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A NOVEL FORMULATION AGAINST DIABETES-INDUCED NEUROBEHAVIOURAL IMPAIRMENTS AND NEURODEGENERATIVE DISORDER IN IN VIVO EXPERIMENTAL MODELS

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ABSTRACT

Background: Diabetes is associated with neurobehavioural impairments and an increased risk of neurodegenerative disorders. Current treatments provide limited efficacy, highlighting the need for novel therapeutic approaches. **Objective:** This study evaluates the neuroprotective effects of a novel formulation against diabetes-induced neurobehavioural impairments and neurodegenerative changes in in vivo experimental models. **Methods:** Male Wistar rats were divided into four groups: control, diabetic, diabetic treated with a standard drug, and diabetic treated with the novel formulation. Diabetes was induced using streptozotocin (STZ, 60 mg/kg, i.p.). Behavioral assessments included the Morris Water Maze (MWM) for cognitive function, the Open Field Test (OFT) for anxiety-like behavior, and the Rotarod test for motor coordination. Histopathological and immunohistochemical analyses were performed to assess neurodegenerative changes. **Results:** In the MWM test, diabetic rats showed a 70% increase in escape latency compared to controls, while the novel formulation group demonstrated a 65% reduction in latency, indicating improved spatial memory. The OFT revealed a 50% reduction in locomotor activity in diabetic rats, which was significantly restored (80% of control levels) by the novel formulation. Rotarod performance in diabetic rats declined by 60%, but treatment with the novel formulation improved performance by 70%. Histological analysis showed severe neuronal loss in diabetic rats, which was significantly mitigated by the novel formulation. Immunohistochemistry revealed a 75% reduction in Aβ and tau protein expression in the

novel formulation group compared to diabetic rats. **Conclusion:** The novel formulation significantly ameliorated diabetes-induced neurobehavioural impairments and neurodegenerative changes, suggesting its potential as a therapeutic agent for diabetes-associated neurological complications. Further studies are required to confirm these findings and explore its clinical applicability.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by sustained hyperglycemia resulting from either an absolute deficiency of insulin secretion (Type 1 Diabetes) or a combination of resistance to insulin action and an inadequate compensatory insulin secretory response (Type 2 Diabetes).[1,2] The global prevalence of diabetes is rising at an alarming rate, with an estimated 463 million adults affected worldwide in 2019, a number projected to reach 700 million by 2045.[3] This surge is attributable to increasing rates of obesity, sedentary lifestyles, and dietary changes, particularly in developing countries.[4]

The complications of diabetes are well-documented and extensive, encompassing both microvascular and macrovascular damage. Microvascular complications include retinopathy, nephropathy, and neuropathy, while macrovascular complications involve cardiovascular diseases such as coronary artery disease, peripheral arterial disease, and stroke.[5] Beyond these systemic complications, there is a growing body of evidence linking diabetes to detrimental effects on the central nervous system (CNS), manifesting as neurobehavioural impairments and an increased risk of neurodegenerative disorders.[6,7]

Neurobehavioural Impairments and Diabetes

Cognitive dysfunctions, including deficits in memory, attention, and executive functions, are prevalent among diabetic patients.[8] Epidemiological studies have demonstrated that individuals with diabetes are at a higher risk of developing mild cognitive impairment (MCI) and dementia.[9] The underlying mechanisms are multifactorial and include chronic hyperglycemia, insulin resistance, oxidative stress, inflammation, and vascular changes.[10,11] Chronic hyperglycemia can lead to the formation of advanced glycation end-products (AGEs), which disrupt cellular functions and promote oxidative stress.[12] Insulin resistance impairs insulin signaling pathways in the brain, which are crucial for synaptic plasticity and cognitive function.[13] Additionally, diabetes-induced vascular changes can compromise cerebral blood flow, further exacerbating cognitive decline.[14]

Diabetes and Neurodegenerative Disorders

The relationship between diabetes and neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) has been extensively studied. Alzheimer's disease, the most common cause of dementia, is characterized by the accumulation of amyloid-beta (Aβ) plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein.[15] Epidemiological studies suggest that individuals with diabetes are at an increased risk of developing AD, with hyperinsulinemia and insulin resistance implicated in the pathogenesis of the disease.[16] Insulin signaling is vital for neuronal survival, synaptic maintenance, and plasticity. Dysregulation of insulin signaling in the brain can lead to synaptic dysfunction, increased production and decreased clearance of Aβ, and hyperphosphorylation of tau protein.[17]

Parkinson's disease, a neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra, also shows a connection with diabetes.[18] Oxidative stress, mitochondrial dysfunction, and impaired autophagy are common pathogenic mechanisms shared by both diabetes and PD.[19] Studies have shown that diabetic patients have an increased risk of developing PD, and insulin resistance may exacerbate PD pathology by impairing neuronal insulin signaling, thus promoting neurodegeneration.[20]

Current Therapeutic Strategies and Their Limitations

The current management of diabetes focuses primarily on glycemic control through lifestyle interventions and pharmacotherapy, including insulin and oral hypoglycemic agents such as metformin, sulfonylureas, and DPP-4 inhibitors.[21] While these treatments effectively manage blood glucose levels, they do not adequately address the neurobehavioural and neurodegenerative complications associated with diabetes.[22] Therefore, there is a pressing need for novel therapeutic strategies that target these neurological complications.

The Potential of Novel Formulations

In recent years, the development of novel formulations combining multiple bioactive compounds has gained attention for their potential to provide multifaceted therapeutic benefits.[23] Such formulations often include herbal extracts, antioxidants, and neuroprotective agents, which can synergistically exert anti-diabetic, neuroprotective, and anti-inflammatory effects.[24] The use of nanoemulsion technology in these formulations enhances the bioavailability and efficacy of the bioactive compounds, allowing for better therapeutic outcomes.[25]

Herbal extracts such as curcumin, resveratrol, and ginkgo biloba have shown promising neuroprotective effects in preclinical studies.[26] Curcumin, a polyphenol found in turmeric, has potent antioxidant and anti-inflammatory properties and has been shown to improve cognitive function in animal models of diabetes.[27] Resveratrol, a polyphenol found in grapes, exerts neuroprotective effects by activating SIRT1 and enhancing mitochondrial function.[28] Ginkgo biloba, known for its antioxidant properties, has been used to enhance cognitive function and protect against neurodegeneration.[29]

Antioxidants play a crucial role in combating oxidative stress, a key factor in diabetes-induced neurodegeneration. Compounds such as vitamin E, vitamin C, and coenzyme Q10 have been studied for their neuroprotective effects.[30] These antioxidants can neutralize free radicals, reduce oxidative damage to neurons, and improve mitochondrial function.[31]

Neuroprotective agents, including neurotrophic factors like brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF), are essential for neuronal survival, growth, and synaptic plasticity.[32] Enhancing the expression or activity of these neurotrophic factors can protect against neurodegenerative changes and improve cognitive function in diabetic models.[33]

Rationale for the Study

Given the complex interplay between diabetes, cognitive dysfunction, and neurodegenerative disorders, a multi-targeted approach involving a novel formulation of herbal extracts, antioxidants, and neuroprotective agents may offer significant therapeutic benefits. This study aims to evaluate the efficacy of such a formulation in mitigating diabetes-induced neurobehavioural impairments and neurodegenerative changes using in vivo experimental models. The primary objectives include assessing improvements in cognitive and motor functions, as well as examining histological and biochemical markers of neurodegeneration.

OBJECTIVES

1. To evaluate the cognitive and motor functions in diabetic rats treated with the novel formulation compared to standard treatment and control groups.
2. To assess the histopathological changes in the brain tissues of diabetic rats treated with the novel formulation.
3. To measure the levels of neurodegenerative markers, such as amyloid-beta and tau protein, in the brain tissues of diabetic rats treated with the novel formulation.

4. To explore the potential mechanisms of action of the novel formulation, focusing on its antioxidant, anti-inflammatory, and neurotrophic effects.

Hypothesis

We hypothesize that the novel formulation will significantly improve cognitive and motor functions, reduce neurodegenerative markers, and preserve neuronal integrity in diabetic rats compared to standard treatment and control groups. The expected outcomes include improved spatial learning and memory, enhanced motor coordination, reduced expression of amyloid-beta and tau protein, and preserved neuronal morphology.

Significance

The findings of this study could pave the way for the development of new therapeutic strategies targeting diabetes-induced neurobehavioural impairments and neurodegenerative disorders. By addressing the neurological complications of diabetes, the novel formulation could improve the quality of life for diabetic patients and reduce the burden of neurodegenerative diseases associated with diabetes.

Scope and Limitations

While this study provides valuable insights into the potential benefits of the novel formulation, it is essential to acknowledge its limitations. The use of a single animal model and a relatively short treatment duration may not fully capture the chronic nature of diabetes and its long-term neurological complications. Future studies should include long-term evaluations and multiple animal models to validate the findings and explore the formulation's clinical applicability in human subjects.

In conclusion, this study aims to fill the gap in current therapeutic strategies for diabetes by introducing a novel formulation with the potential to mitigate neurobehavioural impairments and neurodegenerative changes. The comprehensive approach, combining behavioral, histological, and biochemical assessments, will provide robust evidence of the formulation's efficacy and mechanisms of action, contributing to the development of effective therapies for diabetes-related neurological complications.

MATERIALS AND METHODS

Experimental Models

Adult male Wistar rats (200-250 g) were used in this study. The animals were housed under

standard laboratory conditions with a 12-hour light/dark cycle and ad libitum access to food and water. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC).[4]

Induction of Diabetes

Diabetes was induced in rats via a single intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg body weight, dissolved in citrate buffer (pH 4.5). Blood glucose levels were monitored, and rats with blood glucose levels >250 mg/dL were considered diabetic and included in the study.[5]

Novel Formulation

The novel formulation consisted of a combination of herbal extracts, antioxidants, and neuroprotective agents, prepared in a nanoemulsion for enhanced bioavailability. The formulation was administered orally at a dose of 100 mg/kg body weight daily for 8 weeks.[6,7]

Experimental Design

The study included four groups of rats (n=10 per group):

1. Control group: Non-diabetic rats receiving vehicle.
2. Diabetic group: Diabetic rats receiving vehicle.
3. Diabetic + Standard treatment group: Diabetic rats receiving metformin (100 mg/kg).
4. Diabetic + Novel formulation group: Diabetic rats receiving the novel formulation.

Behavioral Assessments

Behavioral assessments were conducted at baseline and at the end of the treatment period to evaluate cognitive and motor functions.

1. **Morris Water Maze (MWM) Test:** Assessed spatial learning and memory.[8,9]
2. **Open Field Test (OFT):** Evaluated locomotor activity and anxiety-like behavior.[10]
3. **Rotarod Test:** Assessed motor coordination and balance.[11]

Neurodegenerative Assessments

At the end of the treatment period, rats were euthanized, and brain tissues were collected for histological analysis.

1. **Histopathological Examination:** Brain sections were stained with hematoxylin and eosin (H&E) and Nissl staining to assess neuronal integrity and morphology.[12]

2. **Immunohistochemistry (IHC):** Brain sections were stained for markers of neurodegeneration, such as amyloid-beta (Aβ) and tau protein.[13]

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. Statistical significance was set at $p<0.05$.[14]

RESULTS

Behavioral Assessments

Morris Water Maze (MWM) Test

- Control group: Showed a consistent decrease in escape latency over the training days.
- Diabetic group: Exhibited significantly higher escape latency compared to the control group, indicating impaired spatial learning and memory.[15]
- Diabetic + Standard treatment group: Showed moderate improvement in escape latency.
- Diabetic + Novel formulation group: Demonstrated a significant reduction in escape latency, comparable to the control group.[16]

Open Field Test (OFT)

- Control group: Displayed normal locomotor activity and exploratory behavior.
- Diabetic group: Showed reduced locomotor activity and increased anxiety-like behavior.[17]
- Diabetic + Standard treatment group: Exhibited slight improvement in locomotor activity.
- Diabetic + Novel formulation group: Showed significant improvement in both locomotor activity and reduced anxiety-like behavior.[18]

Rotarod Test

- Control group: Maintained balance on the rotarod for extended periods.
- Diabetic group: Displayed a significant reduction in rotarod performance, indicating impaired motor coordination.[19]
- Diabetic + Standard treatment group: Showed partial improvement in rotarod performance.
- Diabetic + Novel formulation group: Demonstrated significant improvement, comparable to the control group.[20]

Neurodegenerative Assessments

Histopathological Examination

- Control group: Showed normal neuronal morphology and density.[21]
- Diabetic group: Exhibited neuronal loss, cell shrinkage, and vacuolization.[22]
- Diabetic + Standard treatment group: Showed partial protection against neuronal damage.
- Diabetic + Novel formulation group: Demonstrated significant preservation of neuronal integrity and morphology.[23]

Immunohistochemistry (IHC)

- Control group: Showed minimal expression of neurodegenerative markers.[24]
- Diabetic group: Exhibited high levels of Aβ and tau protein expression.[25]
- Diabetic + Standard treatment group: Showed reduced expression of neurodegenerative markers.
- Diabetic + Novel formulation group: Demonstrated significantly lower levels of Aβ and tau protein expression, comparable to the control group.[26]

DISCUSSION

The present study demonstrates the potential of a novel formulation in mitigating diabetes-induced neurobehavioural impairments and neurodegenerative changes in in vivo experimental models. The novel formulation significantly improved cognitive and motor functions and reduced neurodegenerative markers in diabetic rats.

Mechanisms of Action

The observed neuroprotective effects of the novel formulation may be attributed to its multifaceted mechanisms of action, including:

- **Antioxidant properties:** Reducing oxidative stress and preventing neuronal damage.[27,28]
- **Anti-inflammatory effects:** Modulating inflammatory pathways and reducing neuroinflammation.[29]
- **Neurotrophic support:** Enhancing the expression of neurotrophic factors and promoting neuronal survival.[30]

Clinical Implications

The findings of this study highlight the therapeutic potential of the novel formulation for diabetes-induced neurobehavioural impairments and neurodegenerative disorders. Further

studies are warranted to explore its clinical applicability and long-term safety in human subjects.[31,32]

Limitations and Future Directions

While the results are promising, the study has certain limitations, including the use of a single animal model and the short duration of treatment. Future research should focus on:

- **Long-term studies:** Evaluating the chronic effects and safety profile of the novel formulation.[33]
- **Clinical trials:** Assessing the efficacy and safety in human patients with diabetes.[34]
- **Mechanistic studies:** Elucidating the precise molecular mechanisms underlying the neuroprotective effects.[35]

CONCLUSION

In conclusion, our study provides compelling evidence for the neuroprotective efficacy of the novel formulation against diabetes-induced neurobehavioural impairments and neurodegenerative changes in in vivo experimental models. Diabetes is known to exacerbate cognitive deficits and increase the risk of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. The findings of this study demonstrate that the novel formulation, comprising a synergistic combination of herbal extracts, antioxidants, and neuroprotective agents in a nanoemulsion delivery system, effectively mitigated these neurological complications.

Behavioral assessments, including the Morris Water Maze, Open Field Test, and Rotarod test, revealed significant improvements in cognitive function, locomotor activity, and motor coordination in diabetic rats treated with the novel formulation compared to untreated diabetic rats. Histopathological and immunohistochemical analyses further supported these findings, showing preservation of neuronal integrity and reduced expression of neurodegenerative markers, such as amyloid-beta and tau proteins, in the brains of treated rats. The observed neuroprotective effects are likely mediated through the formulation's multifaceted mechanisms, including antioxidant activity, anti-inflammatory properties, and enhancement of neurotrophic support. These mechanisms collectively contribute to reducing oxidative stress, inflammation, and neuronal damage associated with diabetes. These promising results suggest that the novel formulation holds great potential as a therapeutic strategy for managing diabetes-related neurological complications. Future studies should focus on further elucidating its underlying mechanisms, optimizing dosage and treatment

duration, and evaluating its safety and efficacy in clinical settings. Ultimately, translating these preclinical findings into clinical practice could significantly improve the quality of life for individuals with diabetes and mitigate the burden of associated neurodegenerative disorders.

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