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# Combined *Andrographis paniculata* and Metformin Therapy Attenuates Hippocampal Demyelination and Apoptosis via BAX/BCL-2 Pathway in Hyperglycemic Rats

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## ABSTRACT

Hyperglycemia is associated with hippocampal neuronal injury, apoptosis, and demyelination; Modulation of apoptotic pathways, particularly Bcl-2 associated X protein (BAX) and B-cell lymphoma 2 (Bcl-2) signaling, represents a potential therapeutic target for neuroprotection. Thirty-five male Wistar rats were divided into five groups (n = 7). Hyperglycemia was induced in Groups B–E using alloxan (120 mg/kg) and nicotinamide (110 mg/kg). Group A served as the non-diabetic control while Group B served as the diabetic control. Group C received A. *paniculata* (400 mg/kg), Group D received metformin (14 mg/kg), and Group E received both. Hyperglycemia induced marked hippocampal degeneration characterized by increased BAX expression, reduced BCL-2 signaling, neuronal vacuolation, loss of Nissl bodies, and disrupted myelin architecture, with CA1 and CA2 showing greater vulnerability. Treatment with *Andrographis paniculata* attenuated apoptotic signaling, preserved neuronal morphology, and improved myelin integrity, while metformin produced moderate neuroprotective effects. Combination therapy demonstrated the most pronounced protection, characterized by strong suppression of BAX, enhancement of BCL-2 expression, and preservation of neuronal and myelin structures across hippocampal subfields. *Andrographis paniculata*, particularly in combination with metformin, confers significant neuroprotection against hyperglycemia-induced hippocampal apoptosis and demyelination through modulation of the BAX/BCL-2 pathway. These findings highlight the therapeutic potential of targeting apoptotic mechanisms to preserve hippocampal integrity under metabolic stress.

**Keywords:** *Andrographis paniculata*, metformin, hippocampus, apoptosis, BAX/BCL-2 pathway, diabetic neurodegeneration

## INTRODUCTION

Hyperglycemia, a hallmark of diabetes mellitus, is increasingly recognized as a significant contributor to central nervous system (CNS) dysfunction, particularly affecting brain regions involved in learning and memory. Accumulating evidence indicates that sustained elevations in blood glucose levels trigger a cascade of neuropathological changes, including oxidative stress, inflammation, mitochondrial dysfunction, and ultimately neuronal death[1,2]. These alterations can severely impair neuronal plasticity and connectivity[3], leading to progressive cognitive decline and increased risk for neurodegenerative diseases like Alzheimer's[4,5].

The pathophysiological mechanisms by which hyperglycemia impairs brain function extend beyond metabolic imbalance. Studies have shown that elevated glucose levels disrupt synaptic homeostasis, reduce neurogenesis, and interfere with neurotransmitter signaling[1,6–8]. Such neurotoxic effects are especially prominent in the hippocampus, a structure critical for memory encoding, spatial navigation, and emotional regulation[9,10]. The hippocampus appears particularly vulnerable due to its high metabolic demand[11] and dense concentration of insulin receptors[3], making it a primary target in diabetic encephalopathy. This vulnerability stems from its central role in neuroplasticity, memory formation, learning, as well as its high energy demand and sensitivity to oxidative and metabolic stress[6,12]. Prolonged hyperglycemia impairs hippocampal synaptic plasticity and reduces dendritic spine density, culminating in spatial memory deficits and cognitive dysfunction commonly observed in both experimental models and diabetic patients[11–13].

Apoptosis, or programmed cell death, is increasingly recognized as a pivotal mechanism underlying neurodegeneration in diabetes-related brain injury. In the hyperglycemic brain, excessive glucose promotes a hostile cellular environment marked by oxidative stress, mitochondrial dysfunction, and inflammation, all of which contribute to the activation of the apoptotic pathway[14]. This is particularly evident in the hippocampus, where neuronal apoptosis significantly contributes to cognitive impairment[15,16].

Central to the intrinsic apoptotic cascade is the balance between pro-apoptotic and anti-apoptotic proteins of the BCL-2 family. BAX (Bcl-2-associated X protein) promotes mitochondrial outer membrane permeabilization and cytochrome c release, triggering caspase activation and neuronal death[17]. In contrast, BCL-2 (B-cell lymphoma 2) counters these processes by stabilizing mitochondrial integrity and inhibiting apoptosis[18]. The BAX/BCL-2 ratio, therefore, serves as a critical molecular switch for neuronal fate under pathological stress[19]. Experimental studies have demonstrated that hyperglycemia upregulates BAX expression and suppresses BCL-2, tipping the balance toward cell death[20,21]. This shift in apoptotic signaling has been correlated with histological signs of hippocampal neurodegeneration and impaired memory function in diabetic models[21,22]. Consequently, targeting the BAX/BCL-2 pathway represents a promising therapeutic strategy for neuroprotection in diabetes-induced cognitive decline.

Metformin remains the cornerstone of pharmacological management for type 2 diabetes mellitus due to its glucose-lowering effects and favorable safety profile[23–25]. Recent studies suggest that beyond glycemic control, metformin may also exhibit neuroprotective properties, including anti-inflammatory effects, enhancement of neurogenesis, and attenuation of oxidative stress[6,13,26–28]. These effects are particularly relevant in diabetic brain injury, where multifactorial cellular insults contribute to neuronal dysfunction and apoptosis.

However, while metformin offers some degree of protection, its efficacy in fully reversing neurodegeneration or cognitive impairment under chronic hyperglycemic conditions remains limited. Notably, long-term use of metformin has been associated with vitamin B12 deficiency[29] and other complications that may exacerbate neurological risks in susceptible populations[30]. These limitations underscore the need for adjunctive or alternative therapies that can more directly address the underlying mechanisms of neuronal damage in diabetes, particularly those involving oxidative stress, mitochondrial dysfunction, and apoptosis. Many medicinal plants are rich in bioactive phytochemicals such as flavonoids, diterpenoids, and polyphenols, which have been shown to counteract oxidative stress, modulate inflammatory responses, and regulate apoptotic pathways, which are key contributors to hyperglycemia-induced neurotoxicity[16].

*Andrographis paniculata*, a medicinal plant widely used in traditional systems of medicine, has attracted attention due to its potent antioxidant and anti-apoptotic properties. The plant's major bioactive constituent, andrographolide, has demonstrated the ability to inhibit BAX expression and upregulate BCL-2 in experimental models of neuronal injury[31]. These effects suggest that *Andrographis paniculata* could directly influence the mitochondrial apoptotic mechanism that underlies hippocampal neurodegeneration in diabetes.

Despite a growing body of research highlighting the deleterious effects of chronic hyperglycemia on the brain, especially the hippocampus, the precise molecular mechanisms driving neuronal death remain incompletely understood. Apoptosis, mediated by the imbalance between pro-apoptotic (BAX) and anti-apoptotic (BCL-2) proteins, is increasingly recognized as a central pathway in diabetic neurotoxicity[14,21]. However, evidence from preclinical models specifically linking hyperglycemia-induced apoptosis in the hippocampus to neuronal outcomes remains sparse.

Therefore, this study aimed to investigate the modulatory effects of *Andrographis paniculata* extract, alone and in combination with metformin, by evaluating the mechanistic role of BAX and BCL-2 in hippocampal apoptosis in a hyperglycemic rat model. The study further sought to elucidate the neuroprotective potential of these treatments in mitigating hippocampal neuronal loss and demyelination under hyperglycemic conditions.

## MATERIALS AND METHODS

### Drug and chemicals

Alloxan Monohydrate (Sigma-Aldrich, USA), which was of analytical grade, was sourced from UGOD lab supplies, Lagos, Nigeria. Nicotinamide (Blackmores, Australia), Metformin (Merck Santé, France), and Accu-Chek glucometer (Roche Diagnostics, Germany) were sourced from Yzee Pharmacy Ltd, Nigeria. All other reagents and chemicals used were of analytical grade.

### Preparation of plant extract

Fresh leaves of *Andrographis paniculata* were obtained from a local herb vendor in Sagamu Local Government Area, Ogun State, Nigeria. The plant was authenticated by a botanist in the Department of Plant Science, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. The leaves were air-dried to constant weight at room temperature and pulverized into fine powder. Approximately 1 kg of the powder was extracted in about 2 liters of 70% ethanol (w/v)

using cold maceration. The extract was filtered and concentrated to dryness using a rotary evaporator (Laborota 4000–efficient, Germany) at a temperature of 40 - 45ºC. The dried extract was stored in airtight containers until use. A stock solution of the extract was prepared at 100 mg/mL by dissolving 2000 mg of the dried extract in 20 mL of distilled water. Qualitative phytochemical screening was performed to identify the secondary metabolites present in the extract. Quantitative estimation of the secondary metabolites was conducted using standard procedures as previously described[32].

**Animal care and ethical approval**

Thirty-five (35) adult male Wistar rats, aged 6–8 weeks and weighing 150–210 g, were obtained from the Animal House, Babcock University, Ilishan-Remo, Ogun State, Nigeria. The animals were housed and managed at the Animal House of the Department of Anatomy, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. They were kept in well-ventilated plastic cages (36 × 24 × 15 cm) under standardized environmental conditions (temperature: 28–31°C; 12 h light/dark cycle), with sawdust bedding and environmental enrichment provided. All animals had free access to clean tap water and standard rat chow (Premier Feed Mills Co., Ltd., Nigeria) *ad libitum*. Animal handling and experimental procedures were conducted in accordance with the Principles of Laboratory Animal Care[33] and the NIH Guide for the Care and Use of Laboratory Animals. The study protocol was approved by the Research Ethics Committee of Olabisi Onabanjo University.

**Induction of hyperglycemia**

Hyperglycemia was induced in Groups B–E following an 18-hour overnight fast. Each rat received an intraperitoneal injection of nicotinamide (110 mg/kg body weight; Horbaach, USA) dissolved in physiological saline, followed 15 minutes later by a single intraperitoneal dose of alloxan monohydrate (120 mg/kg body weight; Sigma-Aldrich, Missouri, St. Louis, USA) prepared in cold citrate buffer (pH 4.5) as previously described[8]. Non-Diabetic control animals received an equivalent volume of citrate buffer via the same route. Fasting blood glucose levels were measured 72 hours post-induction using a glucometer (Accu-Chek, Roche Diagnostics, Germany). Rats with fasting blood glucose levels exceeding 200 mg/dL were considered hyperglycemic and included in the treatment protocol.

**Experimental design and grouping**

Following a two-week acclimatization period, thirty-five (35) adult male Wistar rats were randomly assigned into five groups, with seven animals per group (n = 7): Group A served as the non-diabetic control and consisted of non-hyperglycemic, untreated rats that received distilled water via oral gavage; Group B served as the negative control and included hyperglycemic rats that received no treatment; Group C comprised hyperglycemic rats treated with *Andrographis paniculata* extract at a dose of 400 mg/kg body weight; Group D consisted of hyperglycemic rats administered 14 mg/kg body weight of metformin; Group E received a combination of *Andrographis paniculata* extract (400 mg/kg) and metformin (14 mg/kg).

All treatments were given once daily by oral administration for a duration of four weeks. The dose of *Andrographis paniculata* (400 mg/kg) was selected based on previously published studies in diabetic rat models[8,34]. The metformin dose (14 mg/kg) was calculated as the human-equivalent dose from a standard adult dose of 1000 mg using allometric scaling.

**Animal sacrifice and sample collection**

At the end of the four-week treatment period, all animals were deeply anesthetized with halothane (100 mg/kg) and subsequently sacrificed. Following sacrifice, the brain tissues were carefully excised, weighed, and immediately fixed in 10% neutral buffered formalin for 48 hours. Thereafter, the tissues were processed through a graded series of alcohol for dehydration, cleared in xylene, and embedded in paraffin wax using labelled tissue cassettes.

**Histopathological examination of brain tissues**

Paraffin-embedded brain tissues were sectioned at 5 µm thickness using an RWD S700A rotary microtome. The sections were deparaffinized in xylene, rehydrated through a graded ethanol series (100%, 90%, 80%, 70%, and 50%), and rinsed in distilled water. For general morphological assessment of the cerebral cortex, sections were stained with hematoxylin for 5 minutes, rinsed, and counterstained with eosin. To visualize Nissl substances, Cresyl Fast Violet (CFV) staining was performed according to the method of Zhu *et al*.[35]. Additionally, Luxol Fast Blue staining was employed to evaluate the integrity of the myelin sheath, following the procedure described by García-García *et al*[36]. All stained slides were mounted with DPX and allowed to dry overnight before examination under a light microscope.

**Immunohistochemical staining**

Immunohistochemical staining was performed to assess the expression of pro-apoptotic (BAX) and anti-apoptotic (BCL2) proteins in the hippocampus, following the protocol of Shalaby *et al*.[7] Paraffin-embedded sections were deparaffinized, rehydrated, and treated with 3% hydrogen peroxide to block endogenous peroxidase activity. Non-specific binding was minimized using goat serum as a blocking agent. The sections were then incubated for 60 minutes at room temperature with the following primary antibodies: BAX; Mouse monoclonal antibody (Cat. No. SC-7480, Santa Cruz Biotechnology, USA) and

BCL2; Mouse monoclonal antibody (Cat. No. SC-7382, Santa Cruz Biotechnology, USA). After rinsing, sections were incubated with a biotinylated secondary antibody, followed by treatment with a peroxidase reagent. Visualization was achieved using 3-Amino-9-ethylcarbazole (AEC) chromogen; sections were counterstained with Mayer's hematoxylin, rinsed, and mounted with DPX. Positive immunoreactivity was identified by reddish-brown cytoplasmic staining, while cell nuclei appeared blue.

All stained brain sections (H&E, CFV, LFB, BAX, and BCL2) were examined using a binocular microscope (Leica ICC50 W, Germany), and photomicrographs were captured using an attached camera. An independent, blinded histopathologist conducted qualitative assessments of neuronal structure, Nissl substance, myelin integrity, and immunoreactivity.

**Image quantification**

Quantitative image analysis was performed using ImageJ software (NIH, USA) to measure staining intensity for BAX and BCL2 expressions. Briefly, photomicrographs were captured at 400× magnification under consistent lighting and exposure settings to ensure standardization. Using the Color Deconvolution plugin in ImageJ, chromogenic signals were separated from hematoxylin counterstains. Thresholding was applied uniformly to isolate positive immunoreactive areas. Three non-overlapping regions of interest (ROIs) were randomly selected from the CA1, CA2, CA3, and Dentate Gyrus regions for each sample. For each ROI, the percentage of positively stained area and optical density (OD) were calculated to determine the staining area and staining intensity, respectively.

**Statistical analysis**

The numerical data obtained were expressed as mean ± standard error of the mean (SEM). Statistical comparisons between groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons, using GraphPad Prism version 8.0. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

**Histopathological assessment of hippocampal neurons**

Hematoxylin and Eosin staining (Figure 1) revealed hippocampal histoarchitecture. The non-diabetic control group (Group A) exhibited normal hippocampal cytoarchitecture, characterized by compact, well-aligned pyramidal neurons with prominent hyperchromatic nuclei, indicative of preserved cellular integrity. In contrast, the diabetic control group (Group B) showed disrupted pyramidal layer organization, with less compacted neurons and reduced chromatic intensity relative to the non-diabetic control, suggesting decreased cell density and/or diminished staining affinity. The *Andrographis paniculata*–treated group displayed mild histopathological alterations, including focal vacuolation and subtle chromatin changes when compared with the non-diabetic control, indicative of moderate cellular stress. A comparable morphological pattern was observed in the metformin-treated group, which similarly showed vacuolation and chromatin alterations.

**Histochemical evaluation of hippocampal myelination and Nissl substance expression**

Cresyl Fast Violet (CFV) staining (Figure 2) in the non-diabetic control group revealed a well-preserved pyramidal layer with orderly arrangements of neurons and glial cells and prominent Nissl bodies, indicating intact neuronal integrity. In contrast, the diabetic control group showed marked histopathological alterations characterized by reduced neuronal density and scanty Nissl substances. The *Andrographis paniculata* (AP)–treated group demonstrated a mild loss of Nissl bodies compared with the negative control, while the metformin-treated group exhibited a moderate reduction in Nissl substances. Notably, combined treatment with AP and metformin resulted in evident regeneration of Nissl bodies and improved neuronal organization, approaching the histological appearance of the non-diabetic control.

Similarly, Luxol Fast Blue (LFB) staining (Figure 3) in the non-diabetic control group showed uniform and intense staining of myelinated fibers and neuronal cells, reflecting preserved myelin integrity. The diabetic control group exhibited reduced staining with visible gaps and structural disruptions, indicative of myelin loss and damage. The AP-treated group showed mild LFB staining relative to the negative control, whereas the metformin group demonstrated more uniform staining. The combined AP and metformin treatment group showed the most pronounced improvement, with enhanced and uniform LFB staining among all treatment groups, suggesting restoration of myelinated fibers.

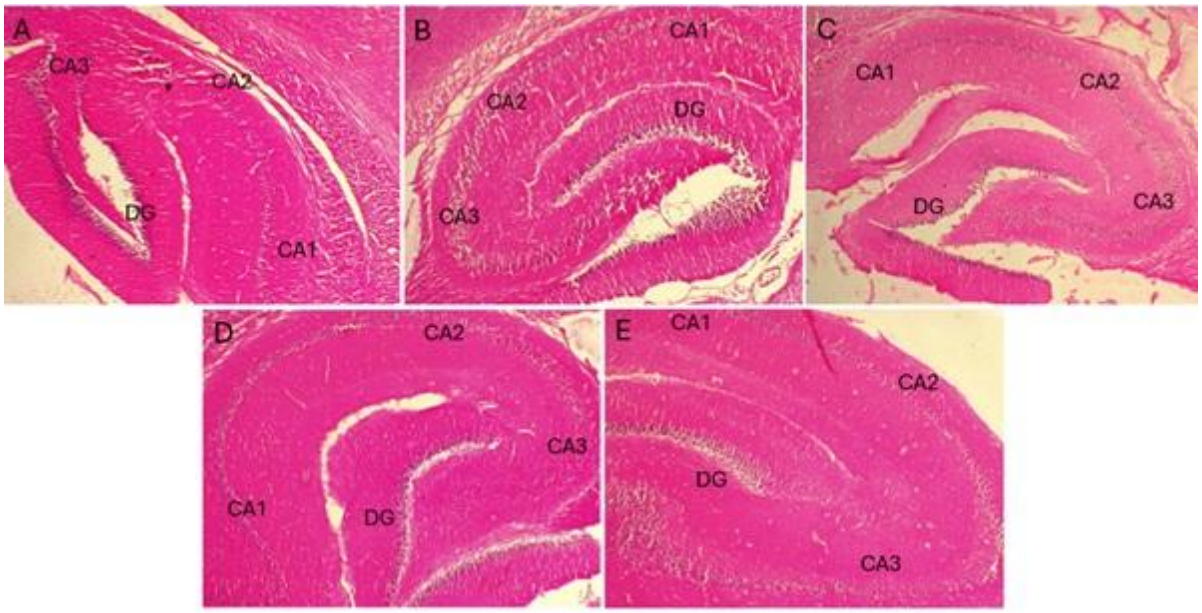


**Figure 1:** Representative photomicrographs (40×) from each group (A-E) showing the general histoarchitecture of the hippocampus. (H&E). A – Non-Diabetic Control, B – Diabetic Control, C – Andrographis paniculata Treated, D – Metformin Treated, E – Andrographis paniculata + Metformin Co-treated. CA - Cornu Ammonis, DG – Dentate Gyrus.

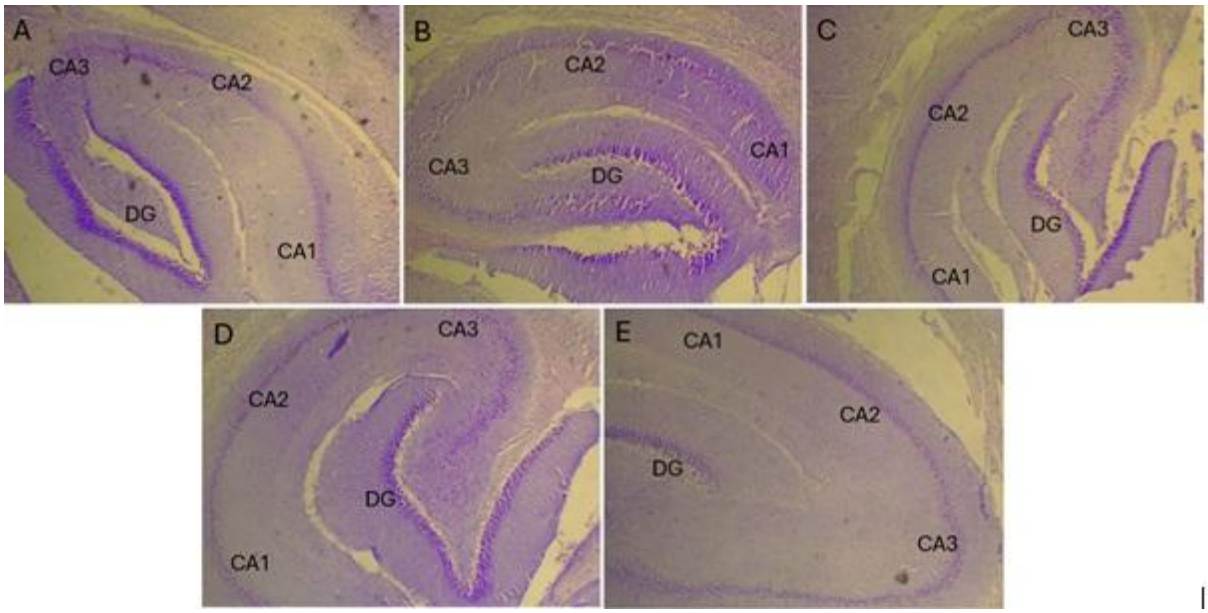


**Figure 2:** Representative photomicrographs (40×) from each group (A-E) showing Nissl substance distribution in the hippocampus. (CFV). A – Non-Diabetic Control, B – Diabetic Control, C – Andrographis paniculata Treated, D – Metformin Treated, E – Andrographis paniculata + Metformin Co-treated. CA - Cornu Ammonis, DG – Dentate Gyrus.

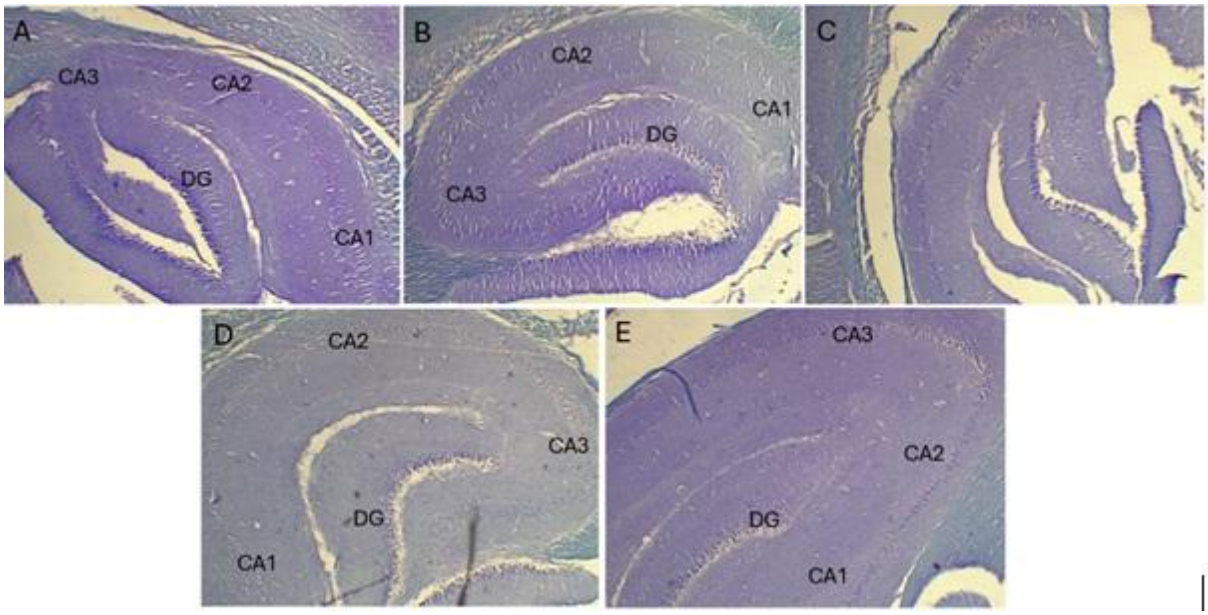


**Figure 3:** Representative photomicrographs (40×) from each group (A-E) showing myelin integrity within the hippocampus. (LFB). A – Non-Diabetic Control, B – Diabetic Control, C – Andrographis paniculata Treated, D – Metformin Treated, E – Andrographis paniculata + Metformin Co-treated. CA - Cornu Ammonis, DG – Dentate Gyrus.

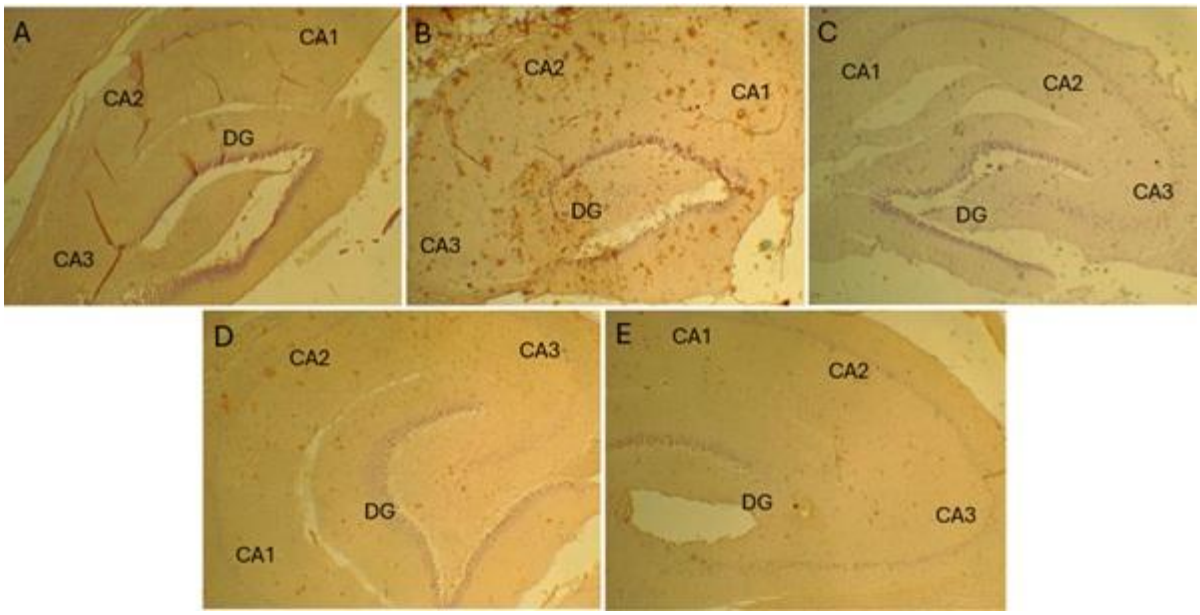


**Figure 4:** Representative photomicrographs (40×) from each group (A-E) showing BAX-positive cells in the hippocampus. (BAX). A – Non-Diabetic Control, B – Diabetic Control, C – Andrographis paniculata Treated, D – Metformin Treated, E – Andrographis paniculata + Metformin Co-treated. CA - Cornu Ammonis, DG – Dentate Gyrus.

**Immunohistochemical analysis of hippocampal apoptosis (BAX and BCL-2)**

BAX immunostaining (Figure 4), indicative of apoptotic activity, was minimal in the non-diabetic control group, reflecting a low basal level of apoptosis. The negative control group exhibited extensive BAX immunoreactivity throughout the hippocampus, consistent with elevated apoptosis. Treatment with *Andrographis paniculata* resulted in reduced BAX-positive cells relative to the negative control, while the metformin-treated group showed moderate BAX immunostaining. Notably, combined *Andrographis paniculata* and metformin treatment displayed the lowest level of BAX staining among all experimental groups, indicating substantial attenuation of apoptosis.

BCL2 immunostaining (Figure 5), indicative of anti-apoptotic signaling, was robust in the non-diabetic control group, reflecting healthy neuronal populations. In contrast, the negative control exhibited markedly reduced BCL2 immunoreactivity, consistent with heightened apoptotic susceptibility. Both *Andrographis paniculata* and metformin treatment groups demonstrated increased BCL2 staining relative to the negative control, with the metformin group exhibiting more pronounced staining. The combined treatment group showed the highest BCL2 immunoreactivity among all groups, suggesting maximal preservation of anti-apoptotic activity.

**Quantitative image analysis of hippocampal subfields**

Quantitative analysis (Figure 6) of BAX immunostaining revealed significant differences across hippocampal subregions (CA1, CA2, CA3, DG) and experimental groups. In the non-diabetic control (Group A), all subfields showed low stained area and uniformly low optical density (OD), with CA3 having the largest stained area. The negative control (Group B) displayed the highest stained area across all regions (CA1 > CA2 > CA3 > DG) and significantly elevated OD compared to Group A ($p < 0.05$). *Andrographis paniculata* treatment (Group C) markedly reduced stained area across all subregions ($p < 0.05$ vs. Group B), with low and uniform OD. Metformin (Group D) exhibited moderate stained areas, higher than Group A but lower than Group B, with relatively uniform OD across regions. The combined treatment (Group E) maintained low stained area and modest OD across all subfields, with CA2 showing the largest area within this group, reflecting effective attenuation of apoptosis.

Conversely, quantitative analysis (Figure 7) of BCL2 staining revealed distinct regional and group-specific patterns. Group A (non-diabetic control) showed generally low stained area across hippocampal subfields, with CA2 and DG slightly higher than CA1 and CA3, and relatively uniform OD. Group B (negative control) displayed significantly higher stained area than Group A ($p < 0.05$), particularly in CA1 and DG, but OD was moderately elevated, indicating less intense staining per unit area. *Andrographis paniculata* treatment (Group C) maintained a low stained area comparable to Group A, with OD significantly higher than Group B in DG and CA3 ($p < 0.05$). Metformin (Group D) showed an elevated stained area in all subfields (CA2 > CA3 > DG > CA1) with the highest average OD across the hippocampus, suggesting intense anti-apoptotic protein expression. The combination group (Group E) exhibited a low stained area relative to Group B with modest OD across all subfields, slightly lower than Group A and significantly lower than Group D ($p < 0.05$), indicating a balanced restoration of anti-apoptotic signaling.

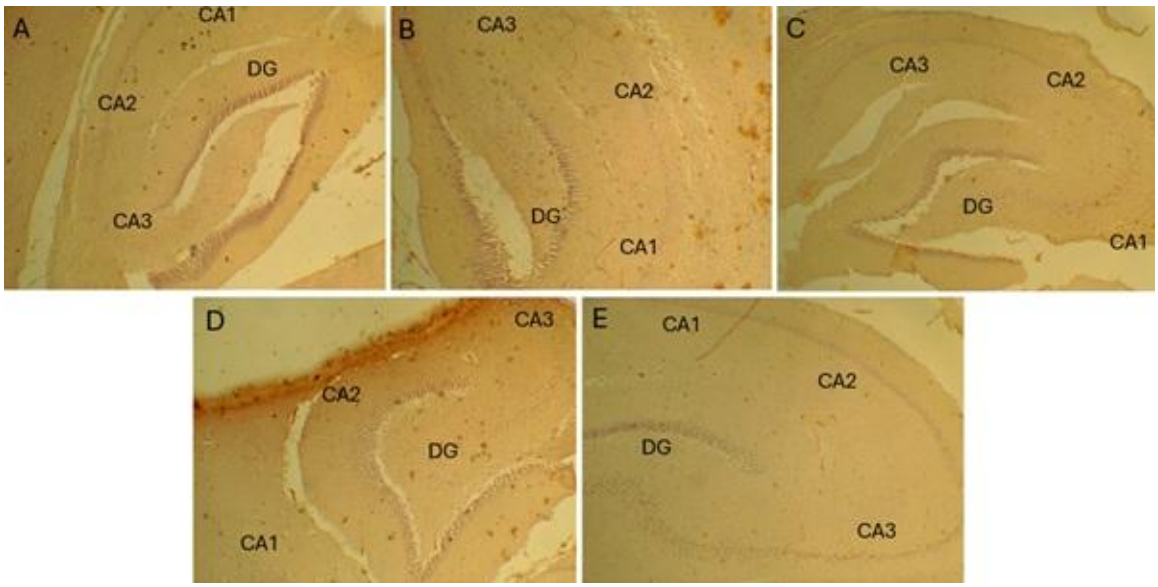


**Figure 5:** Representative photomicrographs (40×) from each group (A-E) showing BCL-2 immunoreactivity in the hippocampus. (BCL-2). A – Non-Diabetic Control, B – Diabetic Control, C – Andrographis paniculata Treated, D – Metformin Treated, E – Andrographis paniculata + Metformin Co-treated. CA - Cornu Ammonis, DG – Dentate Gyrus.

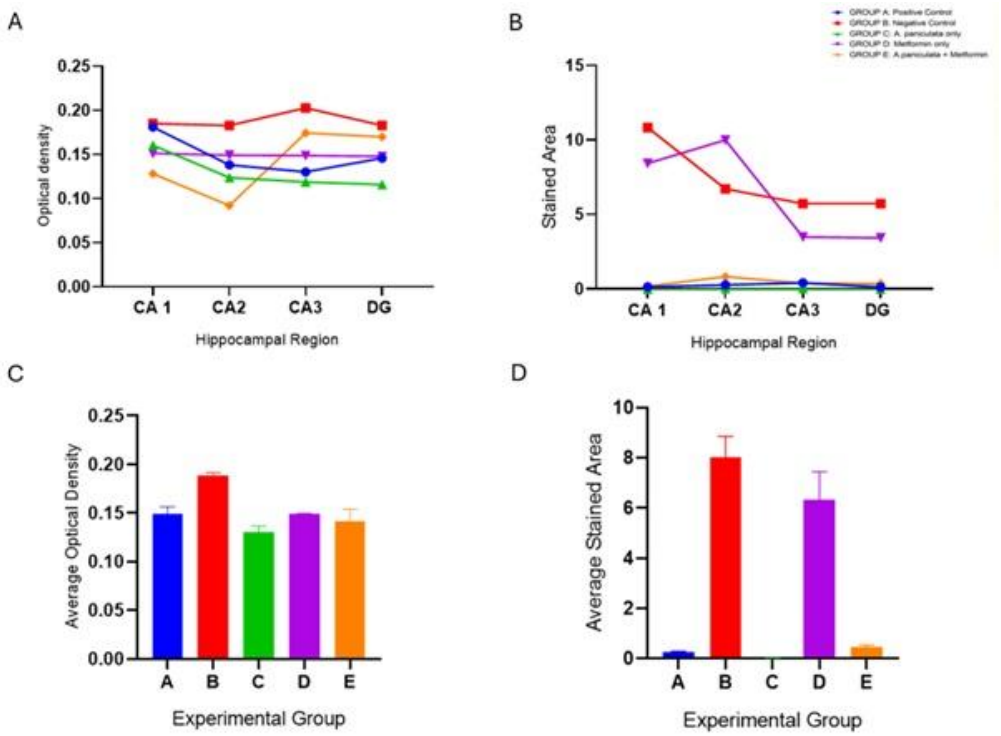


**Figure 6:** Image quantification of BAX immunoreactivity across hippocampal subregions and experimental groups. (A) Optical density of BAX immunostaining for each hippocampal region (CA1, CA2, CA3, and DG). (B) Percentage-stained area of BAX immunoreactivity for each hippocampal region (CA1, CA2, CA3, and DG). (C) Average optical density of BAX staining per experimental group. (D) Average percentage-stained area of BAX immunoreactivity per experimental group.

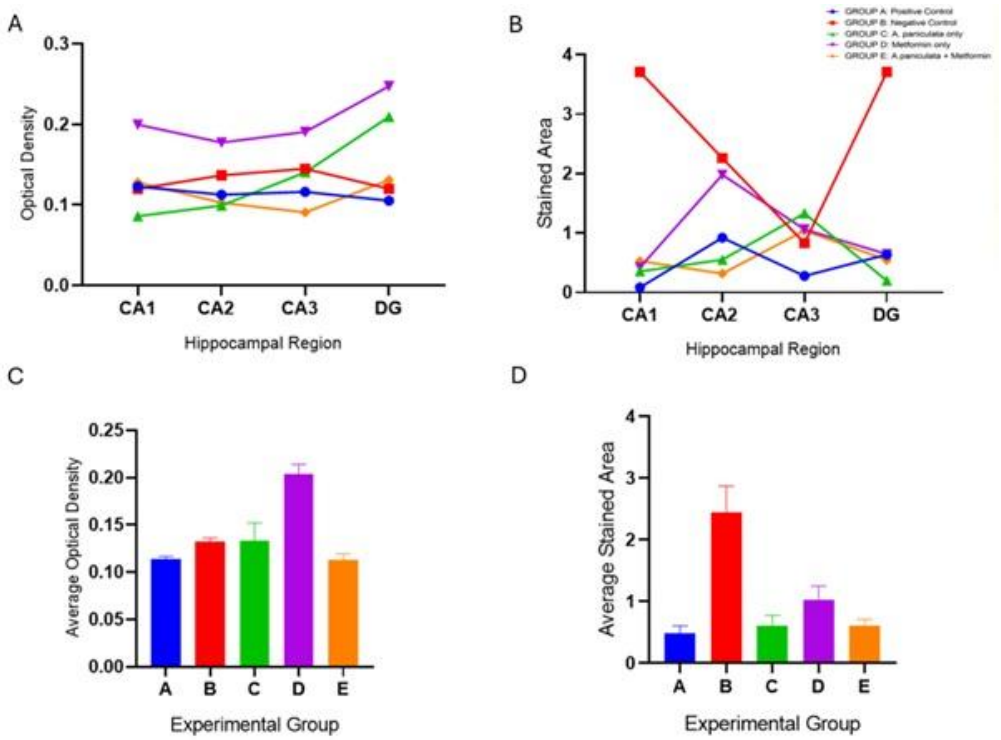


**Figure 7:** Image quantification of BCL-2 immunoreactivity across hippocampal subregions and experimental groups. (A) Optical density of BCL-2 immunostaining for each hippocampal region (CA1, CA2, CA3, and DG). (B) Percentage-stained area of BCL-2 immunoreactivity for each hippocampal region (CA1, CA2, CA3, and DG). (C) Average optical density of BCL-2 staining per experimental group. (D) Average percentage-stained area of BCL-2 immunoreactivity per experimental group.

## DISCUSSION

This study investigated the neuroprotective effects of *Andrographis paniculata* (AP), alone and in combination with metformin, on hippocampal apoptosis and demyelination under hyperglycemic conditions. Our findings demonstrate that hyperglycemia induced marked hippocampal neuronal injury, characterized by elevated BAX expression, reduced BCL-2 levels, vacuolation, karyolysis, pyknosis, loss of Nissl bodies, and disrupted myelin architecture. *Andrographis paniculata* treatment alone effectively attenuated these pathological changes, markedly reducing BAX immunoreactivity, partially restoring BCL-2 expression, and preserving neuronal and myelin integrity. The combination *of Andrographis paniculata* and metformin produced the most significant effects ($p < 0.05$), with near-complete suppression of BAX, substantial enhancement of BCL-2, and robust preservation of both Nissl bodies and myelinated fibres. Quantitative analysis further revealed subregion-specific effects, with CA1 and CA2 exhibiting the highest vulnerability to hyperglycemic insult, while CA3 and the dentate gyrus showed comparatively greater resilience, particularly in response to *Andrographis paniculata* and combination therapy. These results collectively indicate that *Andrographis paniculata*, especially when combined with metformin, confers significant neuroprotection against hyperglycemia-induced hippocampal apoptosis and demyelination.

Hyperglycemia-induced apoptosis and degenerative changes throughout the hippocampus highlight the region's vulnerability to metabolic stress[11,12]. Elevated BAX expression coupled with diminished BCL-2 signaling indicates activation of the intrinsic apoptotic pathway, which likely drives the observed neuronal loss and vacuolation. Morphological disruptions, including nuclear pyknosis, karyolysis, loss of Nissl bodies, and demyelination, reflect the structural consequences of this apoptotic imbalance. Notably, CA1 and CA2 were particularly susceptible, consistent with their known sensitivity to oxidative and metabolic insults, whereas CA3 and the dentate gyrus demonstrated relative resilience. These patterns underscore subfield-specific vulnerability within the hippocampus and suggest that hyperglycemia compromises neuronal survival and myelin integrity[38,39] in regions critical for synaptic integration and cognitive processing[2,3]. Collectively, these findings reinforce the mechanistic link between hyperglycemic stress, intrinsic apoptosis, and structural degeneration, providing a foundation for evaluating interventions aimed at mitigating hippocampal injury.

Administration of *Andrographis paniculata* alone markedly mitigated hyperglycemia-induced hippocampal apoptosis, as evidenced by near-complete suppression of BAX expression and partial restoration of BCL-2 levels. This modulation of the intrinsic apoptotic pathway was accompanied by preservation of neuronal morphology, with pyramidal neurons maintaining compact alignment and hyperchromatic nuclei. CFV staining indicated retention of Nissl bodies, suggesting preserved protein synthesis capacity, while LFB staining demonstrated improved myelin integrity across subfields, particularly in CA3 and DG. These findings suggest that *Andrographis paniculata's* neuroprotective effect involves both the inhibition of pro-apoptotic signaling[31,40] and the maintenance of structural neuronal and myelin integrity[8,41]. The relative subfield-specific preservation, with CA3 and DG responding more robustly than CA1 and CA2, highlights differential vulnerability within the hippocampus and indicates that *Andrographis paniculata* may selectively bolster resilience in regions that are moderately susceptible to hyperglycemic stress. Collectively, these data support a mechanistic link between *Andrographis paniculata*-mediated regulation of BAX/BCL-2[40] and the structural maintenance of neurons and myelinated fibers[41,42].

Metformin treatment conferred moderate neuroprotection against hyperglycemia-induced hippocampal damage, reflected by partial reduction in BAX expression and enhancement of BCL-2 immunoreactivity. Morphologically, pyramidal neurons displayed improved organization and reduced vacuolation, while CFV and LFB staining indicated partial preservation of Nissl bodies and myelin integrity. Unlike *Andrographis paniculata*, metformin's protective effects were more pronounced in CA2 and DG, suggesting a subfield-specific pattern of resilience. However, CA1 remained relatively susceptible, consistent with the region's heightened vulnerability to metabolic stress[43]. When compared to *Andrographis paniculata*, metformin's effect on BAX suppression was less pronounced, though it effectively enhanced BCL-2 expression, indicating that its neuroprotective mechanism may rely more on augmenting anti-apoptotic signaling[44] than on direct inhibition of pro-apoptotic pathways[45]. These findings underscore metformin's potential to partially restore hippocampal structural integrity under hyperglycemic conditions.

The combined administration of *Andrographis paniculata* and metformin produced the most significant effects, surpassing those observed with either treatment alone. BAX expression was effectively suppressed across all hippocampal subfields, while BCL-2 levels were substantially enhanced, indicating robust modulation of the intrinsic apoptotic pathway. Morphological analysis revealed well-preserved pyramidal neurons with

compact alignment and minimal vacuolation, accompanied by restoration of Nissl bodies and uniform myelin integrity as demonstrated by CFV and LFB staining. Subfield-specific analysis showed particularly strong preservation in CA2 and CA3, regions that appeared partially resilient to *Andrographis paniculata* or metformin monotherapy. These findings suggest that the combination therapy exerts additive or synergistic effects, simultaneously attenuating pro-apoptotic signaling and enhancing anti-apoptotic mechanisms, thereby maximizing the preservation of neuronal and myelin structures. The results underscore the potential of combination therapy as a more effective strategy[46,47] to protect the hippocampus against hyperglycemia-induced apoptosis[14–16,20] and demyelination.

The observed structural preservation of hippocampal neurons and myelinated fibers appears closely linked to modulation of the BAX/BCL-2 apoptotic pathway. Suppression of BAX coupled with restoration of BCL-2 in AP-treated and combination-treated groups correlated with the retention of Nissl bodies and uniform myelin architecture, indicating that inhibition of pro-apoptotic signaling directly contributes to the maintenance of neuronal protein synthesis capacity[48] and myelin integrity. Subfield-specific analyses revealed that CA1 and CA2 were the most sensitive to hyperglycemic stress[37,43,], showing pronounced apoptosis and demyelination in untreated conditions, whereas CA3 and the dentate gyrus demonstrated relative resilience, particularly under *Andrographis paniculata* and combination therapy. These findings suggest a mechanistic hierarchy in which BAX/BCL-2 regulation mediates both neuronal survival and myelin preservation, with differential responses among hippocampal subfields. This integrated relationship underscores the dual role of apoptotic modulation in safeguarding both neuronal viability and structural connectivity within the hippocampus under hyperglycemic conditions.

The robust neuroprotective effects observed with *Andrographis paniculata*, particularly in combination with metformin, suggest potential therapeutic strategies for mitigating hyperglycemia-induced hippocampal injury. By modulating the BAX/BCL-2 pathway and preserving neuronal and myelin integrity, these interventions could contribute to the maintenance of hippocampal structure under metabolic stress, which is critical for cognitive processes such as learning and memory[9,10]. While extrapolation to human conditions requires caution, these findings highlight the neuroprotective potential of *Andrographis paniculata*, alone or in combination with conventional anti-hyperglycemic agents, as a candidate for preventing or reducing hippocampal apoptosis and demyelination associated with diabetes or other metabolic disorders. The results also support further investigation into combination therapy approaches that target both metabolic and apoptotic pathways to enhance hippocampal resilience.

## CONCLUSION

Collectively, these findings underscore the therapeutic potential of *Andrographis paniculata* and A. *paniculata* – metformin combination strategies in mitigating metabolic stress–induced hippocampal injury and provide a mechanistic framework for future translational studies aimed at preserving cognitive function under hyperglycemic conditions.

## REFERENCES


1. Adeeyo O, Oyeleke A, Ajibola T, Oguntope E. Carica papaya ameliorates Nissl bodies, grey matter distortion in streptozotocin-induced cerebral hyperglycemia. Achiev J Sci Res. 2023;4(2):1–8.
2. Backeström A, Papadopoulos K, Eriksson S, Olsson T, Andersson M, Blennow K, *et al*. Acute hyperglycemia leads to altered frontal lobe brain activity and reduced working memory in type 2 diabetes. PLoS One. 2021;16(3):e0247753. doi:10.1371/journal.pone.0247753
3. Ansari MA, Al-Jarallah A, Babiker FA. Impaired insulin signalling alters mediators of hippocampal synaptic dynamics/plasticity: a possible mechanism of hyperglycemia-induced cognitive impairment. Cells. 2023;12(13):13. doi:10.3390/cells12131728
4. Dar TA, Sheikh IA, Ganie SA, Ali R, Singh LR, Gan SH, *et al*. Molecular linkages between diabetes and Alzheimer's disease: current scenario and future prospects. CNS Neurol Disord Drug Targets. 2014;13(2):290–8. doi:10.2174/18715273113126660135 PubMed PMID: 24059323.
5. Hiruy EG, Abate TW, Temesgen WA, Bantie B, Yirga GK, Muhamed AN, *et al*. Cognitive impairment and associated factors among adults with type 2 diabetes mellitus in Bahir Dar City referral hospitals, 2021: cross-sectional study. J Alzheimers Dis. 2024;100(1):45–52. doi:10.3233/JAD-230245
6. Sritawan N, Suwannakot K, Naewla S, Chaisawang P, Aranarochana A, Sirichoat A, *et al*. Effect of metformin treatment on memory and hippocampal neurogenesis decline correlated with oxidative stress induced by methotrexate in rats. Biomed Pharmacother. 2021;144:112280. doi:10.1016/j.biopha.2021.112280
7. Shalaby AM, Aboregela AM, Alabiad MA, Tayssir Sadek M. The effect of induced diabetes mellitus on the cerebellar cortex of adult male rat and the possible protective role of oxymatrine: a histological,

immunohistochemical and biochemical study. Ultrastruct Pathol. 2021;45(3):182–96. doi:10.1080/01913123.2021.1926610

8. Onanuga IO, Folarin RO, Muniru ET, Ibrahim RB, Usman IM, Jegede AI, *et al*. Immuno-impaired expression of synaptophysin, GFAP and Nissl substances in the cerebral cortex of diabetic Wistar rats; evaluation of *Andrographis paniculata* effects. Biol Med Nat Prod Chem. 2026;15(1):465–74. doi:10.14421/biomedich.2026.151.465-474
9. Fogwe LA, Reddy V, Mesfin FB. Neuroanatomy, hippocampus. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available from: http://www.ncbi.nlm.nih.gov/books/NBK482171/ PubMed PMID: 29489273.
10. Chauhan P, Jethwa K, Rathawa A, Chauhan G, Mehra S. The anatomy of the hippocampus. In: Pluta R, editor. Cerebral Ischemia [Internet]. Brisbane (AU): Exon Publications; 2021. Available from: http://www.ncbi.nlm.nih.gov/books/NBK575732/ PubMed PMID: 34905307.
11. Shima A, Noguchi-Shinohara M, Shibata S, Usui Y, Tatewaki Y, Thyreau B, *et al*. Glucose metabolism and smaller hippocampal volume in elderly people with normal cognitive function. Npj Aging. 2024;10(1):39. doi:10.1038/s41514-024-00164-2 PubMed PMID: 39251602; PubMed Central PMCID: PMC11384785.
12. Onasanwo SA, Adamaigbo VO, Adebayo OG, Eleazer SE. Moringa oleifera-supplemented diet protects against cortico-hippocampal neuronal degeneration in scopolamine-induced spatial memory deficit in mice: role of oxido-inflammatory and cholinergic neurotransmission pathway. Metab Brain Dis. 2021;36(8):2445–60. doi:10.1007/s11011-021-00855-9
13. Ahmed S, Mahmood Z, Javed A, Hashmi SN, Zerr I, Zafar S, *et al*. Effect of metformin on adult hippocampal neurogenesis: comparison with donepezil and links to cognition. J Mol Neurosci. 2017;62(1):88–98. doi:10.1007/s12031-017-0915-z
14. Ünver Saraydin S, Özdenoğlu Kutlu B, Saraydin D. Effects of diabetes on apoptosis and mitosis in rat hippocampus. Biotech Histochem. 2021;96(6):460–7. doi:10.1080/10520295.2020.1818827 PubMed PMID: 32938250.
15. Li ZG, Zhang W, Grunberger G, Sima AAF. Hippocampal neuronal apoptosis in type 1 diabetes. Brain Res. 2002;946(2):221–31. doi:10.1016/S0006-8993(02)02887-1
16. Sadeghi A, Hami J, Razavi S, Esfandiary E, Hejazi Z. The effect of diabetes mellitus on apoptosis in hippocampus: cellular and molecular aspects. Int J Prev Med. 2016;7(1):57. doi:10.4103/2008-7802.178531
17. Spitz AZ, Gavathiotis E. Physiological and pharmacological modulation of BAX. Trends Pharmacol Sci. 2022;43(3):206–20. doi:10.1016/j.tips.2021.11.001 PubMed PMID: 34848097.
18. Czabotar PE, García-Sáez AJ. Mechanisms of BCL-2 family proteins in mitochondrial apoptosis. Nat Rev Mol Cell Biol. 2023;24(10):732–48. doi:10.1038/s41580-023-00629-4
19. Putri GFT, Nurfatihah ZZ. Bax/Bcl-2 ratio as the golden marker of apoptosis: molecular mechanisms and regulatory pathways. Int J Cell Biomed Sci. 2025;4(10):309–17. doi:10.59278/cbs.v4i10.65
20. Gokalp-Ozkorkmaz E, Kirman G, Pekkolay Z, Asir F, Deveci E. Expression of apoptotic proteins Bax and Bcl-2 in blood cells of type 2 diabetic patients. Proceedings. 2018;2(25):1563. doi:10.3390/proceedings2251563
21. Azab EFE, Mostafa HS. Geraniol ameliorates the progression of high fat-diet/streptozotocin-induced type 2 diabetes mellitus in rats via regulation of caspase-3, Bcl-2, and Bax expression. J Food Biochem. 2022;46:e14142. doi:10.1111/jfbc.14142 PubMed PMID: 35312192.
22. Godam ET, Hamman WO, Oladele S, Samaila MO, Musa SA. Immunohistochemical studies of the upregulation of BCL-2 and downregulation of Bax in the hippocampus of STZ-induced type 1 diabetic rats co-administered melatonin and magnesium. Mathews J Cytol Histol. 2020;4(1):1–10. doi:10.30654/MJCH.10014
23. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017;60(9):1577–85. doi:10.1007/s00125-017-4342-z PubMed PMID: 28776086; PubMed Central PMCID: PMC5552828.
24. Baker C, Retzik-Stahr C, Singh V, Plomondon R, Anderson V, Rasouli N. Should metformin remain the first-line therapy for treatment of type 2 diabetes? Ther Adv Endocrinol Metab. 2021;12:2042018820980225. doi:10.1177/2042018820980225 PubMed PMID: 33489086; PubMed Central PMCID: PMC7809522.
25. Garber AJ. Metformin: mechanisms of antihyperglycemic action, other

pharmacodynamic properties, and safety perspectives. Endocr Pract. 1997;3(6):359–70. doi:10.4158/EP.3.6.359

26. Dehkordi AH, Abbaszadeh A, Mir S, Hasanvand A. Metformin and its anti-inflammatory and anti-oxidative effects; new concepts. J Ren Inj Prev. 2018;8(1):1. doi:10.15171/jrip.2019.11
27. Zhu X, Shen J, Feng S, Huang C, Liu Z, Sun YE, *et al*. Metformin improves cognition of aged mice by promoting cerebral angiogenesis and neurogenesis. Aging (Albany NY). 2020;12(18):17845–62. doi:10.18632/aging.103693 PubMed PMID: 32938817; PubMed Central PMCID: PMC7585073.
28. Asadbegi M, Yaghmaei P, Salehi I, Ebrahim-Habibi A, Komaki A. Neuroprotective effects of metformin against Aβ-mediated inhibition of long-term potentiation in rats fed a high-fat diet. Brain Res Bull. 2016;121:178–85. doi:10.1016/j.brainresbull.2016.02.005
29. Sayedali E, Yalin AE, Yalin S. Association between metformin and vitamin B12 deficiency in patients with type 2 diabetes. World J Diabetes. 2023;14(5):585–93. doi:10.4239/wjd.v14.i5.585 PubMed PMID: 37273250; PubMed Central PMCID: PMC10236989.
30. Infante M, Leoni M, Caprio M, Fabbri A. Long-term metformin therapy and vitamin B12 deficiency: an association to bear in mind. World J Diabetes. 2021;12(7):916–31. doi:10.4239/wjd.v12.i7.916 PubMed PMID: 34326945; PubMed Central PMCID: PMC8311483.
31. Li J, Lu J, Chen G, Li D. Andrographolide protects retinal ganglion cells in rats with glaucoma by regulating the Bcl-2/Bax/caspase-3 signaling pathway. World J Tradit Chin Med. 2022;8(2):241. doi:10.4103/wjtcm.wjtcm_50_21
32. Nagajothi S, Mekala P, Raja A, Raja MJ, Senthilkumar P. *Andrographis paniculata*: qualitative and quantitative phytochemical analysis. J Pharmacogn Phytochem. 2018;7(4):1251–3.
33. Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF. The 1996 Guide for the Care and Use of Laboratory Animals. ILAR J. 1997;38(1):41–8. doi:10.1093/ilar.38.1.41
34. Dandu A, Inamdar M. Evaluation of beneficial effects of antioxidant properties of aqueous leaf extract of *Andrographis paniculata* in STZ-induced diabetes. Pak J Pharm Sci. 2009;22:49–52.
35. Zhu Y, Liu F, Zou X, Torbey M. Comparison of unbiased estimation of neuronal number in the rat hippocampus with different staining methods. J Neurosci Methods. 2015;254:73–9. doi:10.1016/j.jneumeth.2015.07.022
36. García-García ÓD, Weiss T, Chato-Astrain J, Raimondo S, Carriel V. Staining methods for normal and regenerative myelin in the nervous system. In: Pellicciari C, Biggiogera M, Malatesta M, editors. Histochemistry of Single Molecules: Methods and Protocols [Internet]. New York, NY: Springer US; 2023. p. 187–203. doi:10.1007/978-1-0716-2675-7_15
37. Wang X, Pal R, Chen XW, Limpeanchob N, Kumar KN, Michaelis EK. High intrinsic oxidative stress may underlie selective vulnerability of the hippocampal CA1 region. Mol Brain Res. 2005;140(1):120–6. doi:10.1016/j.molbrainres.2005.07.018
38. Wang WT, Lee P, Yeh HW, Smirnova IV, Choi IY. Effects of acute and chronic hyperglycemia on the neurochemical profiles in the rat brain with streptozotocin-induced diabetes detected using in vivo $^{1}$H MR spectroscopy at 9.4 T. J Neurochem. 2012;121(3):407–17. doi:10.1111/j.1471-4159.2012.07698.x
39. Morozova MP, Savinkova IG, Gorbacheva LR. Peculiarities of brain cell functioning in hyperglycemia and diabetes mellitus. J Evol Biochem Physiol. 2024;60(4):1531–45. doi:10.1134/s0022093024040215
40. Thai QK, Huynh P, Tran TT, Nguyen BH, Nguyen HTT. Investigating *Andrographis paniculata* compounds for apoptosis induction in cancer. Asian Pac J Cancer Prev. 2025;26(7):2657–67. doi:10.31557/APJCP.2025.26.7.2657 PubMed PMID: 40729089; PubMed Central PMCID: PMC12530664.
41. Radhika P, Annapurna A, Rao SN. Immunostimulant, cerebroprotective and nootropic activities of *Andrographis paniculata* leaves extract in normal and type 2 diabetic rats. Indian J Med Res. 2012;135(5):636.
42. Otong ES, Makena W, Solomon AY, Bazabang SA, Aminu A, Henry R. *Andrographis paniculata* protects against brain hippocampus and cerebellum from mercury chloride-induced damage by attenuating oxidative stress. Environ Anal Health Toxicol. 2022;37(4):e2022027. doi:10.5620/eaht.2022027 PubMed PMID: 36916040.
43. Bartsch T, Döhring J, Reuter S, Finke C, Rohr A, Brauer H, *et al*. Selective neuronal vulnerability of human hippocampal CA1 neurons: lesion evolution, temporal course, and pattern of hippocampal damage in diffusion-weighted MR imaging. J Cereb Blood Flow

Metab. 2015;35(11):1836–45. doi:10.1038/jcbfm.2015.137 PubMed PMID: 26082014; PubMed Central PMCID: PMC4635239.

44. Chen D, Xia D, Pan Z, Xu D, Zhou Y, Wu Y, *et al*. Metformin protects against apoptosis and senescence in nucleus pulposus cells and ameliorates disc degeneration in vivo. Cell Death Dis. 2016;7(10):e2441. doi:10.1038/cddis.2016.334

45. Chukkapalli V, Gordon LI, Venugopal P, Borgia JA, Karmali R. Metabolic changes associated with metformin potentiate Bcl-2 inhibitor, Venetoclax, and CDK9 inhibitor, BAY1143572, and reduce viability of lymphoma cells. Oncotarget. 2018;9(30):21166–81. doi:10.18632/oncotarget.24989 PubMed PMID: 29765528; PubMed Central PMCID: PMC5940409.

46. Prabhakar PK, Kumar A, Doble M. Combination therapy: a new strategy to manage diabetes and its complications. Phytomedicine. 2014;21(2):123–30. doi:10.1016/j.phymed.2013.08.020

47. Xie X, Wu C, Hao Y, Wang T, Yang Y, Cai P, *et al*. Benefits and risks of drug combination therapy for diabetes mellitus and its complications: a comprehensive review. Front Endocrinol. 2023;14:1301093. doi:10.3389/fendo.2023.1301093

48. Gorman AM. Neuronal cell death in neurodegenerative diseases: recurring themes around protein handling. J Cell Mol Med. 2008;12(6a):2263–80. doi:10.1111/j.1582-4934.2008.00402.x PubMed PMID: 18624755; PubMed Central PMCID: PMC4514105.