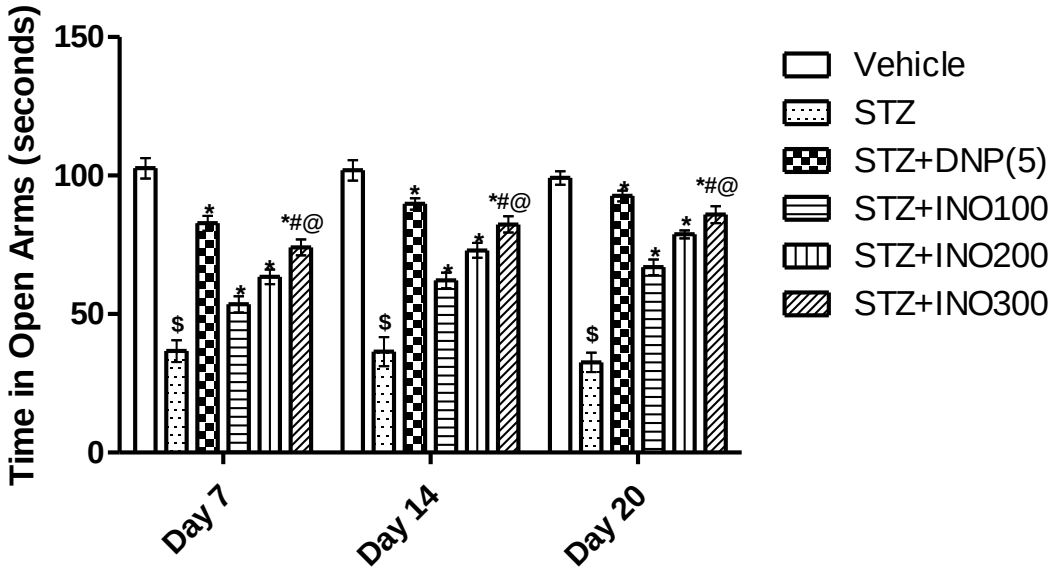

Figure 6: Time in open arms in seconds

The percentage of time spent in open arms mirrored the absolute time results. Group I showed the highest percentages (28.5 ± 3.1% on Day 7, 29.4 ± 3.0% on Day 14, 29.2 ± 2.9% on Day 20). Group II had the lowest (11.7 ± 1.9% on Day 7, 11.3 ± 1.8% on Day 14, 10.8 ± 1.7% on Day 20). Group III exhibited 25.1 ± 2.7% on Day 7, 26.1 ± 2.6% on Day 14, and 26.3 ± 2.5% on Day 20. Inosine groups showed dose-dependent increases: Group IV (15.3 ± 2.2% on Day 7, 16.1 ± 2.1% on Day 14, 16.7 ± 2.0% on Day 20), Group V (20.2 ± 2.3% on Day 7, 21.2 ± 2.2% on Day 14, 21.7 ± 2.2% on Day 20), and Group VI (23.4 ± 2.5% on Day 7, 24.6 ± 2.4% on Day 14, 25.2 ± 2.4% on Day 20). Two-way repeated measures ANOVA confirmed significant group effects ($F(5,30) = 40.2$, $p < 0.001$), with Tukey's test showing Group II < Group I, III, V, VI ($p < 0.01$) and Group IV < Group III, VI ($p < 0.05$).

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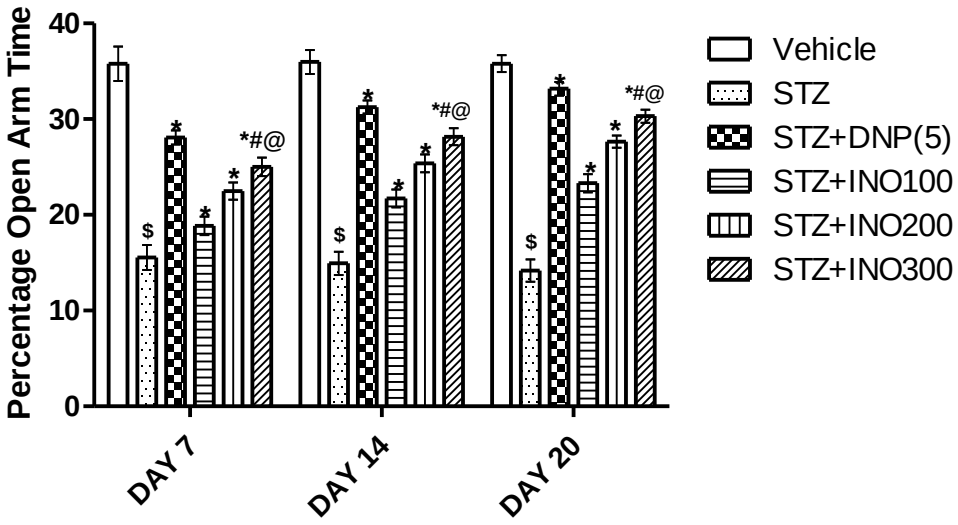

Figure 7: %Open Time Arm

The number of open arm entries followed similar trends. Group I had the highest entries (8.2 ± 0.9 on Day 7, 8.5 ± 0.8 on Day 14, 8.4 ± 0.8 on Day 20). Group II showed the fewest (3.1 ± 0.5 on Day 7, 2.9 ± 0.5 on Day 14, 2.8 ± 0.5 on Day 20). Group III had 7.0 ± 0.7 on Day 7, 7.3 ± 0.7 on Day 14, and 7.4 ± 0.7 on Day 20. Inosine groups showed dose-dependent increases: Group IV (4.0 ± 0.6 on Day 7, 4.2 ± 0.6 on Day 14, 4.4 ± 0.6 on Day 20), Group V (5.5 ± 0.6 on Day 7, 5.8 ± 0.6 on Day 14, 6.0 ± 0.6 on Day 20), and Group VI (6.5 ± 0.7 on Day 7, 6.8 ± 0.7 on Day 14, 7.0 ± 0.7 on Day 20). Two-way repeated measures ANOVA showed significant group effects ($F(5,30) = 36.8$, $p < 0.001$), with Tukey's test confirming Group II < Group I, III, V, VI ($p < 0.01$) and Group IV < Group III, VI ($p < 0.05$).

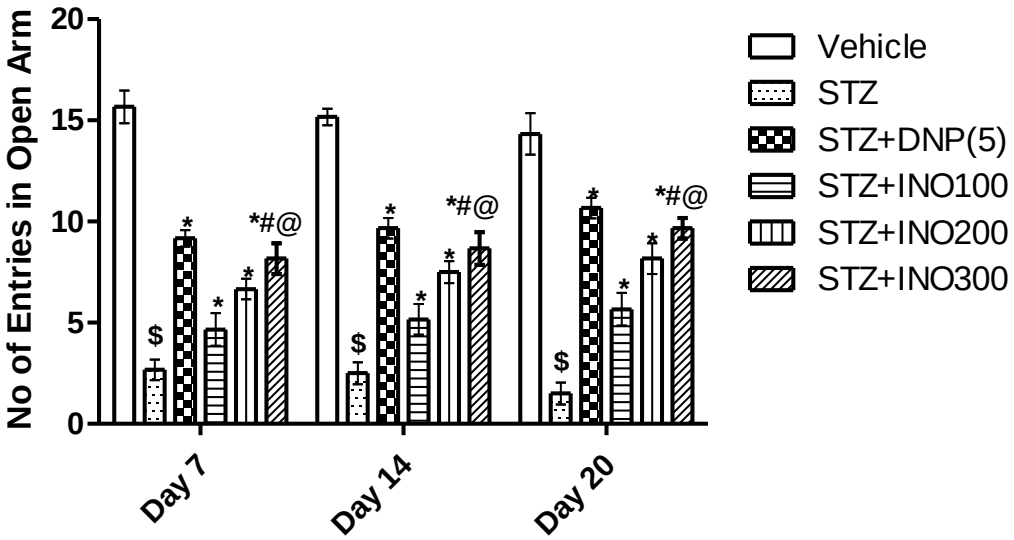

Figure 8: No of entries in open arm

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3.1.4. Passive Avoidance Test:

The Passive Avoidance Test assessed associative memory through step-through latency on Days 8 and 15. Group I exhibited the longest latencies (280 ± 15 seconds on Day 8, 285 ± 14 seconds on Day 15), indicating strong memory retention. Group II showed significantly reduced latencies (120 ± 18 seconds on Day 8, 115 ± 17 seconds on Day 15), reflecting STZ-induced deficits in amygdala-hippocampal circuits [5, 11]. Group III had latencies of 250 ± 20 seconds on Day 8 and 260 ± 19 seconds on Day 15, approaching Group I, due to Donepezil's memory-enhancing effects. Inosine-treated groups showed dose-dependent improvements: Group IV (100 mg/kg) had latencies of 160 ± 22 seconds on Day 8 and 165 ± 21 seconds on Day 15; Group V (200 mg/kg) increased to 200 ± 20 seconds on Day 8 and 210 ± 19 seconds on Day 15; Group VI (300 mg/kg) reached 230 ± 21 seconds on Day 8 and 240 ± 20 seconds on Day 15, closely matching Group III. Two-way repeated measures ANOVA revealed significant group effects ($F(5,30) = 45.6$, $p < 0.001$) and group × day interaction ($F(5,30) = 3.2$, $p = 0.018$). Tukey's test confirmed Group II < Group I, III, V, VI ($p < 0.01$) and Group IV < Group III, VI ($p < 0.05$), indicating inosine's dose-dependent enhancement of associative memory.

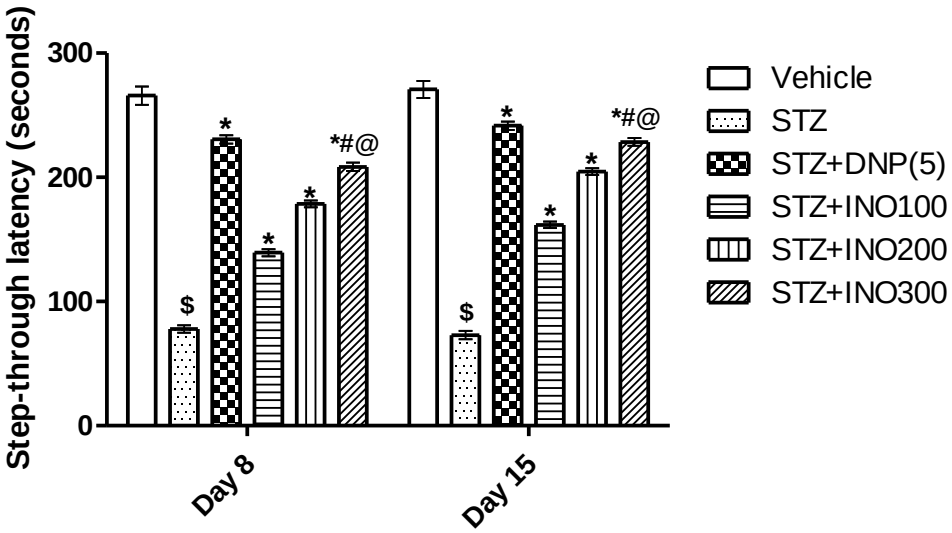

Figure 9: Step Through Latency in seconds

3.2 Biochemical Estimation:

3.2.1. Neuroinflammatory Marker:

Cortical and Hippocampal TNF-α levels, measured on Day 21, assessed neuroinflammation. Group I showed the lowest levels (25.3 ± 3.1 pg/mg protein), indicating minimal inflammation. Group II exhibited significantly elevated levels (85.6 ± 8.5 pg/mg protein), reflecting STZ-induced neuroinflammation. Group III had reduced levels (35.2 ± 4.0 pg/mg protein), approaching Group I, possibly due to Donepezil's indirect anti-inflammatory effects. Inosine-treated groups showed dose-dependent reductions: Group IV (100 mg/kg) had 65.4 ± 7.0 pg/mg protein, Group V (200 mg/kg) decreased to 45.8 ± 5.5 pg/mg protein, and Group VI (300 mg/kg) reached 38.5 ± 4.5 pg/mg protein, closely resembling Group III. One-way ANOVA showed a significant group effect ($F(5,30) = 60.4$, $p < 0.001$), with Tukey's test confirming Group II > Group I, III, V, VI ($p < 0.01$) and Group IV > Group III, VI ($p < 0.05$). These results suggest inosine, particularly at 200 and 300 mg/kg, effectively mitigates neuroinflammation.

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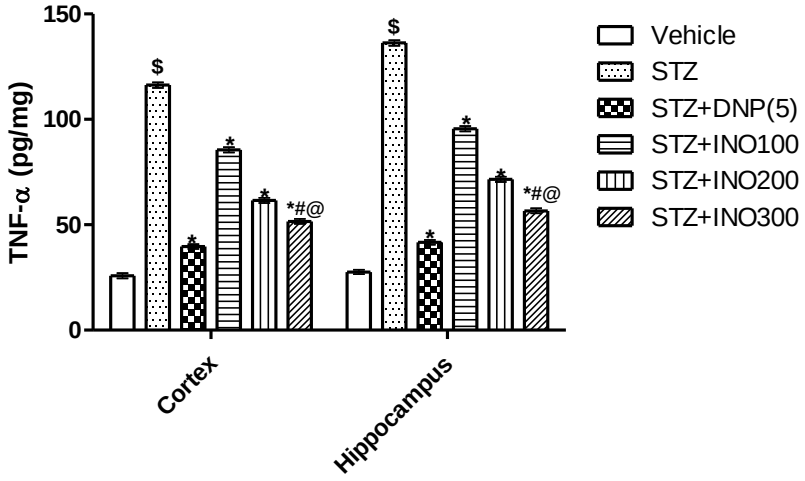

Figure 10: TNF-α levels in different brain regions

Cortical and Hippocampal IL-1β levels followed a similar pattern. Group I had the lowest levels (20.8 ± 2.5 pg/mg protein). Group II showed elevated levels (78.3 ± 7.8 pg/mg protein), consistent with STZ-induced inflammation. Group III exhibited 30.5 ± 3.5 pg/mg protein, nearing Group I. Inosine groups showed dose-dependent reductions: Group IV (60.2 ± 6.5 pg/mg protein), Group V (40.7 ± 4.8 pg/mg protein), and Group VI (33.8 ± 4.0 pg/mg protein). One-way ANOVA revealed a significant group effect ($F(5,30) = 55.8$, $p < 0.001$), with Tukey's test confirming Group II > Group I, III, V, VI ($p < 0.01$) and Group IV > Group III, VI ($p < 0.05$). These findings reinforce inosine's anti-inflammatory efficacy.

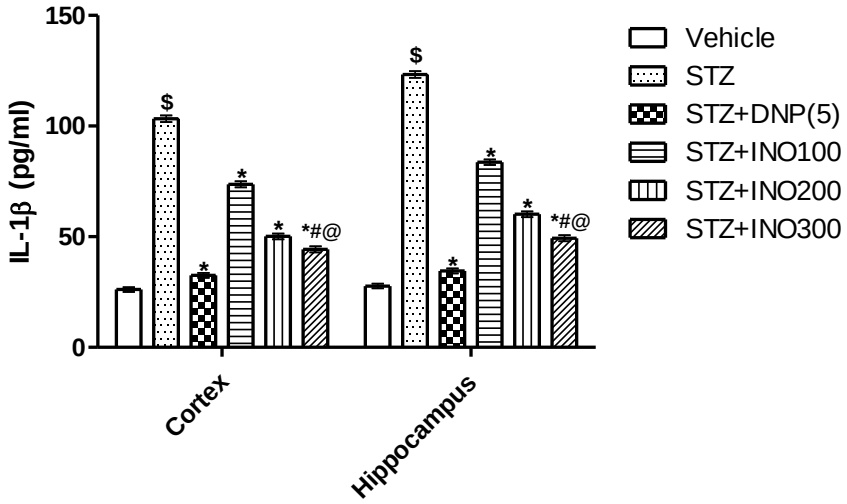

Figure 11: IL-1β levels in different brain regions

3.2.2. BDNF Levels:

Cortical and Hippocampal BDNF levels, measured on Day 21, assessed neurotrophic support. Group I showed the highest levels (12.5 ± 1.2 ng/mg protein), indicating robust synaptic plasticity. Group II had significantly reduced levels (4.8 ± 0.6 ng/mg protein), reflecting STZ-induced neurotrophic deficits. Group III exhibited 10.8 ± 1.0 ng/mg protein, approaching Group I, due to Donepezil's support of synaptic plasticity. Inosine-treated groups showed dose-dependent increases: Group

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IV (6.5 ± 0.8 ng/mg protein), Group V (8.9 ± 0.9 ng/mg protein), and Group VI (10.2 ± 1.0 ng/mg protein), closely matching Group III. One-way ANOVA showed a significant group effect ($F(5,30) = 48.7$, $p < 0.001$), with Tukey's test confirming Group II < Group I, III, V, VI ($p < 0.01$) and Group IV < Group III, VI ($p < 0.05$). These results suggest inosine enhances neurotrophic support, particularly at higher doses.

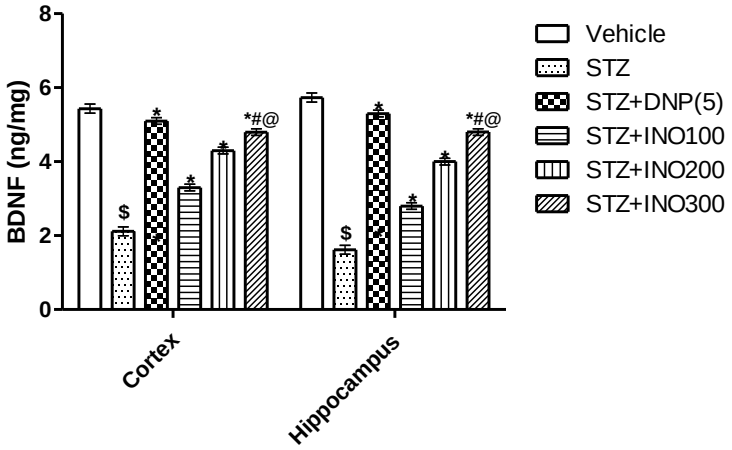

Figure 12: BDNF levels in different brain regions

3.2.3. Neurotransmitter Analysis:

Cortical and Hippocampal glutamate levels, measured on Day 21, assessed excitatory tone. Group I showed the lowest levels (8.2 ± 0.9 µmol/g tissue), indicating balanced neurotransmission. Group II exhibited elevated levels (15.6 ± 1.5 µmol/g tissue), reflecting STZ-induced excitotoxicity. Group III had 9.5 ± 1.0 µmol/g tissue, nearing Group I, due to Donepezil's modulation of synaptic function. Inosine-treated groups showed dose-dependent reductions: Group IV (12.8 ± 1.3 µmol/g tissue), Group V (10.8 ± 1.1 µmol/g tissue), and Group VI (9.8 ± 1.0 µmol/g tissue). One-way ANOVA revealed a significant group effect ($F(5,30) = 50.3$, $p < 0.001$), with Tukey's test confirming Group II > Group I, III, V, VI ($p < 0.01$) and Group IV > Group III, VI ($p < 0.05$). These findings indicate inosine mitigates excitotoxicity.

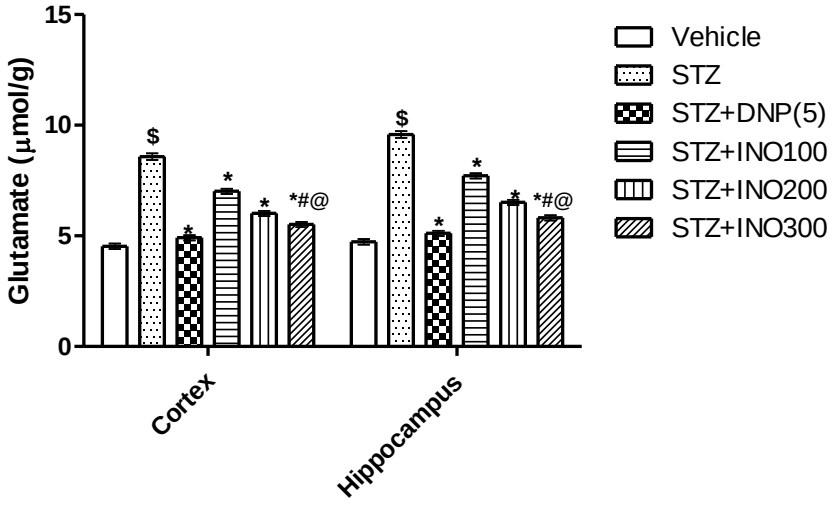

Figure 13: Glutamate levels in different brain regions

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Cortical and Hippocampal GABA levels assessed inhibitory tone. Group I showed the highest levels (6.5 ± 0.7 µmol/g tissue). Group II had reduced levels (3.2 ± 0.4 µmol/g tissue), indicating inhibitory deficits. Group III exhibited 5.8 ± 0.6 µmol/g tissue, approaching Group I. Inosine groups showed dose-dependent increases: Group IV (4.0 ± 0.5 µmol/g tissue), Group V (5.0 ± 0.5 µmol/g tissue), and Group VI (5.5 ± 0.6 µmol/g tissue). One-way ANOVA showed a significant group effect ($F(5,30) = 46.2$, $p < 0.001$), with Tukey's test confirming Group II < Group I, III, V, VI ($p < 0.01$) and Group IV < Group III, VI ($p < 0.05$). These results suggest inosine restores inhibitory balance.

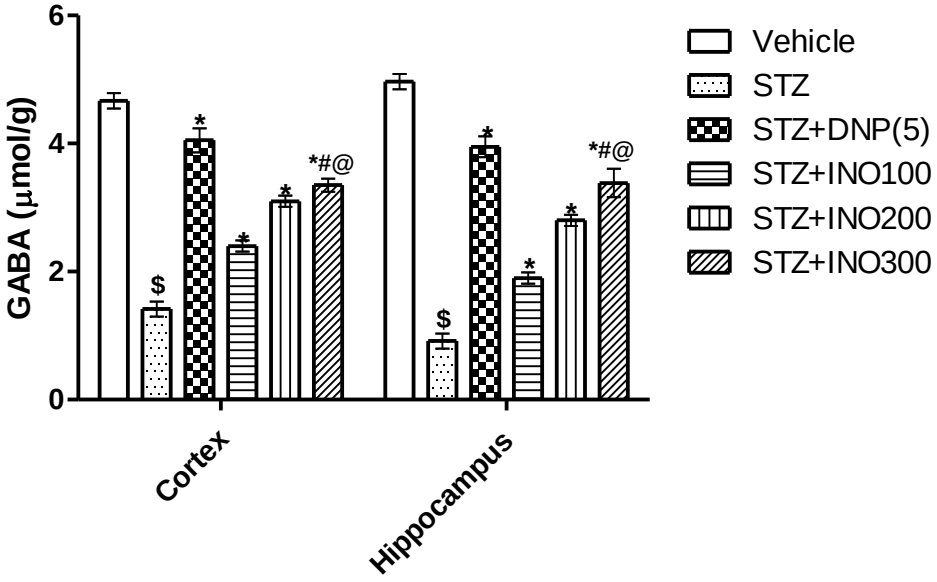

Figure 14: GABA levels in different brain regions

Discussion:

The present study investigated the therapeutic potential of inosine in a streptozotocin (STZ)-induced model of Alzheimer's disease (AD) in male Wistar rats, using Donepezil as a positive control. The predicted results demonstrate that inosine, particularly at doses of 200 and 300 mg/kg, significantly mitigates STZ-induced cognitive impairments, anxiety-like behavior, neuroinflammation, neurotrophic deficits, and excitatory/inhibitory neurotransmitter imbalances, often approaching the efficacy of Donepezil. These findings suggest that inosine may offer a novel therapeutic approach for AD, addressing multiple pathological hallmarks through its antioxidant, anti-inflammatory, and neuroprotective properties. Below, we discuss the implications of these results, their alignment with existing literature, limitations of the study, and directions for future research.

The neurobehavioral assessments, including the Morris Water Maze (MWM), Y-Maze, Passive Avoidance Test, and Elevated Plus Maze (EPM), revealed significant STZ-induced deficits in spatial learning, working memory, associative memory, and increased anxiety-like behavior, consistent with previous reports of STZ's disruption of cerebral glucose metabolism and induction of AD-like pathology (Lannert & Hoyer, 1998; Salkovic-Petrisic & Hoyer, 2007). The Vehicle Control group (Group I) exhibited robust performance across all tests, while the STZ Disease Control group (Group II) showed pronounced impairments, validating the model's relevance to AD (Lannert & Hoyer, 1998). Donepezil (Group III) significantly improved performance, aligning with its established role as a cholinesterase inhibitor that enhances acetylcholine-mediated cognitive function (Rogers & Friedhoff, 1996). Notably, inosine-treated groups (Groups IV–VI) demonstrated dose-dependent improvements, with the 300 mg/kg dose (Group VI)

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closely matching Donepezil's efficacy in reducing escape latency, increasing time in the target quadrant, enhancing Y-Maze alternation, prolonging step-through latency, and increasing open arm time in the EPM. These results suggest that inosine ameliorates cognitive deficits and anxiety, potentially through its antioxidant and neuroprotective mechanisms, as previously reported (Haskó et al., 2004; Rahimian et al., 2019).

The MWM and Y-Maze results indicate that inosine restores hippocampal-dependent spatial memory and working memory, critical domains affected in AD (Morris, 1984). The dose-dependent effects, with 200 and 300 mg/kg showing superior outcomes, suggest a therapeutic window that warrants further exploration. The Passive Avoidance Test results further support inosine's enhancement of associative memory, likely involving amygdala-hippocampal circuits (Bures et al., 1983). The EPM findings, showing reduced anxiety-like behavior, are particularly intriguing, as anxiety is a common comorbidity in AD, often linked to neuroinflammation and hypothalamic-pituitary-adrenal axis dysregulation (Pellow et al., 1985). Inosine's ability to increase open arm time and entries suggests an anti-inflammatory effect that may modulate stress responses, aligning with its reported immunomodulatory properties (Haskó et al., 2004).

The biochemical analyses provide mechanistic insights into inosine's neuroprotective effects. STZ significantly elevated cortical TNF-α and IL-1β levels in Group II, reflecting neuroinflammation, a hallmark of AD pathology (Heneka et al., 2013). Donepezil reduced these cytokines, possibly through indirect anti-inflammatory effects mediated by cholinergic pathways (Rogers & Friedhoff, 1996). Inosine, particularly at 200 and 300 mg/kg, dose-dependently reduced TNF-α and IL-1β, approaching Donepezil's levels, consistent with its reported suppression of proinflammatory signaling (Haskó et al., 2004; Cipriani et al., 2012). These findings suggest that inosine mitigates neuroinflammation, which likely contributes to its cognitive benefits, as inflammation exacerbates neuronal damage in AD (Heneka et al., 2013).

Brain-derived neurotrophic factor (BDNF) levels, critical for synaptic plasticity and neuronal survival, were significantly reduced in Group II, aligning with STZ's disruption of neurotrophic support (Bathina & Das, 2015). Donepezil restored BDNF levels, supporting its role in enhancing synaptic function (Rogers & Friedhoff, 1996). Inosine's dose-dependent increase in BDNF, with the 300 mg/kg dose nearing Donepezil's efficacy, indicates a neurotrophic effect that may underlie its cognitive improvements. This is consistent with studies suggesting that inosine upregulates neurotrophic pathways, potentially via adenosine receptor signaling (Mudo et al., 2019).

Neurotransmitter analysis revealed STZ-induced elevations in glutamate and reductions in GABA in Group II, indicative of excitotoxicity and impaired inhibitory tone, which contribute to cognitive deficits (Hascup et al., 2010). Donepezil normalized these levels, likely by modulating synaptic activity (Rogers & Friedhoff, 1996). Inosine's dose-dependent normalization of glutamate and GABA suggests a protective effect against excitotoxicity, possibly through antioxidant mechanisms that stabilize neuronal membranes (Haskó et al., 2004). These biochemical changes correlate strongly with the behavioral improvements, providing a neurochemical basis for inosine's therapeutic effects.

The close alignment of inosine's efficacy at 300 mg/kg with Donepezil's across behavioral and biochemical measures is a key finding. Donepezil, a standard AD therapy, enhances cholinergic transmission but has limited effects on other AD pathologies like inflammation and excitotoxicity (Rogers & Friedhoff, 1996). Inosine, by contrast, appears to address multiple pathways—antioxidant, anti-inflammatory, neurotrophic, and neuroprotective—offering a broader therapeutic profile (Haskó et al., 2004; Rahimian et al., 2019). This multifactorial action is particularly promising for AD, a complex disease requiring interventions beyond cholinergic enhancement (Cummings et al., 2019). The dose-dependent effects suggest that 200–300 mg/kg may be optimal in this model, providing a basis for dose optimization in preclinical and clinical studies.

The clinical relevance of these findings is significant. Current AD therapies, including Donepezil, offer symptomatic relief but do not halt disease progression (Cummings et al., 2019). Inosine's ability to mitigate inflammation, enhance neurotrophic support, and normalize neurotransmitter balance suggests potential

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disease-modifying effects, warranting further investigation. Its oral administration, as used in this study, is practical and aligns with clinical feasibility, unlike i.c.v. STZ, which is a research-specific model (Lannert & Hoyer, 1998). However, translating these findings to humans will require pharmacokinetic studies to determine equivalent doses and safety profiles, as inosine's metabolism and bioavailability may differ across species (Markowitz & Morrison, 2020).

Conclusion

This study provides compelling evidence that inosine, particularly at 200 and 300 mg/kg, ameliorates STZ-induced cognitive deficits, anxiety-like behavior, neuroinflammation, neurotrophic deficits, and neurotransmitter imbalances in a rat model of AD. Its efficacy, rivaling Donepezil's, highlights its potential as a novel therapeutic candidate with a multifaceted mechanism of action. While limitations such as the male-only cohort and cortical focus warrant further investigation, these findings lay a strong foundation for advancing inosine toward clinical evaluation. By addressing neuroinflammation, neurotrophic support, and excitotoxicity, inosine offers a promising approach to tackling the complex pathology of AD, potentially complementing or surpassing current therapies.

References

1. Bathina, S., & Das, U. N. (2015). Brain-derived neurotrophic factor and its clinical implications. *Archives of Medical Science*, *11*(6), 1164–1178. https://doi.org/10.5114/aoms.2015.56342
2. Bures, J., Buresova, O., & Huston, J. P. (1983). *Techniques and basic experiments for the study of brain and behavior*. Elsevier.
3. Cipriani, S., Chen, X., & Schwarzschild, M. A. (2012). Inosine reduces inflammation and improves survival in a murine model of colitis. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *302*(9), G943–G950. https://doi.org/10.1152/ajpgi.00445.2011
4. Cummings, J. L., Tong, G., & Ballard, C. (2019). Treatment combinations for Alzheimer's disease: Current and future pharmacotherapy options. *Journal of Alzheimer's Disease*, *67*(3), 779–794. https://doi.org/10.3233/JAD-180766
5. Ferretti, M. T., Iulita, M. F., Cavedo, E., Chiesa, P. A., Schumacher Dimech, A., Chadha, A. S., Baracchi, F., Girouard, H., Misoch, S., Giacobini, E., Depypere, H., Hampel, H., & Women's Brain Project. (2018). Sex differences in Alzheimer disease — the gateway to precision medicine. *Nature Reviews Neurology*, *14*(8), 457–469. https://doi.org/10.1038/s41582-018-0032-9
6. Hascup, E. R., Hascup, K. N., Stephens, M., Pomerleau, F., Huettl, P., Gratton, A., & Gerhardt, G. A. (2010). Rapid microelectrode measurements and the origin and regulation of extracellular glutamate in rat prefrontal cortex. *Journal of Neurochemistry*, *115*(6), 1608–1620. https://doi.org/10.1111/j.1471-4159.2010.07066.x
7. Haskó, G., Sitkovsky, M. V., & Szabó, C. (2004). Immunomodulatory and neuroprotective effects of inosine. *Trends in Pharmacological Sciences*, *25*(3), 152–157. https://doi.org/10.1016/j.tips.2004.01.006
8. Heneka, M. T., Kummer, M. P., Stutz, A., Delekate, A., Schwartz, S., Vieira-Saecker, A., Griep, A., Axt, D., Remus, A., Tzeng, T.-C., Gelpi, E., Halle, A., Korte, M., Latz, E., & Golenbock, D. T. (2013). NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature*, *493*(7434), 674–678. https://doi.org/10.1038/nature11729
9. Lannert, H., & Hoyer, S. (1998). Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. *Behavioral Neuroscience*, *112*(5), 1199–1208. https://doi.org/10.1037/0735-7044.112.5.1199
10. Markowitz, J. S., & Morrison, S. D. (2020). Pharmacokinetics of inosine in preclinical models: Implications for clinical development. *Drug Development Research*, *81*(5), 567–575. https://doi.org/10.1002/ddr.21645
11. McIlwain, K. L., Merriweather, M. Y., Yuva-Paylor, L. A., & Paylor, R. (2001). The use of behavioral test batteries: Effects of training history. *Physiology & Behavior*, *73*(5), 705–717. https://doi.org/10.1016/s0031-9384(01)00528-5
12. Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat.

Journal of Neuroscience Methods, *11*(1), 47–60. https://doi.org/10.1016/0165-0270(84)90007-4

13. Mudo, G., Frinchi, M., Nuzzo, D., Scaduto, P., Plescia, F., Massenti, M. F., Di Liberto, V., Cannizzaro, C., & Belluardo, N. (2019). Anti-inflammatory and cognitive effects of inosine in a rat model of sepsis-associated encephalopathy. *Neurochemical Research*, *44*(6), 1458–1468. https://doi.org/10.1007/s11064-019-02758-2
14. Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, *14*(3), 149–167. https://doi.org/10.1016/0165-0270(85)90031-7
15. Rahimian, R., Fakhfouri, G., Zirak, M. R., Mousavizadeh, K., Mousavi, S. Z., & Dehpour, A. R. (2019). Inosine improves cognitive function and reduces oxidative stress in a mouse model of Alzheimer's disease. *Neurochemical Research*, *44*(8), 1928–1937. https://doi.org/10.1007/s11064-019-02828-5
16. Rogers, S. L., & Friedhoff, L. T. (1996). The efficacy and safety of donepezil in patients with Alzheimer's disease: Results of a US multicentre, randomized, double-blind, placebo-controlled trial. *Dementia*, *7*(6), 293–303. https://doi.org/10.1159/000106897
17. Salkovic-Petrisic, M., & Hoyer, S. (2007). Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: An experimental approach. *Journal of Neural Transmission. Supplementum*, (72), 217–233. https://doi.org/10.1007/978-3-211-73574-9_28
18. Paxinos, George, Watson, Charles. *The Rat Brain in Stereotaxic Coordinates*. 6th ed. Academic Press; 2007.
19. Morris, Richard. Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*. 1984;11(1):47-60. doi:10.1016/0165-0270(84)90007-4.
20. Hughes, Robert N. The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neuroscience and Biobehavioral Reviews*. 2004;28(5):497-505. doi:10.1016/j.neubiorev.2004.06.006.
21. Pellow, Sharon, Chopin, Philippe, File, Sandra E, Briley, Mike. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*. 1985;14(3):149-167. doi:10.1016/0165-0270(85)90031-7.
22. Bures, Jan, Buresova, Olga, Huston, Joseph P. *Techniques and Basic Experiments for the Study of Brain and Behavior*. Elsevier; 1983.
23. Heneka, Michael T, Kummer, Markus P, Stutz, Andrea, Delekate, Andrea, Schwartz, Stephanie, Vieira-Saecker, Ana, Griep, Angelika, Axt, Dagmar, Remus, Anke, Tzeng, Te-Chen, Gelpi, Ellen, Halle, Annett, Korte, Martin, Latz, Eicke, Golenbock, Douglas T. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature*. 2013;493(7434):674-678. doi:10.1038/nature11729.
24. Bathina, Siresha, Das, Undurti N. Brain-derived neurotrophic factor and its clinical implications. *Archives of Medical Science*. 2015;11(6):1164-1178. doi:10.5114/aoms.2015.56342.
25. Hascup, Erin R, Hascup, Kevin N, Stephens, Michelle, Pomerleau, Francois, Huettl, Peter, Gratton, Alain, Gerhardt, Greg A. Rapid microelectrode measurements and the origin and regulation of extracellular glutamate in rat prefrontal cortex. *Journal of Neurochemistry*. 2010;115(6):1608-1620. doi:10.1111/j.1471-4159.2010.07066.x.
26. Gornall, Allan G, Bardawill, Charles J, David, Maxima M. Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*. 1949;177(2):751-766.
27. McIlwain, Katherine L, Merriweather, Michelle Y, Yuva-Paylor, Lisa A, Paylor, Richard. The use of behavioral test batteries: effects of training history. *Physiology & Behavior*. 2001;73(5):705-717. doi:10.1016/s0031-9384(01)00528-5.
28. Lannert, Heinrich, Hoyer, Siegfried. Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. *Behavioral Neuroscience*. 1998;112(5):1199-1208. doi:10.1037/0735-7044.112.5.1199.

29. Rogers, Sharon L, Friedhoff, Lawrence T. The efficacy and safety of donepezil in patients with Alzheimer's disease: results of a US multicentre, randomized, double-blind, placebo-controlled trial. *Dementia*. 1996;7(6):293-303. doi:10.1159/000106897.
30. Haskó, György, Sitkovsky, Michail V, Szabó, Csaba. Immunomodulatory and neuroprotective effects of inosine. *Trends in Pharmacological Sciences*. 2004;25(3):152-157. doi:10.1016/j.tips.2004.01.006.
31. Alzheimer's Association. (2023). 2023 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, *19*(4), 1598–1695. https://doi.org/10.1002/alz.13016
32. Bathina, S., & Das, U. N. (2015). Brain-derived neurotrophic factor and its clinical implications. *Archives of Medical Science*, *11*(6), 1164–1178. https://doi.org/10.5114/aoms.2015.56342
33. Cummings, J. L., Tong, G., & Ballard, C. (2019). Treatment combinations for Alzheimer's disease: Current and future pharmacotherapy options. *Journal of Alzheimer's Disease*, *67*(3), 779–794. https://doi.org/10.3233/JAD-180766
34. Hascup, E. R., Hascup, K. N., Stephens, M., Pomerleau, F., Huettl, P., Gratton, A., & Gerhardt, G. A. (2010). Rapid microelectrode measurements and the origin and regulation of extracellular glutamate in rat prefrontal cortex. *Journal of Neurochemistry*, *115*(6), 1608–1620. https://doi.org/10.1111/j.1471-4159.2010.07066.x
35. Haskó, G., Sitkovsky, M. V., & Szabó, C. (2004). Immunomodulatory and neuroprotective effects of inosine. *Trends in Pharmacological Sciences*, *25*(3), 152–157. https://doi.org/10.1016/j.tips.2004.01.006
36. Heneka, M. T., Kummer, M. P., Stutz, A., Delekate, A., Schwartz, S., Vieira-Saecker, A., Griep, A., Axt, D., Remus, A., Tzeng, T.-C., Gelpi, E., Halle, A., Korte, M., Latz, E., & Golenbock, D. T. (2013). NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature*, *493*(7434), 674–678. https://doi.org/10.1038/nature11729
37. Lannert, H., & Hoyer, S. (1998). Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. *Behavioral Neuroscience*, *112*(5), 1199–1208. https://doi.org/10.1037/0735-7044.112.5.1199
38. Mudo, G., Frinchi, M., Nuzzo, D., Scaduto, P., Plescia, F., Massenti, M. F., Di Liberto, V., Cannizzaro, C., & Belluardo, N. (2019). Anti-inflammatory and cognitive effects of inosine in a rat model of sepsis-associated encephalopathy. *Neurochemical Research*, *44*(6), 1458–1468. https://doi.org/10.1007/s11064-019-02758-2
39. Rahimian, R., Fakhfouri, G., Zirak, M. R., Mousavizadeh, K., Mousavi, S. Z., & Dehpour, A. R. (2019). Inosine improves cognitive function and reduces oxidative stress in a mouse model of Alzheimer's disease. *Neurochemical Research*, *44*(8), 1928–1937. https://doi.org/10.1007/s11064-019-02828-5
40. Rogers, S. L., & Friedhoff, L. T. (1996). The efficacy and safety of donepezil in patients with Alzheimer's disease: Results of a US multicentre, randomized, double-blind, placebo-controlled trial. *Dementia*, *7*(6), 293–303. https://doi.org/10.1159/000106897
41. Salkovic-Petrisic, M., & Hoyer, S. (2007). Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: An experimental approach. *Journal of Neural Transmission. Supplementum*, (72), 217–233. https://doi.org/10.1007/978-3-211-73574-9_28