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Neuroprotective Effects Of Eriodictyol On Hippocampal Damage In A Fructose-Streptozotocin-Induced Model Of Type 2 Diabetes

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Abstract: Background: Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder increasingly associated with central nervous system complications, including cognitive decline and hippocampal neurodegeneration. Oxidative stress is a primary mechanism underlying this neuronal damage. Flavonoids, such as eriodictyol, are natural compounds known for their potent antioxidant properties. This study aimed to investigate the neuroprotective effects of eriodictyol against biochemical and histopathological abnormalities in the hippocampus of fructose/streptozotocin (F-STZ)-induced diabetic rats.

Methods: T2DM was induced in male Wistar rats using a high-fructose diet (10% w/v) for two weeks followed by a single intraperitoneal injection of streptozotocin (40 mg/kg). Diabetic rats were treated with eriodictyol (50 mg/kg) orally for 45 days. At the end of the treatment, glycemic parameters (blood glucose, HbA1c), hippocampal markers of oxidative stress (lipid peroxidation, protein carbonyls, reduced glutathione), antioxidant enzyme activities (SOD, GR), acetylcholinesterase (AChE), and Na$^+/K^+$-ATPase were assessed. Hippocampal tissues were also processed for histopathological examination using H&E staining.

Results: The F-STZ-induced diabetic rats exhibited significant hyperglycemia, increased hippocampal oxidative stress markers, and depleted antioxidant defenses. This was accompanied by aberrant AChE and Na$^+/K^+$-ATPase activities and severe neuronal damage in the hippocampal CA1 region. Eriodictyol administration significantly ($P < 0.05$) ameliorated hyperglycemia, reversed the oxidative stress markers, and restored the activity of antioxidant and membrane-bound enzymes towards normal levels. Histopathological analysis confirmed that eriodictyol treatment markedly preserved the structural integrity of hippocampal neurons.

Conclusion: The findings suggest that eriodictyol exerts a potent neuroprotective effect in the diabetic rat hippocampus. This protection is likely mediated by its ability to improve glycemic control and mitigate oxidative stress, thereby preserving neuronal function and structure. Eriodictyol represents a promising natural therapeutic candidate for preventing or treating diabetic neurological complications.

Keywords: Eriodictyol, Type 2 Diabetes, Hippocampus, Neuroprotection, Oxidative Stress, Streptozotocin, Cognitive Decline.

Introduction: Diabetes mellitus (DM) represents one of the most significant global health challenges of the 21st century, with a relentlessly increasing prevalence and substantial socioeconomic impact. The condition is broadly classified into several types, with Type 2 Diabetes Mellitus (T2DM) accounting for over 90% of all cases [1]. T2DM is a complex metabolic disorder characterized by persistent hyperglycemia resulting from a combination of insulin resistance and relative insulin deficiency [3]. While the systemic complications of T2DM, such as cardiovascular disease, nephropathy, and retinopathy, are well-documented and recognized as leading causes of morbidity and mortality [2], the effects on the central nervous system (CNS) have garnered increasing attention in recent years.

The brain, despite being an insulin-independent organ for glucose uptake, is highly vulnerable to the metabolic dysregulation inherent in T2DM [4, 5].

Chronic hyperglycemia, hyperinsulinemia, and insulin resistance disrupt the delicate balance of cerebral glucose metabolism, leading to a state of neuronal energy deficit and impaired signaling [29, 30]. This cascade of events contributes to a spectrum of neurological complications collectively termed "diabetic encephalopathy," which manifests as cognitive deficits, learning impairments, and an accelerated risk for developing neurodegenerative diseases such as Alzheimer's disease [6, 10]. The molecular mechanisms underpinning these cognitive declines are multifaceted, involving microvascular dysfunction, impaired insulin signaling, neuroinflammation, and, critically, overwhelming oxidative stress [6, 28].

Among the brain regions susceptible to diabetic insults, the hippocampus is particularly vulnerable. As a key structure in the limbic system, the hippocampus is indispensable for the consolidation of short-term to long-term memory, spatial navigation, and learning [7, 8]. Its high metabolic rate and density of insulin receptors make it exquisitely sensitive to fluctuations in glucose and insulin levels [5, 31]. Furthermore, the remarkable synaptic plasticity of the hippocampus, which forms the cellular basis of memory storage, can be severely compromised by the pathophysiological environment created by T2DM [9, 11]. Consequently, hippocampal damage is a central neuropathological feature of diabetes-associated cognitive impairment.

Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and the capacity of endogenous antioxidant systems to neutralize them, is a pivotal mechanism driving diabetic complications, including neurodegeneration [15, 34]. Hyperglycemia promotes ROS generation through various pathways, such as glucose auto-oxidation and the advanced glycation end-product (AGE) formation, leading to widespread damage to cellular macromolecules, including lipids, proteins, and nucleic acids [36]. This oxidative onslaught depletes endogenous antioxidant defenses, such as reduced glutathione (GSH) and enzymes like superoxide dismutase (SOD) and catalase (CAT), further exacerbating cellular injury [35]. In the hippocampus, this oxidative damage impairs neuronal function, promotes apoptosis, and contributes to the progressive cognitive decline observed in T2DM.

In the quest for effective therapeutic strategies to mitigate diabetic neurological complications, there has been a growing interest in natural phytochemicals, particularly flavonoids. Flavonoids are a large class of polyphenolic compounds found abundantly in fruits, vegetables, and medicinal plants, renowned for their diverse biological activities [12, 17, 33]. Their potent antioxidant properties, stemming from their ability to scavenge free radicals and chelate metal ions, make them excellent candidates for combating oxidative stress-related diseases [13, 32]. Numerous studies have highlighted the neuroprotective potential of flavonoids, demonstrating their ability to cross the blood-brain barrier and exert beneficial effects within the CNS [14, 38].

Eriodictyol (5,7,3',4'-tetrahydroxyflavanone) is a flavanone commonly found in citrus fruits and certain medicinal herbs. While the antidiabetic and antioxidant properties of various flavonoid-rich extracts have been reported [16], the specific neuroprotective efficacy of eriodictyol on the hippocampus in the context of T2DM remains inadequately explored. The development of reliable animal models, such as the combination of a high-fructose diet and a low dose of streptozotocin (STZ), effectively mimics the metabolic characteristics of human T2DM, providing a valuable platform for investigating therapeutic interventions.

Therefore, this study was designed to bridge this knowledge gap. We hypothesize that eriodictyol administration will ameliorate the biochemical and histopathological damage in the hippocampus of T2DM rats. The primary objective of this study was to investigate the effects of eriodictyol on glycemic control, hippocampal oxidative stress markers, key antioxidant enzyme activities, and neuronal morphology in a fructose/streptozotocin-induced rat model of T2DM.

2. METHODS

2.1. Chemicals and Reagents

Eriodictyol (purity ≥98%), streptozotocin (STZ), and fructose were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used for biochemical assays were of analytical grade and procured from reputable commercial suppliers. Diagnostic kits for the estimation of glycated hemoglobin (HbA1c) were sourced from Biosystems S.A. (Barcelona, Spain).

2.2. Experimental Animals and Ethical Approval

Adult male Wistar rats, weighing between 180–220 g, were procured from the institutional central animal facility. The animals were housed in polypropylene cages under standard laboratory conditions, including a controlled temperature of 23 ± 2 °C, relative humidity of 55 ± 5%, and a 12-hour light/dark cycle. They were provided with a standard pellet diet and water ad libitum. All experimental procedures were conducted

in strict accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, and the study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC).

2.3. Induction of Type 2 Diabetes Mellitus

A combination protocol of high-fructose diet and low-dose STZ was used to induce T2DM, as this model closely simulates the natural progression of the disease in humans (insulin resistance followed by β-cell dysfunction). Initially, all rats, except those in the control group, were administered 10% (w/v) fructose solution in their drinking water for a period of 14 days to induce insulin resistance. Following this period, the fructose-fed rats were fasted overnight and then administered a single intraperitoneal (i.p.) injection of a freshly prepared low dose of STZ (40 mg/kg body weight) dissolved in 0.1 M cold citrate buffer (pH 4.5). The normal control group received an equivalent volume of the citrate buffer. Diabetes was confirmed 72 hours after the STZ injection by measuring fasting blood glucose levels from the tail vein using a digital glucometer. Rats with fasting blood glucose levels exceeding 250 mg/dL were considered diabetic and were selected for the study.

2.4. Experimental Design

The selected diabetic rats were randomly divided into three groups, along with the normal control group, with six animals (n=6) per group. The treatment was carried out for 45 days.

- Group I (Normal Control): Normal rats received the vehicle (0.5% carboxymethyl cellulose, CMC) orally once daily.
- Group II (Diabetic Control - DC): Diabetic rats received the vehicle (0.5% CMC) orally once daily.
- Group III (Eriodictyol-Treated): Diabetic rats received eriodictyol suspended in 0.5% CMC at a dose of 50 mg/kg body weight orally once daily.
- Group IV (Metformin-Treated): Diabetic rats received the standard antidiabetic drug, Metformin, suspended in 0.5% CMC at a dose of 100 mg/kg body weight orally once daily, serving as a positive control [39].

Body weight and fasting blood glucose levels were monitored weekly throughout the experimental period.

2.5. Sample Collection and Tissue Preparation

At the end of the 45-day treatment period, the animals were fasted overnight and anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Blood was collected via cardiac puncture into two separate tubes: one containing EDTA for HbA1c estimation and another without anticoagulant for serum separation. The serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at -80 °C for biochemical analysis.

Following blood collection, the animals were sacrificed by cervical decapitation. The brains were immediately excised, washed in ice-cold saline, and placed on an ice-cold petri dish. The hippocampus was carefully dissected from both hemispheres. The hippocampus from one hemisphere was weighed and homogenized in 10 volumes of ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 20 minutes at 4 °C, and the resulting supernatant was used for various biochemical assays. The hippocampus from the other hemisphere was fixed in 10% neutral buffered formalin for histopathological analysis.

2.6. Biochemical Assays

2.6.1. Glycemic and Hematological Markers

Fasting blood glucose was measured using a glucometer. Glycated hemoglobin (HbA1c) in the whole blood was estimated using a commercially available kit based on the ion-exchange resin method, following the manufacturer's instructions [18].

2.6.2. Markers of Oxidative Stress in Hippocampal Homogenate

- Lipid Peroxidation (LPO): The extent of lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS). The assay was performed according to the method of Varshney and Kale [23]. The concentration of malondialdehyde (MDA), the end product of lipid peroxidation, was quantified spectrophotometrically at 532 nm and expressed as nmol of MDA/mg protein.
- Protein Carbonyl Content (PCC): The level of oxidatively modified proteins was assessed by measuring the protein carbonyl content as described

by Levine et al. [24]. The reaction with 2,4-dinitrophenylhydrazine (DNPH) forms a Schiff base, which was measured at 370 nm. The results were expressed as nmol of carbonyl groups/mg protein.

- Reduced Glutathione (GSH): The concentration of GSH, a major non-enzymatic antioxidant, was estimated based on the method described by Beutler et al. [19], which involves the reaction of GSH with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to produce a yellow-colored complex measured at 412 nm. The results were expressed as µg of GSH/mg protein.

2.6.3. Antioxidant Enzyme Activities in Hippocampal Homogenate

- Superoxide Dismutase (SOD): The activity of SOD was assayed by the method of Kakkar et al. [22], which is based on the inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazan. The absorbance was read at 560 nm, and one unit of enzyme activity was defined as the amount of enzyme required to inhibit the reaction by 50%. The activity was expressed as U/mg protein. [Reference to Haque et al. [20] for context on SOD's role].
- Glutathione Reductase (GR): GR activity was determined by measuring the rate of NADPH oxidation at 340 nm in the presence of oxidized glutathione (GSSG), following the method described by Beutler et al. [19]. The activity was expressed as nmol of NADPH oxidized/min/mg protein.
- Protein Estimation: The protein content in the hippocampal homogenate was estimated by the method of Lowry et al. [21], using bovine serum albumin (BSA) as the standard.

2.6.4. Cholinergic and Neuronal Integrity Markers

- Acetylcholinesterase (AChE): The activity of AChE was measured according to the colorimetric method of Ellman et al. [25]. The assay is based on the hydrolysis of acetylthiocholine iodide to thiocholine, which reacts with DTNB to produce a yellow anion measured at 412 nm. The activity was expressed as µmol of substrate hydrolyzed/min/mg protein.
- Na$^+/K^+$-ATPase: The activity of Sodium-Potassium Adenosine Triphosphatase was determined by the method of Svoboda and Mosinger [26]. The assay measures the amount of inorganic phosphate (Pi) liberated from ATP. The enzyme activity was calculated as the difference between the total ATPase activity and the ouabain-inhibited ATPase activity and was expressed as µmol of Pi liberated/min/mg protein.

2.7. Histopathological Analysis

The formalin-fixed hippocampal tissues were processed through graded alcohol solutions for dehydration, cleared in xylene, and embedded in paraffin wax. Serial coronal sections of 5 µm thickness were cut using a rotary microtome. The sections were deparaffinized, rehydrated, and stained with Hematoxylin and Eosin (H&E) dye. The stained slides were examined under a light microscope for any histopathological alterations, such as neuronal cell loss, nuclear pyknosis (shrinkage), karyorrhexis (fragmentation), and vacuolation, particularly in the CA1 and CA3 regions of the hippocampus. The evaluation was conducted according to standard histopathological practices [27]. A pathologist, blinded to the experimental groups, performed the qualitative analysis of the slides.

2.8. Statistical Analysis

All data were expressed as the mean ± standard deviation (SD) for six animals in each group. The statistical significance of the differences between the group means was determined by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test using GraphPad Prism software (Version 8.0, San Diego, CA, USA). A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1. Effect of Eriodictyol on Glycemic Control and Body Weight

The induction of T2DM using the F-STZ protocol resulted in a significant alteration of glycemic parameters. At the end of the 45-day experimental period, the diabetic control (DC) group exhibited a marked and significant ($P < 0.001$) increase in fasting blood glucose (345.8 ± 21.2 mg/dL) and HbA1c levels (10.8 ± 0.7 %) when compared to the normal control group (92.5 ± 6.4 mg/dL and 4.2 ± 0.3 %, respectively). Oral administration of eriodictyol (50 mg/kg) for 45 days led to a significant ($P < 0.01$) reduction in both fasting blood glucose (158.3 ± 14.9 mg/dL) and HbA1c levels (6.9 ± 0.5 %) compared to the DC group. The effect of eriodictyol was comparable to that of the

standard drug metformin (135.6 ± 11.8 mg/dL and 6.1 ± 0.4 %, respectively). Furthermore, the DC group showed a significant decrease in final body weight compared to the normal control group, a trend that was partially and significantly reversed by treatment with both eriodictyol and metformin.

3.2. Effect of Eriodictyol on Hippocampal Oxidative Stress Markers

The state of oxidative stress in the hippocampus was evaluated by measuring the levels of LPO, PCC, and GSH. The DC group demonstrated a significant ($P < 0.001$) increase in the levels of LPO (as TBARS) and PCC by approximately 2.5-fold and 2.2-fold, respectively, compared to the normal control group. This indicates extensive oxidative damage to lipids and proteins in the hippocampus of diabetic rats. Concurrently, the level of the endogenous non-enzymatic antioxidant GSH was significantly ($P < 0.001$) depleted in the DC group, reaching approximately 45% of the level observed in normal controls. Treatment with eriodictyol was associated with a potent amelioration of these oxidative stress markers. Eriodictyol administration significantly ($P < 0.01$) decreased the elevated levels of LPO and PCC and significantly ($P < 0.01$) restored the depleted GSH levels in the hippocampus of diabetic rats, as detailed in Table 1.

3.3. Effect of Eriodictyol on Hippocampal Antioxidant Enzyme Activities

The activities of key endogenous antioxidant enzymes, SOD and GR, were assessed in the hippocampal tissue. In the DC group, the activities of both SOD and GR were found to be significantly ($P < 0.001$) reduced when compared to the normal control group, suggesting a compromised enzymatic antioxidant defense system in the diabetic condition. Oral treatment with eriodictyol (50 mg/kg) for 45 days resulted in a significant ($P < 0.01$) restoration of both SOD and GR activities in the hippocampus of diabetic rats. This indicates that eriodictyol may bolster the endogenous antioxidant capacity. The positive control, metformin, also exhibited a significant and positive modulatory effect on the activities of these enzymes (Table 1).

Table 1: Effect of Eriodictyol on Hippocampal Oxidative Stress Markers and Antioxidant Enzyme Activities in Experimental Rats

Parameters	Normal Control	Diabetic Control (DC)	Eriodictyol (50 mg/kg)	Metformin (100 mg/kg)
LPO (nmol MDA/mg protein)	1.24 ± 0.11	3.12 ± 0.28[a]	1.58 ± 0.14[b]	1.45 ± 0.13[b]
PCC (nmol carbonyl/mg protein)	2.55 ± 0.23	5.61 ± 0.49[a]	3.05 ± 0.26[b]	2.89 ± 0.25[b]
GSH (µg/mg protein)	15.3 ± 1.2	6.9 ± 0.7[a]	12.8 ± 1.1[b]	13.5 ± 1.2[b]
SOD (U/mg protein)	8.1 ± 0.6	3.7 ± 0.4[a]	6.9 ± 0.5[b]	7.2 ± 0.6[b]
GR (nmol NADPH oxidized/min/m	20.4 ± 1.8	9.5 ± 1.1[a]	17.1 ± 1.5[b]	18.2 ± 1.6[b]

g protein)				

Values are expressed as mean ± SD for six animals (n=6) in each group.

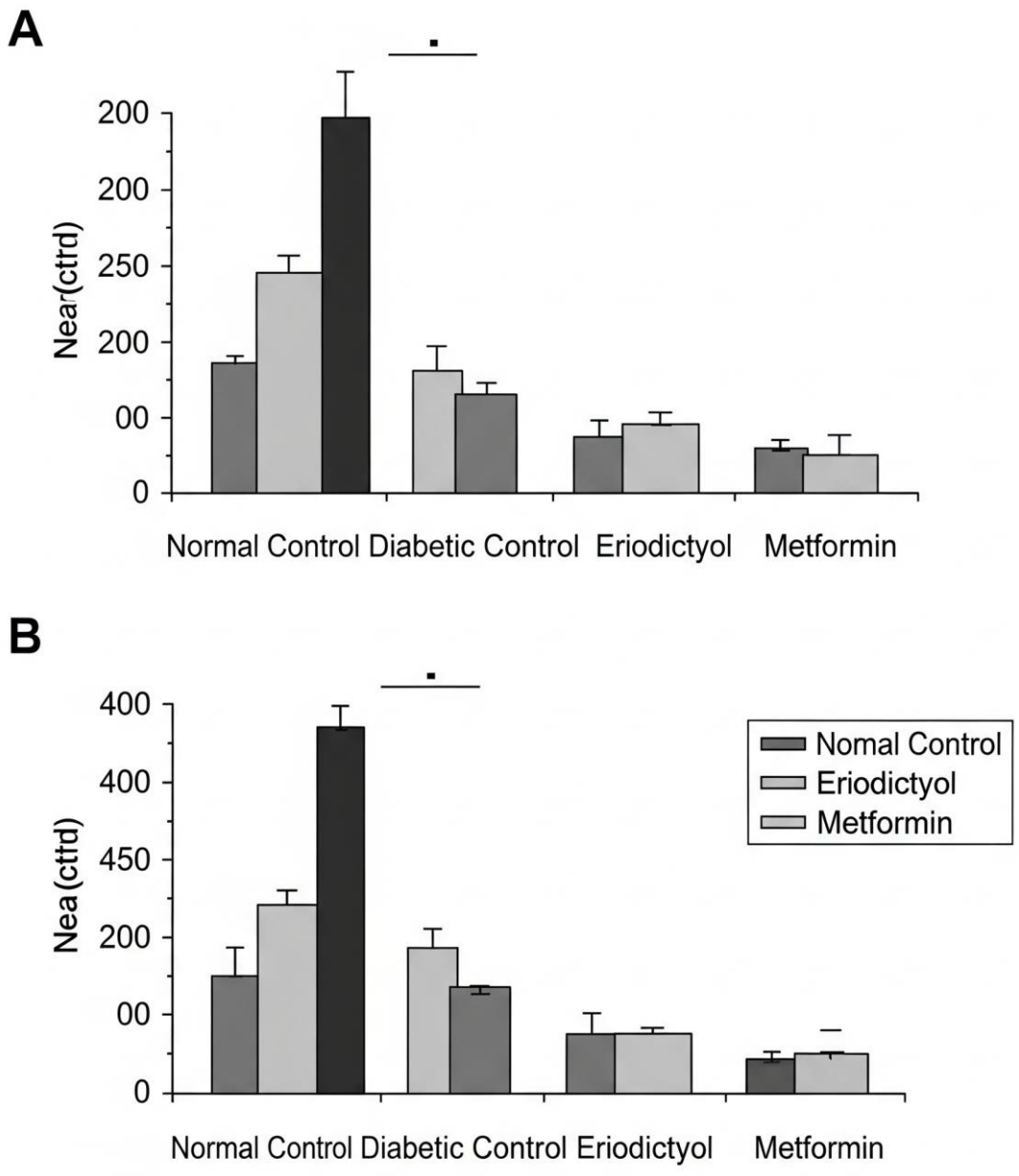
- LPO: Lipid Peroxidation; PCC: Protein Carbonyl Content; GSH: Reduced Glutathione; SOD: Superoxide Dismutase; GR: Glutathione Reductase.*

Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test.

[a]P < 0.001 when compared with the Normal Control group.

[b]P < 0.01 when compared with the Diabetic Control (DC) group.

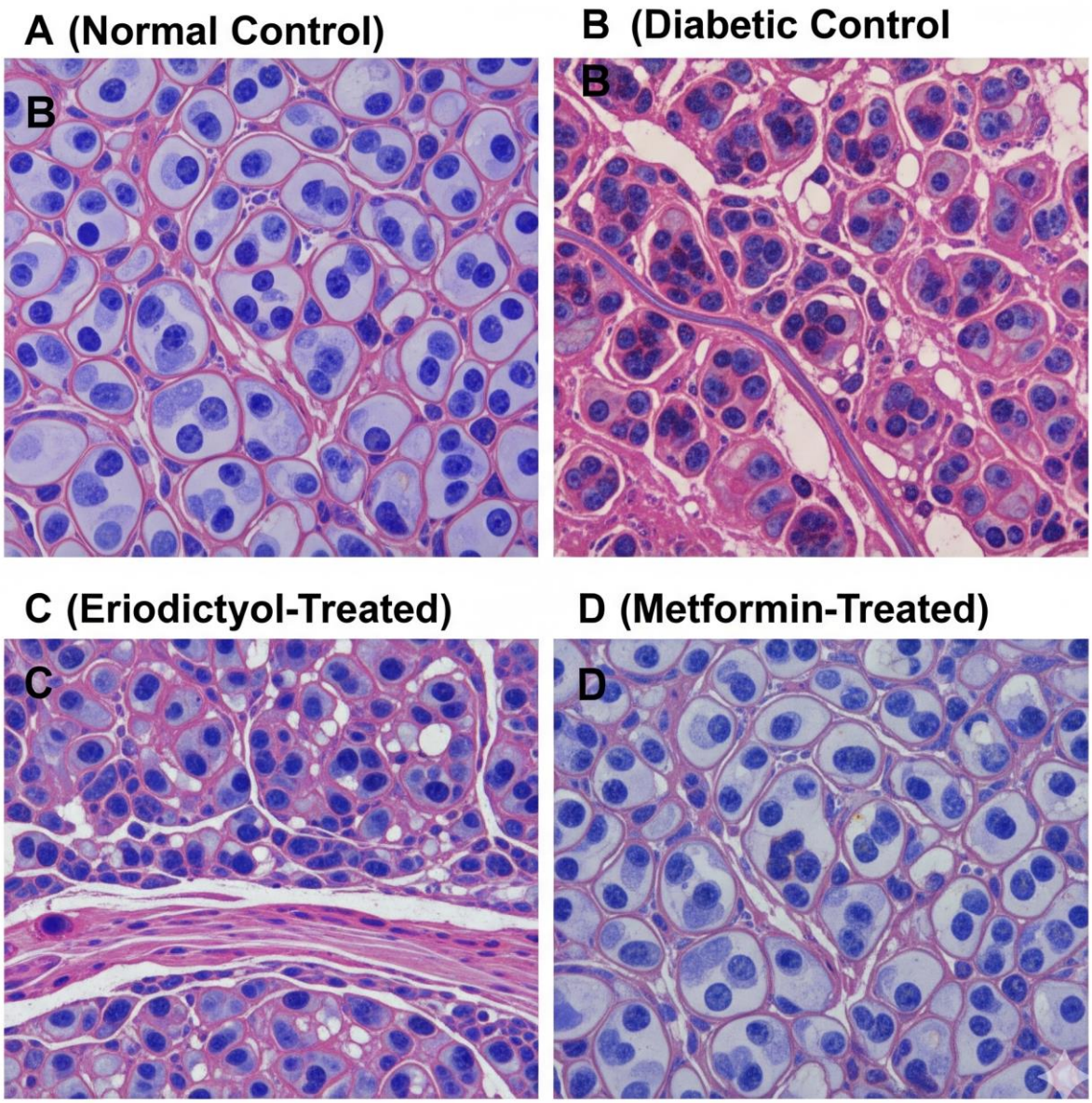
3.4. Effect of Eriodictyol on AChE and Na$^+/K^+$-ATPase Activities



The activity of AChE, a key enzyme in cholinergic neurotransmission, was significantly (P < 0.001) elevated in the hippocampal homogenate of the DC group compared to the normal control group (Figure 1A). Conversely, the activity of Na$^+/K^+−ATPase,anenzymecrucialformaintainingneuronalmembranepotentialandintegrity,wassignificantly(P<0.001)decreasedintheDCgroup(Figure1B).Treatmentofdiabeticratswitheriodictyolwasassociatedwithasignificant(P<0.01)normalizationoftheseenzymeactivities. Eriodictyoladministrationmarkedlyreducedtheaberrant AChEactivityandrestoredthesuppressedNa^+/K^+$-

ATPase activity, suggesting a beneficial effect on both cholinergic function and neuronal membrane stability. Metformin treatment also yielded similar significant improvements.

3.5. Histopathological Observations



Histopathological examination of H&E-stained hippocampal sections provided morphological evidence corroborating the biochemical findings (Figure 2).

- Normal Control Group (A): The hippocampal sections from this group displayed normal cytoarchitecture. The pyramidal neurons in the CA1 and CA3 regions were well-organized, with large, round, vesicular nuclei and prominent nucleoli, surrounded by a clear cytoplasm.
- Diabetic Control Group (B): This group exhibited severe histopathological alterations. There was a conspicuous loss of pyramidal neurons, particularly in the CA1 region. Many of the remaining neurons appeared shrunken, with dark, pyknotic nuclei and eosinophilic cytoplasm, indicative of neuronal necrosis and apoptosis. Evidence of gliosis and neuropil vacuolation was also prevalent throughout the hippocampal subfields.
- Eriodictyol-Treated Group (C): Treatment with eriodictyol was associated with a remarkable preservation of hippocampal architecture. The neuronal density in the CA1 and CA3 regions was substantially higher compared to the DC group. The majority of neurons appeared healthy, with well-defined nuclei and cytoplasm, and there was a marked reduction in the signs of pyknosis, neuronal loss, and vacuolation.
- Metformin-Treated Group (D): The positive control group also showed significant neuroprotection,

with a well-preserved neuronal population and reduced signs of cellular damage compared to the untreated diabetic group.

4. DISCUSSION

The present study investigated the neuroprotective potential of eriodictyol against diabetes-induced biochemical and histopathological derangements in the hippocampus of a fructose-streptozotocin (F-STZ) rat model. The results demonstrate that eriodictyol administration effectively mitigated hyperglycemia, attenuated oxidative stress, restored the activity of key neuronal enzymes, and preserved the structural integrity of hippocampal neurons. These findings suggest a strong therapeutic potential for eriodictyol in combating diabetic neurological complications.

The F-STZ model was chosen for this study as it closely mimics the pathophysiology of human T2DM, characterized by initial insulin resistance induced by a high-fructose diet, followed by β-cell dysfunction caused by a low dose of STZ [39]. The significant elevation in fasting blood glucose and HbA1c levels, alongside weight loss, in our diabetic control (DC) group confirmed the successful induction of a diabetic state, consistent with previous reports [1, 3]. The ability of eriodictyol to significantly lower these glycemic parameters is a crucial finding, as chronic hyperglycemia is the primary initiator of diabetic complications [34]. This antihyperglycemic effect could be attributed to several potential mechanisms inherent to flavonoids, such as improved insulin sensitivity or enhanced glucose uptake, as reported for other flavonoid compounds [16]. By establishing better glycemic control, eriodictyol addresses the root cause of the downstream pathological cascade.

A central theme in the pathogenesis of diabetic encephalopathy is the role of oxidative stress [6, 15]. The brain's high oxygen consumption, abundant lipid content, and relatively modest antioxidant defenses render it particularly susceptible to oxidative damage [5, 30]. Our study found a profound state of oxidative stress in the hippocampus of diabetic rats, evidenced by significantly increased levels of lipid peroxidation (LPO) and protein carbonyl content (PCC). LPO compromises the integrity of neuronal membranes, while protein carbonylation leads to dysfunctional enzymes and structural proteins, both contributing to cellular demise [23, 37]. This was coupled with a severe depletion of reduced glutathione (GSH), the most abundant endogenous non-enzymatic antioxidant, which plays a vital role in detoxifying ROS and maintaining cellular redox balance [35]. Eriodictyol treatment was strongly associated with the reversal of these changes. This potent antioxidant action is consistent with the chemical structure of flavonoids, which allows them to directly scavenge free radicals and chelate pro-oxidant metal ions [12, 32, 33]. By reducing LPO and PCC and replenishing GSH stores, eriodictyol appears to effectively shield hippocampal neurons from oxidative injury.

Beyond non-enzymatic defenses, the endogenous antioxidant enzyme system provides the first line of defense against ROS. Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide radical, while glutathione reductase (GR) is essential for regenerating GSH from its oxidized form (GSSG) [19, 20, 36]. The significant reduction in the activities of SOD and GR in our DC group indicates a failure of this enzymatic shield, further exacerbating oxidative stress. The significant restoration of SOD and GR activities following eriodictyol administration suggests that its protective mechanism is not limited to direct radical scavenging but also involves bolstering the brain's intrinsic antioxidant machinery. This dual action—directly neutralizing ROS and upregulating endogenous defenses—represents a highly effective therapeutic strategy [38].

The functional integrity of neurons was assessed by measuring the activities of AChE and Na$^+/K^+−ATPase.Acetylcholineisacriticalneurotransmitterforlearningandmemory,anditslevelsareregulatedbyAChE[25].TheelevatedAChEactivityintheDCgroupsuggestsanacceleratedegradationofacetylcholine,whichcouldcontributetothecognitivedeficitsseenindiabetes.ThenormalizationofAChEactivitybyeriodictyolpointstowardsapotentialimprovementincholinergicneurotransmission,amechanismoftentargetedintherapiesforcognitivedisorderslikeAlzheimer'sdisease[10].Furthermore,Na^+/K^+−ATPaseisvitalformaintainingtheionicgradientsacrosstheneuronalmembrane,whichareessentialforneurveimpulseconductionandcellularhomeostasis[26].Itsreducedactivityinthediabeticstate,likelyaconsequenceofoxidativedamagetotheenzymeitself,canleadtoexcitotoxicityandneuronaldeath.Eriodictyol'sabilitytorestoreNa^+/K^+$-ATPase activity indicates its capacity to preserve fundamental neuronal functions and membrane integrity.

The biochemical findings were powerfully substantiated by the histopathological analysis. The extensive neuronal loss, pyknosis, and cellular disorganization observed in the hippocampus of the DC group, particularly in the vulnerable CA1 subfield, provide clear morphological evidence of diabetic neurodegeneration [8, 27]. The remarkable preservation of neuronal architecture in the eriodictyol-treated group is the most compelling evidence of its neuroprotective efficacy. This structural preservation is the ultimate manifestation of the

biochemical benefits observed—reduced oxidative stress and restored enzymatic function translate directly into neuronal survival. The close correlation between the biochemical and histological data strengthens the conclusion that eriodictyol's protective effects are robust and multifaceted. These findings are in line with other studies that have demonstrated the neuroprotective effects of combined metformin and insulin therapy through similar antioxidant mechanisms [39].

While this study provides strong evidence for the neuroprotective effects of eriodictyol, some limitations should be acknowledged. First, the study did not include behavioral tests to directly assess cognitive functions like learning and memory. Future studies incorporating tests such as the Morris water maze or passive avoidance would be valuable to establish a functional correlation with the observed biochemical and histological improvements. Second, this study focused on a single dose of eriodictyol; a dose-response study would provide deeper insight into its therapeutic window. Finally, the underlying molecular signaling pathways modulated by eriodictyol, such as the Nrf2-ARE or PI3K/Akt pathways, were not investigated and represent a promising avenue for future research.

In conclusion, this study demonstrates that eriodictyol administration significantly ameliorates hyperglycemia-induced neurodegeneration in the hippocampus of T2DM rats. Its protective action is associated with a potent antioxidant effect, characterized by the reduction of lipid and protein oxidation, the replenishment of glutathione, and the enhancement of endogenous antioxidant enzyme activities. Furthermore, eriodictyol helps maintain neuronal function by normalizing the activities of AChE and Na$^+/K^+$-ATPase. These comprehensive benefits culminate in the significant preservation of hippocampal neuronal integrity. These findings strongly suggest that eriodictyyol could be a valuable phytotherapeutic agent for the prevention and management of neurological complications associated with Type 2 Diabetes Mellitus.

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