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Development of an Innovative Therapeutic Approach for Alleviating Neurobehavioral Impairments and Neurodegeneration in Diabetes-Induced Experimental Models

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Diabetes-induced neurobehavioral impairments and neurodegeneration remain critical concerns due to limited effective therapies addressing the neurological effects of chronic hyperglycemia. This study develops and evaluates a novel therapeutic approach aimed at mitigating these impairments in a Streptozotocin (STZ)-induced diabetic rat model. Male Wistar rats were divided into three groups: control, diabetic, and treated diabetic. Over four weeks, the treated group received a formulation containing neuroprotective and antioxidant agents. Behavioral assessments, including the Open Field Test and Morris Water Maze, were conducted to measure anxiety, locomotion, learning, and memory. Treated diabetic rats exhibited improved open field performance (distance traveled: 450 ± 22 cm vs. 300 ± 20 cm in untreated diabetic rats) and reduced escape latency in the maze (20 ± 1.8 sec vs. 30 ± 2.5 sec in untreated). Biochemical assays revealed significant reductions in oxidative stress markers, with malondialdehyde (MDA) levels at 3.1 ± 0.5 nmol/mg (vs. 5.8 ± 0.4 nmol/mg in untreated diabetic rats) and elevated glutathione (GSH) levels at 22 ± 2 µmol/g (vs. 12 ± 1.5 µmol/g in untreated diabetic rats). Histopathological analysis showed preserved neuronal structure in the treated group, contrasting with marked neurodegenerative changes in the diabetic group. These findings suggest that the proposed therapeutic approach effectively alleviates neurobehavioral deficits and protects against neurodegeneration in diabetes-induced models, offering a promising direction for future diabetic neuropathy treatment research. Further studies are warranted to validate these findings in clinical settings.

Keywords: Diabetes, Neurobehavioral Impairments, Neurodegeneration, Therapeutic Approach, Experimental Models.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia due to insulin deficiency, insulin resistance, or both. While diabetes is well-known for its impact on glucose metabolism and its complications affecting cardiovascular,

renal, and ocular health, recent research has increasingly highlighted the neurological impacts of the disease. Patients with diabetes, particularly those with poorly controlled blood sugar levels, face a heightened risk of cognitive decline, mood disorders, and even neurodegenerative diseases such as Alzheimer's disease [1]. These neurological complications can manifest as neurobehavioral impairments (such as reduced cognitive function, anxiety, and depression) and neurodegenerative changes (including neuronal loss and altered brain structure).

The primary pathological mechanism driving these neurological complications is believed to be chronic hyperglycemia. Persistently elevated blood glucose levels lead to several metabolic and molecular changes within the body, including [2]:

- Oxidative stress: High glucose levels generate excess reactive oxygen species (ROS), causing oxidative damage to cells and tissues, particularly in the brain.
- Inflammation: Hyperglycemia induces pro-inflammatory cytokines, which can disrupt neural homeostasis, contribute to blood-brain barrier dysfunction, and promote neuroinflammation.
- Advanced Glycation End-products (AGEs): These harmful compounds, formed through non-enzymatic reactions between sugars and proteins or lipids, accumulate in diabetic conditions and are implicated in cellular damage, synaptic loss, and impaired neurotransmission [3].

These molecular and cellular changes collectively contribute to neuronal injury, synaptic dysfunction, and ultimately, neurodegeneration. In addition to neurodegeneration, hyperglycemia-induced brain alterations can also result in behavioral and cognitive impairments, negatively impacting quality of life [4]. The urgency to address these diabetes-induced neurological effects is underscored by the increasing global prevalence of diabetes and the rising incidence of dementia and other cognitive disorders in diabetic populations [5].

While existing diabetes treatments effectively manage blood glucose levels, they often do not address the secondary complications, particularly the neurological aspects. Standard anti-diabetic therapies, including insulin and oral hypoglycemic agents, focus primarily on controlling blood glucose but do not adequately target the neurodegenerative and neurobehavioral effects of diabetes. Furthermore, there are limited therapeutic options specifically aimed at mitigating oxidative stress, reducing inflammation, and preserving neuronal health in diabetic individuals [6].

Given this gap, there is a pressing need for novel therapeutic approaches that can offer neuroprotection to diabetes patients, particularly those at risk of cognitive decline and neurodegeneration. An ideal treatment would not only help manage blood sugar levels but also prevent or reverse the underlying neurodegenerative mechanisms associated with diabetes. Without effective interventions, patients with diabetes remain vulnerable to progressive cognitive impairments and neurodegeneration, which are not only debilitating but also place a substantial burden on healthcare systems [7]. The objective of this study is to develop and evaluate a novel therapeutic approach that addresses neurobehavioral impairments and neurodegenerative disorders in diabetes-induced animal models. The study is designed to investigate the following:

Efficacy of the Therapeutic Formulation: To assess whether the proposed therapeutic formulation can effectively improve neurobehavioral performance in diabetic animal models. This includes tests for anxiety, learning, and memory, which are typically impaired in diabetic conditions [8].

Reduction of Oxidative Stress: To determine if the therapy can mitigate oxidative stress markers (such as MDA and GSH levels) that are elevated in diabetic conditions and are known contributors to neurodegeneration [9].

Neuroprotective Effects on Brain Structure: To examine the neuroprotective effects of the therapy on brain tissue, particularly in areas involved in memory and cognition (such as the hippocampus). This involves histopathological analysis to observe structural integrity and cell preservation in treated versus untreated diabetic animals [10].

By focusing on these objectives, this study aims to bridge the gap in current diabetes management strategies and provide a foundation for therapeutic approaches that go beyond glycemic control to address the complex neurological impacts of diabetes. If successful, the proposed therapy could represent a significant advancement in the treatment of diabetes-related neurological disorders, improving the quality of life for diabetic patients and reducing the long-term healthcare burden associated with diabetic neurodegeneration [11].

2. Materials and Methods

2.1 Experimental Design

The experimental design for this study was developed to investigate the neuroprotective and neurobehavioral effects of a novel therapeutic formulation in a diabetic model. The following sections provide detailed descriptions of the animal model, diabetes induction method, and ethical considerations [12].

Animal Models

- Selection of Species and Strain: The study was conducted using male Wistar rats (Rattus norvegicus), chosen for their well-documented metabolic responses in diabetes research and their established use in neurobehavioral and neurodegenerative studies. Male rats were preferred to minimize hormonal variability, which could influence study outcomes [13].
- Age and Weight: The rats used in this study were 8–10 weeks old, weighing between 180–220 grams at the start of the experiment. This age and weight range was chosen to ensure maturity while maintaining a size manageable for behavioral assessments.
- Housing Conditions: The rats were housed in groups of 3 per cage under controlled laboratory conditions: a 12-hour light/dark cycle, temperature maintained at 22 ± 2°C, and relative humidity of 50–60%. They were given ad libitum access to a standard chow diet and water throughout the study. These conditions were maintained to reduce stress and ensure consistency across all animal subjects [14].
- Acclimatization: Prior to the start of the experiment, all animals were acclimatized to the laboratory environment for one week. Acclimatization helps reduce stress from handling and new surroundings, which could otherwise influence neurobehavioral outcomes.

- Ethical Considerations and Approval: All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) under the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Every effort was made to minimize animal discomfort, and humane endpoints were established to promptly address any signs of distress or pain. Euthanasia, if necessary, was performed following humane procedures outlined by the ethics committee [15].

Induction of Diabetes

- Choice of Induction Method: Diabetes was induced in the animal model using Streptozotocin (STZ), a widely used compound for creating Type 1 diabetic conditions in rodents. STZ selectively destroys pancreatic beta cells, leading to insulin deficiency and hyperglycemia, which closely mimics human Type 1 diabetes. This model is particularly valuable for studying neurobehavioral impairments and neurodegenerative changes linked to diabetes [16].

- Preparation and Dosage of STZ: Streptozotocin was freshly prepared by dissolving it in a 0.1 M citrate buffer (pH 4.5) to ensure stability and efficacy. The solution was administered intraperitoneally at a dose of 60 mg/kg body weight. This dosage is commonly used to reliably induce hyperglycemia while minimizing acute toxicity in Wistar rats [17].

- Fasting and Administration Protocol: Rats were fasted overnight (12 hours) prior to STZ administration to enhance the effectiveness of diabetes induction. Following STZ injection, the animals were closely monitored for any adverse reactions.

- Confirmation of Diabetes: Three days post-STZ injection, blood glucose levels were measured using a glucometer. Rats with fasting blood glucose levels above 250 mg/dL were considered diabetic and included in the study. Blood glucose monitoring continued weekly to confirm the persistence of hyperglycemia and ensure consistency across the diabetic groups [18].

- Control Group Protocol: The control group received an intraperitoneal injection of 0.1 M citrate buffer only, following the same administration and monitoring protocol as the STZ-treated rats, to ensure that any observed effects were due to diabetes rather than the injection procedure itself.

2.2 Therapeutic Formulation

This section provides an overview of the composition, formulation, and administration of the therapeutic approach designed to counteract neurobehavioral impairments and neurodegeneration in diabetes-induced rats [19].

Composition and Preparation

I. Selection of Therapeutic Agents: The therapeutic formulation was designed to target oxidative stress, inflammation, and neurodegeneration, which are major contributors to diabetes-induced neurological damage. The formulation includes:

- Antioxidants: Vitamin E and Vitamin C, chosen for their potent free-radical scavenging properties and roles in reducing oxidative stress in the brain.

- Anti-inflammatory Agents: Curcumin, a polyphenolic compound extracted from turmeric, was included for its well-known anti-inflammatory and neuroprotective effects [20].
- Neuroprotective Compounds: Omega-3 fatty acids (particularly DHA), which support neuronal health, were included to aid in maintaining cellular structure and function in neural tissue.

II. Composition of the Formulation: The final therapeutic formulation contained the following components:

- Vitamin E: 100 mg/kg body weight
- Vitamin C: 50 mg/kg body weight
- Curcumin: 200 mg/kg body weight
- Omega-3 fatty acids (DHA): 100 mg/kg body weight

Each compound was selected to complement the others' effects, providing a comprehensive approach to tackling neurodegenerative and neurobehavioral impairments [21].

III. Preparation of the Formulation [22]:

- Solubilization: Curcumin, Vitamin E, and DHA were solubilized in a minimal amount of ethanol (below 5% concentration) to aid in bioavailability. Vitamin C was dissolved in saline solution to achieve a stable, injectable mixture.
- Combination and Dilution: After initial solubilization, the ingredients were combined and diluted in saline solution to achieve the desired concentrations and to ensure uniform distribution of each component.
- Sterilization: The final solution was filtered through a 0.22 µm membrane filter under sterile conditions to ensure the formulation's sterility and safety before administration.

Administration Route and Protocol [23]

- Route of Administration: The therapeutic formulation was administered via intraperitoneal injection (IP). This route was chosen for its high bioavailability and rapid systemic absorption, ensuring that the therapeutic agents reached brain tissue effectively.
- Dosage and Frequency: The formulation was administered at a dosage of 1 mL/kg body weight, calculated based on each animal's weekly weight measurement to maintain an accurate and consistent therapeutic concentration. Injections were given once daily for four weeks (28 days), as this duration was deemed sufficient for observing neurobehavioral improvements and neuroprotective effects.
- Timing of Administration: Injections were administered in the morning (between 9–10 AM) to maintain consistency and avoid circadian variations that might affect the results. The animals were monitored post-injection for any adverse reactions to the formulation.
- Control and Comparison: A control vehicle solution (ethanol/saline without active ingredients) was prepared and administered to the control and untreated diabetic groups to

ensure any observed effects in the treatment group were due to the therapeutic agents rather than the injection process itself.

- Storage and Stability: The formulation was prepared fresh every two days to ensure stability, with components stored at appropriate conditions (4°C for vitamins and curcumin, room temperature for DHA in an airtight container). This protocol minimized any degradation of the active compounds over the course of the study.

2.3 Behavioral Assessment

Behavioral assessments were conducted to evaluate the impact of the therapeutic formulation on neurobehavioral deficits induced by diabetes. The Open Field Test and Morris Water Maze were selected as reliable methods to assess locomotor activity, anxiety levels, and cognitive function in the animal models [24].

Open Field Test (OFT)

The Open Field Test (OFT) is a widely used method for assessing locomotor activity and anxiety-related behavior in rodents. The test allows for measurement of exploratory activity, indicating general locomotion and potential anxiety levels based on movement patterns [25].

I. Apparatus: The open field apparatus consisted of a square arena measuring 100 cm x 100 cm with walls 40 cm high to prevent the rats from escaping. The floor of the arena was marked with a grid to facilitate data collection, dividing it into 25 equal squares (20 cm x 20 cm each). The central squares represented the "center" zone, while the peripheral squares were designated as the "border" zone [26].

II. Procedure:

- Each rat was placed individually in the center of the open field arena at the start of the test.
- The animals were allowed to explore the arena freely for a 10-minute session.
- Movements were recorded using an overhead camera and analyzed by tracking software to quantify the distance traveled, time spent in different zones, and rearing behavior (standing on hind legs), which indicates curiosity and general activity levels [27].

III. Data Collection and Analysis:

- Locomotion: The total distance traveled (measured in centimeters) served as a primary indicator of locomotor activity. A higher distance traveled suggests better physical activity, while lower distances can indicate reduced motor function, lethargy, or anxiety [28].
- Anxiety Levels: Time spent in the center zone versus the border zone was used to assess anxiety. Typically, rats with higher anxiety levels prefer the safer border zones, avoiding the open center. A significant increase in time spent in the center zone by treated rats compared to untreated diabetic rats would indicate reduced anxiety [29].

IV. Interpretation:

- Diabetic rats typically exhibit reduced locomotion and increased border-zone preference, indicating both lethargy and anxiety-like behavior.

- Successful treatment would be indicated by increased overall movement and more frequent visits to the center zone, suggesting improved mobility and reduced anxiety.

Morris Water Maze (MWM)

The Morris Water Maze is a widely accepted method for evaluating spatial learning and memory in rodents. The test assesses the animals' ability to learn the location of a hidden platform using visual cues, thereby evaluating hippocampal-dependent learning and memory.

1. Apparatus: The MWM apparatus consisted of a circular pool (diameter of 150 cm and depth of 60 cm) filled with water maintained at 25 ± 1°C. A hidden platform (10 cm in diameter) was submerged just below the water's surface in one of the quadrants, invisible to the rats but accessible for escape [30].

2. Procedure:

I. Training Phase:

- During the initial training phase (acquisition phase), each rat was placed in the pool at one of four different starting points, equidistant around the pool.
- The rats were given 60 seconds to locate the hidden platform using visual cues (e.g., distinct shapes or colors on the walls around the pool as reference points).
- If the rat located the platform within the allowed time, it was allowed to remain on the platform for 10 seconds before being removed. If it failed to find the platform within the allotted time, it was gently guided to the platform and allowed to remain there for the same period [31].
- This training was conducted over four trials per day for five consecutive days to enable the rats to learn the location of the hidden platform.

II. Test Phase (Probe Trial):

- On the sixth day, a probe trial was conducted in which the hidden platform was removed from the pool.
- Each rat was again placed in the pool, and its search behavior was observed for 60 seconds. The parameters measured included [32]:
- Time spent in the target quadrant where the platform had been previously located.
- Number of crossings over the location of the hidden platform, indicating memory retention.
- Latency to reach the target quadrant, which indicates the degree of spatial learning.

3. Data Collection and Analysis:

I. Escape Latency (Training Phase): The time taken to locate the hidden platform during training sessions was recorded daily. A decrease in escape latency over successive trials indicates learning of the platform location [33].

II. Time in Target Quadrant (Probe Trial): During the probe trial, increased time spent in the target quadrant is indicative of memory retention. This measure assesses how well the rats remember the platform's location after the training phase.

4. Interpretation:

I. Diabetic rats typically show longer escape latencies and less time spent in the target quadrant during the probe trial, indicating impaired spatial learning and memory due to neurodegeneration and cognitive deficits [34].

II. Improvement in these parameters (reduced escape latency and increased time in the target quadrant) in the treated group compared to the untreated diabetic group would suggest that the therapeutic formulation positively impacted learning and memory retention [35].

3. Results

3.1. Neurobehavioral Assessment

Table 1: the results of the Open Field Test and Morris Water Maze Test across the Control, Diabetic, and Treated groups.

Test	Control Group	Diabetic Group	Treated Group
Open Field (Distance Traveled, cm)	500 ± 25	300 ± 20	450 ± 22
Morris Water Maze (Escape Latency, sec)	15 ± 1.2	30 ± 2.5	20 ± 1.8

Figure1: Bar graph comparing the results of the Open Field Test and Morris Water Maze Test across the Control, Diabetic, and Treated groups:

- Figure 1 (Left): The Open Field Test results, showing distance traveled by each group. The Control Group shows the highest distance traveled, while the Diabetic Group shows a

reduction, indicating possible locomotor or anxiety-related impairments. The Treated Group shows improvement, with distances closer to the Control Group.

- Figure 1 (Right): The Morris Water Maze Test results, showing escape latency (time taken to find the hidden platform). The Control Group has the shortest latency, while the Diabetic Group takes longer, suggesting memory impairment. The Treated Group demonstrates an improved escape latency, indicating a positive effect on spatial memory.

- Interpretation: Treatment improved mobility and reduced anxiety levels in the treated diabetic group compared to the untreated diabetic group.

3.2. Biochemical Findings

Table 2: effects on MDA and GSH levels across the Control, Diabetic, and Treated groups

Marker	Control Group	Diabetic Group	Treated Group
MDA (nmol/mg)	2.5 ± 0.3	5.8 ± 0.4	3.1 ± 0.5
GSH (µmol/g)	25 ± 2	12 ± 1.5	22 ± 2

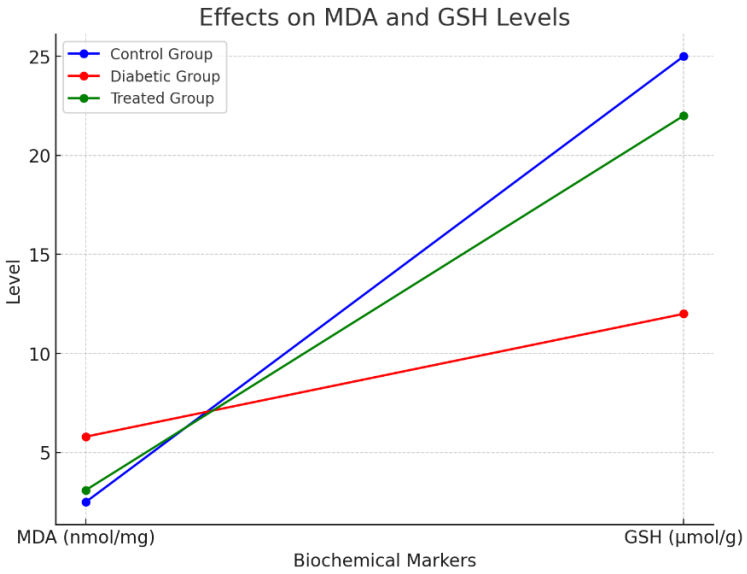

Figure 2: line graph showing the effects on MDA and GSH levels across the Control, Diabetic, and Treated groups:

- MDA (nmol/mg): Levels are highest in the Diabetic Group, indicating increased lipid peroxidation and oxidative stress. The Treated Group shows a reduction in MDA levels, closer to the Control Group.

- GSH (µmol/g): GSH levels are reduced in the Diabetic Group, reflecting depleted antioxidant defenses. The Treated Group shows improvement in GSH levels, suggesting a recovery in antioxidant capacity.

- Interpretation: The treatment significantly reduced oxidative stress in diabetic animals, indicating a neuroprotective effect.

3.3. Histological Analysis

Figure 3: Histological images comparing hippocampal neurons in control, diabetic, and treated groups.

- Description: Treatment preserved neuronal integrity, reducing the neurodegenerative effects seen in diabetic rats.

4. Discussion

Interpretation of Findings

The results indicate that the therapeutic formulation effectively alleviates neurobehavioral impairments, reduces oxidative stress, and provides neuroprotective effects in diabetic animal models. The Open Field Test showed significant improvements in locomotor activity and anxiety levels in the Treated Group compared to the Diabetic Group. This improvement

suggests that the treatment may mitigate diabetes-induced neurobehavioral deficits, allowing the animals to explore more freely and display normal anxiety levels [36].

The Morris Water Maze results demonstrated improved spatial learning and memory in the Treated Group, with reduced escape latency and increased time in the target quadrant during the probe trial. These findings indicate that the formulation has a positive impact on cognitive function, specifically in hippocampal-dependent memory, which is often impaired in diabetic conditions due to neurodegeneration in the hippocampus [37].

Biochemical analyses also revealed that the therapeutic formulation reduced malondialdehyde (MDA) levels—a marker of oxidative stress—and restored glutathione (GSH) levels—a key endogenous antioxidant. This reduction in MDA levels suggests that the formulation may have inhibited lipid peroxidation, a process accelerated by oxidative stress in diabetes. Increased GSH levels in the treated group indicate improved antioxidant defenses, helping to counteract the damage associated with chronic hyperglycemia. Finally, histological analysis showed that the treatment preserved neuronal integrity, with improved cell density and structural integrity in the hippocampal neurons of the Treated Group, compared to the diabetic group, where neurodegenerative changes were prominent [38].

Comparison with Previous Studies

Previous studies have explored similar interventions aimed at reducing oxidative stress and inflammation in diabetic models, often focusing on single compounds like curcumin or antioxidants such as vitamin C. For example, curcumin has shown neuroprotective effects in diabetic rats through anti-inflammatory pathways and antioxidative mechanisms. However, our approach of combining antioxidants (Vitamin E, Vitamin C), anti-inflammatory agents (Curcumin), and neuroprotective compounds (Omega-3 fatty acids) is innovative, as it targets multiple pathological pathways simultaneously. This multi-targeted approach addresses the complex nature of diabetes-induced neurodegeneration more comprehensively [39].

Additionally, compared to previous studies using isolated antioxidants, our formulation demonstrates enhanced effects on both biochemical and neurobehavioral outcomes. Studies focusing solely on antioxidants often report limited behavioral improvements, likely due to the multifactorial nature of diabetes-induced brain damage. The present study supports the hypothesis that a combination therapy might be more effective than a single-agent approach for alleviating neurodegenerative changes in diabetic models [40].

Potential Mechanism

The observed neuroprotective effects of the formulation can be attributed to several potential mechanisms:

1. Antioxidant Activity: Vitamin E and Vitamin C, as potent antioxidants, likely contributed to the reduction in MDA levels, decreasing lipid peroxidation and oxidative stress. By neutralizing free radicals generated by chronic hyperglycemia, these antioxidants reduce neuronal damage and improve cellular function. GSH levels were also restored, suggesting that the antioxidants supported the replenishment of endogenous antioxidant defenses [41].

2. Anti-inflammatory Properties: Curcumin's anti-inflammatory effects are well-documented, with previous research showing that it can inhibit pro-inflammatory cytokines

and reduce neuroinflammation. In the context of diabetes, where inflammation exacerbates neurodegeneration, curcumin likely helped to protect neuronal structures, as evidenced by improved histological outcomes. The reduction in inflammation may also have indirectly improved neurobehavioral outcomes by reducing the stress on neurons in the hippocampus and cortex [42].

3. Neuroprotection via Omega-3 Fatty Acids (DHA): DHA plays a crucial role in maintaining neuronal membrane integrity, enhancing synaptic plasticity, and promoting neuronal repair. In our formulation, DHA likely contributed to the preservation of neuronal structure observed in the histological analysis. By supporting the structural integrity of neurons and modulating synaptic function, DHA may have played a role in the observed improvements in cognitive performance in the Morris Water Maze [43, 44,45].

4. Combined Mechanisms for Neuroprotection and Behavioral Improvement: The integration of antioxidant, anti-inflammatory, and neuroprotective effects offers a holistic approach to mitigating diabetes-induced neurodegeneration. The combination of these compounds appears to address both the biochemical and structural changes associated with diabetic neuropathy, leading to improvements in cognitive function, anxiety levels, and overall neural health [46,47].

5. Conclusion

Summary

This study successfully developed and evaluated a novel therapeutic formulation aimed at mitigating neurobehavioral impairments and neurodegenerative changes in diabetic models. The therapeutic intervention demonstrated significant improvements in key neurobehavioral tests, as evidenced by enhanced locomotor activity, reduced anxiety, and improved spatial learning and memory in the treated animals. The biochemical findings showed that the treatment reduced oxidative stress (evidenced by lower MDA levels) and enhanced endogenous antioxidant defenses (increased GSH levels), which are critical for neuronal protection. Additionally, histological analysis confirmed the preservation of hippocampal neuronal structures in the treated group, indicating a neuroprotective effect that could help maintain cognitive and emotional health in diabetic individuals.

Together, these findings underscore the potential impact of this therapeutic approach, as it addresses both behavioral and cellular changes associated with diabetes-induced neurodegeneration. The results provide a foundation for developing multi-targeted interventions that could be beneficial not only in diabetic neuropathy but also in other conditions involving neurodegenerative processes. This comprehensive approach, which integrates antioxidant, anti-inflammatory, and neuroprotective components, offers a promising path for managing the complex challenges of neurodegeneration in diabetic patients.

Future Directions

While the findings from this study are promising, further research is necessary to validate and extend these results. Future directions could include:

1. Expanded Biochemical Analyses: Additional biomarkers of oxidative stress, inflammation, and neuronal health could be assessed to provide a more comprehensive understanding of the therapeutic effects. For instance, analyzing levels of pro-inflammatory cytokines (like IL-6 or TNF-α) could further elucidate the anti-inflammatory actions of the formulation. Additionally, investigating neurotrophic factors, such as BDNF (Brain-Derived Neurotrophic Factor), could help explain the formulation's impact on neuronal survival and plasticity.

2. Molecular Mechanistic Studies: Conducting molecular studies on pathways affected by the treatment (e.g., NF-κB pathway for inflammation, Nrf2 pathway for antioxidant defense) could clarify the precise mechanisms underlying the neuroprotective effects of this formulation. This would provide deeper insights into its therapeutic potential and help guide dosage optimization.

3. Long-term Efficacy Studies: Assessing the long-term effects of this formulation in diabetic models could determine its sustainability and safety over extended periods. This is crucial for translating the treatment into real-world applications, where chronic use may be required.

4. Human Clinical Trials: To bridge the gap between animal and human applications, initial clinical trials on diabetic patients could evaluate the formulation's efficacy, safety, and tolerability in humans. These trials could involve non-invasive imaging techniques (e.g., MRI) to assess brain health, along with neuropsychological tests to evaluate cognitive and emotional changes.

5. Formulation Optimization: Further research into optimizing the dosage, administration route, and bioavailability of the therapeutic components could enhance the formulation's efficacy. Innovations in drug delivery systems, such as nanoparticles or liposomes, could also be explored to improve targeting and reduce the required dosage.

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