The extract of Sclerocarya birrea, Nauclea latifolia, and Piper longum mixture ameliorates diabetes-associated cognitive dysfunction

Jean Philippe Djientcheu Tientcheu (philippedjientstar@gmail.com)
University of Yaounde I: Universite de Yaounde I https://orcid.org/0000-0002-3651-4762
Florence Ngueguim Tsofack
University of Yaounde I: Universite de Yaounde I https://orcid.org/0000-0002-5382-9184
Gounoue Kamkumo Gounoue
University of Yaounde I: Universite de Yaounde I
Michel Arnaud Mbock
University of Yaounde I: Universite de Yaounde I
Rodrigue Fifen
University of Yaounde I Faculty of Sciences: Universite de Yaounde I Faculte des Sciences
Antoine Kandeda Kavaye
University of Yaounde I Faculty of Sciences: Universite de Yaounde I Faculte des Sciences
Théophile Dimo
University of Yaounde I Faculty of Sciences: Universite de Yaounde I Faculte des Sciences

Research Article

Keywords: Fructose, Streptozotocin, Diabetes, Memory dysfunction, Hippocampal neurons, Neuroprotection

Posted Date: June 29th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2908660/v1

Version of Record: A version of this preprint was published at Metabolic Brain Disease on October 12th, 2023. See the published version at https://doi.org/10.1007/s11011-023-01291-7.

Abstract

Diabetes-associated cognitive dysfunction is linked to chronic hyperglycemia, oxidative stress, inflammation, cholinergic dysfunction, and neuronal degeneration. We investigated the antidiabetic and neuroprotective activity of a mixture of *Sclerocarya birrea, Nauclea latifolia,* and *Piper longum* (SNP) in type 2 diabetic (T2D) rat model-induced memory impairment. Fructose (10%) and streptozotocin (35 mg/kg) were used to induce T2D in male Wistar rats. Diabetic animals received distilled water, metformin (200 mg/kg), or SNP mixture (75, 150, or 300 mg/kg). HPLC-MS profiling of the mixture was performed. Behavioral testing was conducted using the Y-maze, NORT, and Morris water mazes to assess learning and memory. Biochemical markers were evaluated, including carbohydrate metabolism, oxidative/nitrative stress, pro-inflammatory markers, and acetylcholinesterase activity. Histopathological examination of the pancreas and hippocampus was also performed. Fructose/STZ administration resulted in T2D, impaired short- and long-term memory, significantly increased oxidative/nitrative stress, pro-inflammatory cytokine levels, acetylcholinesterase activity (AChE), hippocampal neuronal loss and degeneration in CA1 and CA3 subfields, and neuronal vacuolation in DG. SNP mixture at 150 and 300 mg/kg significantly improved blood glucose and memory function in diabetic rats. The mixture reduced oxidative/nitrative stress and increased endogenous antioxidant levels. It also reduced serum IL-1β, INF-γ and TNF-α levels and ameliorated AChE activity. Histologically, SNP protected hippocampus neurons against T2D-induced neuronal necrosis and degeneration. We conclude that the aqueous extract of SNP mixture has antidiabetic and neuroprotective activities thanks to active metabolites identified in the plant mixture, which consequently normalized blood glucose, protected hippocampus neurons, and improved memory function in diabetic rats.

Highlights

- The aqueous extract of the mixture of *Sclerocarya birrea*, *Nauclea latifolia*, and *Piper longum* (SNP) demonstrated neuroprotective and memory-strengthening properties by protecting hippocampus neurons in diabetic rats;
- Antidiabetic properties of the SNP mixture were exhibited via normalization of blood glucose levels and pancreas protection;
- SNP activity was due to the main naturally-occurring substances identified.

1. Introduction

As a diverse metabolic condition, diabetes mellitus is defined by hyperglycemia brought on by ineffective insulin secretion, ineffective insulin action, or both (Punthakee *et al.*, 2018). Worldwide, diabetes is becoming more common. It is one of the 21st century's fastest growing worldwide health emergencies. According to the International Diabetes Federation (IDF), 536.6 million individuals worldwide (diagnosed or undiagnosed) had diabetes in 2021. By 2045, that figure is expected to rise by 46%, reaching 783.2 million (Sun et al., 2022). Diabetes is related with many organs damage and severe life-threatening

health problems due to chronic uncontrolled high blood sugar. These consequences mostly impact the central and peripheral neurological systems, as well as organs such as the kidneys and eyes, resulting in cognitive decline, peripheral neuropathy, diabetic nephropathy, and diabetic retinopathy (Tang et al., 2012; Zhong et al., 2016).

Diabetes-related central nervous system (CNS) problems are referred to as "diabetic encephalopathy" with memory loss being the pathology's hallmark (Duff et al., 2015). The brain is the most susceptible organ to variations in glucose levels. Even minor variations in glucose levels can result in irreversible brain damage and cognitive impairment (Moheet et al., 2015; Papanas and Ziegler, 2015). Diabetic encephalopathy in type 2 diabetic patients is associated with hippocampal formation atrophy (a locus specifically involved in learning and memory processing in mammals (Umegaki, 2014)) caused by chronic hyperglycemia, oxidative stress, and cholinergic dysfunction (Bhutada et al., 2010). Animal studies indicate that streptozotocin-induced chronic hyperglycemia reduces acetylcholine synthesis and release in the brain (Castro et al., 2021; Welsh and Wecker, 1991), as well as a significant loss of cortical neurons, resulting in cognitive impairment, as memory is thought to be "stored" in the cortex (Redish and Touretzky, 1997). Inflammatory alterations in the brain are also linked to cognitive loss in type 2 diabetes. The production of various pro-inflammatory cytokines due to diabetes-induced ROS production, including interleukin IL-1β,IL-6, interferon (INF-γ) and tumor necrosis factor (TNF-α) increases inflammation and insulin resistance, and has been linked to CNS complications in diabetes (Zilliox *et al.*, 2016). TNF-α production *in vivo* has been demonstrated to increase in chronic hyperglycemia, potentially exacerbating insulin resistance and contributing to the development of diabetic complications (Akash et al., 2018). According to a study conducted by Cozachenco and colleagues, elevated IFN-γ levels reported in diabetic individuals may enhance the incidence of dementia and may represent the plausible link between diabetes mellitus and dementia (Cozachenco et al., 2019). In addition, IL-1β has been implicated in perpetuating immune responses (neuroinflammation) and contributing to diabetes brain complications (Mendiola and Cardona, 2018).

Although cognitive deficiency does not affect quality of life in the early stages, diabetes-related cognitive decline can progress to and raise the risk of dementia (Driscoll et al., 2016). Though some antidiabetics, insulin sensitizing agents, anti-oxidants and acetylcholinesterase inhibitors have demonstrated beneficial effects in animal models (Bhutada et al., 2010; Ryan and Deci, 2006), no specific medications for the prevention and/or management of diabetes-associated cognitive dysfunction are currently available.

Sclerocarya birrea is a tree with a rounded top and 8 to 12 m high belonging to the Anacardiaceae family. Also called African yellow plum, its leaves are alternate, reaching 20 cm long with 6 to 10 pairs of opposite leaflets. *Sclerocarya birrea* is a medicinal plant known for the treatment of several diseases including epilepsy, hypertension, inflammation and memory disorders in Cameroonian folk medicine (Nonmarmbaye et al., 2021). One of their uses is the management of diabetes mellitus (Dimo et al., 2007). Studies conducted on the methanolic extract of *Sclerocarya birrea* stem bark have demonstrated its hypoglycemic properties (Dimo et al., 2007). The secondary metabolites contained in the stembark

were shown to be responsible for its antioxidant, antihyperglycemic and diuretic activities (Chukwuma et al., 2019; Dimo et al., 2007).

Nauclea latifolia (the African peach tree) is a plant species in the Rubiaceae family, native to sub-Saharan Africa. It is an evergreen shrub, very branched, up to 12 meters high. *Nauclea latifolia* is highly prized by traditional practitioners as a remedy for many diseases. It is used in the symptomatic treatment of various ailments such as malaria, fever, diabetes, high blood pressure and abdominal pain. The antipyretic and anti-inflammatory activities of hydroalcoholic extracts of the roots and leaves of *Nauclea latifolia* have been tested and proven in rats (Amouzoun et al., 2008). Studies conducted in mice have demonstrated the anti-convulsive and anxiolytic properties of root bark decoction (Ngo Bum et al., 2009). The hypoglycemic and antihyperglycemic potentials of *Nauclea latifolia* leaves aqueous extract have also been demonstrated(Gidado et al., 2008).

Piper longum, commonly known as long pepper, is a fruit of a vine native to India, growing wild in the foothills of the Himalayas. It belongs to the Piperaceae family which produces pepper. Its flavor is warmer, slightly sweet, and less strong than black pepper. Pepper is a spice almost universally consumed. Its pungent flavor is due to piperidine amides. The fruits of this plant have been used traditionally to treat a variety of diseases. Reported activities include antibacterial, antidepressant, hepatoprotective and anti-inflammatory properties, bioavailability enhancement, larvicidal activity, radio protective activity, cardioprotective activity, antifertility activity, and antioxidant activity (Kilari et al., 2015). The aqueous extract of *Piper longum* roots shows antidiabetic and antihyperlipidemic properties (Nabi et al., 2013). These properties would be attributed to secondary metabolites acting through an improvement of insulin secretion, peripheral glucose utilization and a reduction of gastrointestinal glucose absorption (Kumar et al., 2011).

Local populations in Cameroon's Far North region utilize an empirical combination of these three macerated plants (*Sclerocarya birrea* and *Nauclea latifolia* stem barks, and *Piper longum* fruits) to treat diabetes. Despite being consumed empirically by local community, this plant mixture has never been the subject of any scientific study to prove its efficacy in the treatment of diabetes and its consequences. Furthermore, to the best of our knowledge, no study has shown its efficacy in an animal model of type 2 diabetes associated with memory impairment. Given the different properties of these plants, particularly their anti-diabetic and brain function enhancing properties, we hypothesize that the combination of these plants could correct the memory disorders brought on by type 2 diabetes by restoring normal blood sugar and insulin levels, scavenging free radicals, reducing inflammation, stabilizing neurons, and ultimately improving memory.

The current study sought to determine the antidiabetic and antiamnesic activity of the aqueous extract of the mixture of *Sclerocarya birrea* and *Nauclea latifolia* stem barks, and *Piper longum* fruits in a type 2 diabetes rat model-induced memory impairment.

2. Materials and methods

2.1. Plant material, preparation of extract and phytochemical profile

The stem barks of *Sclerocarya birrea* and *Nauclea latifolia* and fruits of *Piper longum* were harvested in Cameroon (Maga district, Far North Cameroon, January 2019). The identity, as well as the authenticity of the plants was made from a botanist at the National Herbarium of Cameroon in comparison to different specimens deposited under the voucher numbers: N°7770/SRF/CAM, N°47652/HNC and N°34187/HNC respectively for *Sclerocarya birrea* and *Nauclea latifolia* stem barks and *Piper longum* fruits. The aqueous extract of the mixture of each plant parts was prepared according to the traditional healer's recommendations. Briefly, different parts of fresh plants were cleaned and dried in the shade at room temperature, cut into small pieces and powdered to yield 200g of *Sclerocarya birrea* powder, 200g of *Nauclea latifolia* powder and 90g of *Piper longum* powder. Each powder was stored in opaque jars at room temperature (23 ± 2° C). Then, mixed, the combined plants powder was macerated in 10L of distilled water for 48 hours, filtered and dried in a thermostat oven at 45° C. This made it possible to obtain a raw dry extract (34g), yielding 6.93% (w/w). The dry extract obtained was then stored at room temperature in airtight boxes until needed for the main experiments.

1. **LC-MS analysis of the aqueous extract of the combination of Sclerocarya birrea, Nauclea latifolia and Piper longum**

Analytical LC-MS was performed on an Agilent 6220 ToF-MS with a Dual ESI-source, 1200 HPLC system with autosampler, degasser, binary pump, column oven, diode array detector and a Hypersil Gold C18 column (1.9 _m, 50 x 2.1 mm) with a gradient (in 11 min from 0% B to 98%B, back to 0%B in 0.5 min, total run time 15 min) at a flow rate of 300 _L/min and column oven temperature of 40°C. HPLC solvent A consists of 94.9% water, 5% acetonitrile and 0.1% formic acid, solvent B of 5% water, 94.9% acetonitrile and 0.1% formic acid. The mass spectra were recorded in both profile and centroid mode with the Mass Hunter Workstation Acquisition B.04.00 software (Agilent Technologies, Santa Clara, CA, USA). Mass Hunter Qualitative Analysis B.07.00 software (Agilent Technologies, Santa Clara, CA, USA) was used for processing and averaging of several single spectra. Identification of peaks was performed by comparing the peaks in the samples with those of data reported from the literature available in SciFinder database about *Sclerocarya, Nauclea* and *Piper* genius.

2.3. Drugs and reagents

Fructose was purchased from Markal-ZA (France) and metformin tablets (Glucophage) from Merck S.L. Poligono (Barcelona, Spain). Streptozotocin (STZ), and ELISA kits were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trichloroacetic acid, thiobarbituric acid, glacial acetic acid, ether, Ellman reagent, Griess reagent, and formalin were also purchased from Sigma-Aldrich, while glucometer and test strips (Accu-Chek Active, Roche Diagnostic Corporation, Germany) were purchased from a local pharmacy.

2.4. Experimental animals and housing conditions

50 Adult male Wistar rats that were normoglycemic (80–100 mg/dL) and aged between 45 to 60 days at the start of the study (weighing 180-200g) were raised in the animal facilities of the Laboratory of Animal Physiology (University of Yaoundé I, Cameroon) under standard light (12-hour day/night cycle), temperature (24 ± 2°C), and humidity (45 ± 3°C) with access to standard animal diet and tap water ad libitum. The animals were housed in groups of 5 rats per cages (40 cm x 40 cm). All the experiments were conducted in accordance with the approval of the Cameroon National Ethical Committee (Ref No. FwIRb00001954, 04/09/2006), which adopted the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals. All animal experiments also comply with the ARRIVE guidelines 2.0. For behavioral experiments, animals were randomly selected. At the end of the study, rats were euthanized by decapitation under anesthesia (ether) to avoid animal distress.

2.5. Experimental induction of type 2 diabetes mellitus

Type 2 diabetes (T2D) was achieved via the method described by Sadeghi and colleagues, modified by fructose feeding (10% w/v) ad libitum for 6 weeks and streptozotocin (35 mg/kg, *i.v.*) to rats(Sadeghi et al., 2017). Thus, to develop diabetic animal model, all rats were divided into 2 groups:

(i): a normal control group of 10 rats was given tap water

(ii): a fructose-treated group (Fruct.10) of 40 rats received tap water supplemented with 10% fructose.

Firstly, insulin resistance was induced by feeding rats with 10% fructose water (Fruct 10) for 6 weeks while normal control remained on tap water. Secondly, induction of late onset T2D was carried out by administering after 12h fasting, a low dose STZ (35 mg/kg, *i.v.*) to fructose-treated rats and a saline solution to normal control group. The STZ-injected rats subsequently had 10% glucose solution for 24 h to prevent death from STZ-induced hypoglycemic shock. All animals were then observed for 2 weeks followed by blood glucose level measurement using a or table glucometer (Accu-Check Active; Roche Diagnostic Corporation, Germany) in the blood collected from tail vein. Animals with fasting blood glucose level between 200 and 350 mg/dL were considered as diabetic and used for the study (Liu et al., 2022). After 4 weeks of development of diabetes, 20 diabetic rats were randomly selected and subjected to neurobehavioral studies to assess learning and memory impairments (Mehta and Banerjee, 2017). During these 4 weeks fasting blood glucose levels were measured every 2 weeks. Behavioral tests included the Y-maze test, the novel object recognition test (NORT) and the Morris water maze test (MWM) and were performed for 10 days before and after diabetes treatment.

2.6. Experimental design and treatment

Diabetic rats were divided into 5 groups of 5 animals each as follow:

(i): a diabetic control group that received distilled water (10 mL/kg, *p.o.*)

(ii): a positive control group (Met 200) that received metformin (200 mg/kg, *p.o.*)

(iii): three test groups (SNP 75, SNP 150, SNP 300) that received *p.o.* the aqueous extract of SNP mixture at the doses of 75, 150, and 300 mg/kg, respectively.

A normal control group was added as the 6th group (n = 5) and treated with distilled water (10 ml/kg, *p.o.*). Treatment was given to animals for 21 consecutive days and fasting blood glucose levels were measured every week, and then, cognitive decline or its improvement was assessed with the same behavioral tests used before diabetes treatment (Y-maze, NORT and MWM). After completion of the behavioral procedures, rats were subjected to the oral glucose tolerance test (OGTT) and sacrificed under anesthesia. Serum samples were collected. Brain and pancreas tissue samples were reconstituted and used for biochemical analysis and histopathological studies.

2.7. Behavioral studies

Learning and memory functions were evaluated before and after diabetes treatment (Fig. 1) and lasted 10 days as follow: Y-maze test on day 1, NORT on days 2–4 and MWM on days 5–10. All testing was recorded and analyzedby ANY-maze video tracking system (Stoelting, Wood Dale, IL, USA) with a Logitech camera.

2.7.1. Y-maze test

The Y-maze test was used to assess spatial working memory in animals by recording spontaneous alternations (Kraeuter et al., 2019). The maze used was a wooden Y-shaped compartment with (21 x 7 x 15.5 cm) with equal length arms. The walls of each arm were decorated with different patterns to differentiate them and labeled as A, B, and C. All rats were placed to the testing room 1 week prior to the experiment so they can acclimatize to the conditions. During 8 min, rats were allowed to explore the maze undisturbed and the number of entries in each arm of the maze was recorded. The apparatus (walls and floor) was cleaned with 70% ethanol between each passage to avoid odors. A spontaneous alternation (SA) was defined as three successive entries in three different arms, for example, ABC, CAB, or BCA. The percentage of SA was used as an index of working memory and calculated according to the following formula (Kraeuter et al., 2019) : Percentage of SA = [(number of SA)/(total number of arms visited − 2)] ∗100.

2.7.2. Novel object recognition test (NORT)

The object recognition test was used for assessing non spatial memory (recognition memory) in rats by recording the recognition index as a percentage time investigating the novel object. The test environment was an open field constructed of wooden material (80cm length x 80cm width x 60cm height). On day 1, each rat was placed in the middle of the open field and allow to explore the field for 10 min. On day 2, 2 non movable identical objects were placed at the northwest and southeast pole of the test field while the rats were allowed to explore the test area. On day 3, one of the identical objects was replaced with a novel object and day two experiment was repeated for each rat. During the intertrial interval (24h), the objects and Open-field apparatus was cleaned with 70% ethanol to avoid a confounding error induced by the influence of odour. The total of time which each rat spent exploring each object on day 3 was recorded

and calculated as a recognition index as follow (Mathiasen and DiCamillo, 2010): Recognition index = [T*novel*/ (T *familiar* + T*novel*)] ∗100

Where T *novel* = time spent to explore the novel object and T *familiar* = time spent to explore the familiar object.

2.7.3. Morris water maze test (MWM)

Spatial learning and long-term memory impairments were assessed using the Morris water maze test (Kouémou et al., 2017) with a circular tank of 150 cm diameter and 60 cm height filled to 40 cm with water at 25°C. The tank was divided into four equal quadrants with a white refuge platform of 8 cm diameter and 30 cm height placed in the center of one of the quadrants 1 cm below the water surface. The pool was in a room with multiple visual cues. On the 1st day of the test, i.e., during the habituation phase, each rat was acclimated for 120 s without the platform. The acquisition phase started on the 2nd day, during which all rats received 3 swimming trials per day for 4 consecutive days to strengthen their memory, the starting positions during trials were randomly chosen from different quadrants with animal's head facing the wall (Harandi et al., 2015).The water was clouded by adding liquid milk so that the platform was invisible from the water surface. The session time for each animal to find the platform was 120 s, and the time interval between sessions was 5 min. When an animal found the platform, it was allowed to stay for 20 otherwise the experimenter helped rat found it to stay on it for 20 s. During each session of the acquisition phase, the latency time to find the platform was recorded for each animal while the failed trainers were considered as 120 s. The effectiveness of learning was then assessed in the retention phase on the 6th day. During this phase, which lasted 60 s, the platform was removed from the tank. Thus, the latency time to find the proper quadrant where the platform was previously, the number of entries and swim distance to the target quadrant and the time spent in this compartment were recorded.

2.7.4. Oral glucose tolerance test

An Oral glucose tolerance test (OGTT) was performed at the end of the treatment period. After an overnight fast (12 h), rats were orally dosed with a D-glucose solution (3 g/kg b.w.) and blood glucose concentrations were subsequently measured using glucometer at 0 (just before oral glucose dosing), 30, 60, 120, and 180 min after the oral dosing of glucose.

2.7.5. Blood and tissue sampling

Following the behavioral assessments and OGTT, animals were immediately sacrificed by decapitation under anesthesia with ether. The blood was collected in dry tubes and serum samples were prepared by allowing blood samples to stand for 1 hour at room temperature (25 ± 1°C) and then centrifuged at 3000 rpm, 4°C for 15 min. The supernatant (serum) was collected, aliquoted and stores in Eppendorf tubes at −20°C for biochemical assays. For the preparation of tissue homogenates, the liver from each rat was removed, washed in cold saline solution (0.9% NaCl) and a part was taken and used to estimate glycogen content. Then from pancreas tissue, a small portion was homogenized in Tris-HCl buffer (50 mM, pH 7.4) at a ratio of 20% (w/v) to reconstitute homogenate for biochemical analysis while the other half was

fixed in 10% neutral buffered formalin. The brain of each rat was rapidly removed and divided into two hemispheres. The hippocampi and prefrontal cortex were isolated from one hemisphere, washed in ice-cold saline, and blotted to dryness. They were then weighed and homogenized in cold Tris-HCl buffer at a ratio of 5% (w/v). After centrifugation of each homogenate at 3000 rpm, 4°C for 25 min, the supernatant was collected into tubes and stored at − 20°C for further biochemical evaluation. The other hemisphere of the brain was fixed for later histological analysis.

2.8. Biochemical analysis

2.8.1. Serum insulin and pro-inflammatory marker levels

Serum insulin, TNF-α, INF-γ and IL-1β levels were estimated using Rat enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer protocol. The wells were already coated with a specific capture (primary) antibody for each cytokine or insulin. Briefly, once standard was reconstituted, 100 µL of standard or samples were added to each pre-coated well. After gently patting the frame plate, the microplates were covered and incubated for 2 hours at room temperature. After incubation, each well was carefully washed 4 times with 1X wash solution (300 µL) and dry against clean absorbent paper. Then, 100 µL of prepared detection antibody was added to each well. The preparation was covered and incubated for 1 hour at room temperature with gentle shaking and then washed 4 times as previously described. 100 µL of prepared Streptavidin solution was added to each well and the preparation was incubated for 45 minutes at room temperature with gentle shaking and then washed 4 times as previously described. 100 µL of TBM substrate reagent was added to each well, covered and incubated for 30 minutes at room temperature in the dark with gentle shaking. Then, 50 µL of stop solution (sulfuric acid) was added to each well to stop the reaction and absorbances were read at 450 nm immediately with a microplate reader. Insulin concentration was expressed in µIU/ml while cytokine levels were expressed in pg/ml and determined from corresponding calibration curves.

2.8.2. Homeostasis model assessment (HOMA), serum glucose and glycogen content

Homeostasis model assessment (HOMA-IR index) was calculated using the formula:

HOMA-IR = (fasting glucose (mg/dL) x fasting insulin (µIU/mL))/405(Matthews et al., 1985).

Homeostasis model assessment of β-cell function (HOMA-B score) was calculated using the formula:

HOMA-B = (360 x fasting insulin (µIU/mL)) / (fasting glucose (mg/dl) – 63) (Matthews et al., 1985)

Serum glucose level was determined by colorimetric method using commercial kit according to the manufacturer's instructions. Liver glycogen content was assayed following the method described by Handel (Van Handel, 1965).

2.8.3. Evaluation of acetylcholinesterase activity in brain samples

Acetylcholinesterase (AChE) activity was estimated by colorimetric method to mimic the cholinergic function in rats' brain(Ellman et al., 1961). AChE activity was measured at 412 nm against the blank at 30 and 90 seconds after reaction with Ellman's reagent and acetylthiocholine iodide. AChE activity was expressed in µM of hydrolyzed acetylthiocholine iodide/g of tissue/minute according to the Beer-Lambert law(Ellman et al., 1961).

2.8.4. Oxidative and nitrative stress markers

The Wilbur method was used to calculate malondialdehyde (MDA) in brain tissue homogenates. The MDA content was measured at 530 nm against the blank after its reaction with trichloroacetic acid and thiobarbituric acid. The MDA concentration was measured in µmol per g of organ (Wilbur et al., 1949). Nitric levels were estimated by measuring the amount of nitrite using the Griess reagent. 50 µL of homogenate and 200 µL of distilled water were mixed in tubes and 250 µL of Griess reagent (0.1% naphthyl ethylenediamine, 1% sulfanilamide and 5% phosphoric acid), and the absorbance was measured at 570 nm against the blank (Tsikas, 2007). The amount of nitrite was calculated by absorbance data extrapolation using a standard curve with sodium nitrite, and estimated in µmol per g of organ.

2.8.5. Endogenous antioxidants

The concentration of reduced glutathione (GSH) was determined using the Ellman method (Ellman, 1959). The GSH content was measured at 412 nm against the blank after its reaction with Ellman reagent (2,2-Dithio-5,5'-dinitrobenzoic acid). The amount of GSH was expressed in µmol per g of organ. Catalase activity was measured using the Sinha technique (Sinha, 1972), based on the principle of hydrogen peroxide (H_2O_2) degradation in the presence of catalase enzyme. The concentration of undecomposed H_2O_2 was calculated using a calibration curve established from a standard solution (50 mM H_2O_2). Tissue catalase activity was represented as mM of H_2O_2 per g of organ.

2.9. Histopathological analysis of pancreas and brain tissues

The brains and pancreas were fixed in neutral buffered formalin (4%). Thereafter, washing, dehydration in alcohol, and embedding in paraffin blocks were carried out. For histopathological examination, tissues were cut with 5 µm thick coronal sections on a microtome, then dewaxed with xylene and rehydrated with ethanol at gradient concentrations. After washing twice with distilled water, all sections were stained with hematoxylin-eosin and/or Cresyl violet, and then mounted slides were observed at various magnifications using a Scientico STM-50 optical microscope (HSIDC Industrial Estate, Haryana, India) equipped with a Celestron 44421 digital camera connected to a computer (HP Pavilion, g series). Pancreatic islet size was obtained by measuring pancreatic islet areas over four different fields on each section. The pyknotic

index in the CA1 subfield of the hippocampus was calculated as a percentage of pyknotic pyramidal cells out of the total number of cells counted. Neuronal density in the CA3 layer of the hippocampus was determined by counting the number of surviving neurons per 400 μm^2. Each set of data was obtained from five independent rats in each group, and five random sections of each rat.

2.10. Statistical analysis

The results are presented as mean ± standard deviation (SD). Statistical analysis and presentation of data were performed using GraphPad Prism (version 8.00; GraphPad Software, Inc., San Diego, CA, USA). Data were analyzed using one-way ANOVA followed by the Tukey's *post hoc* test. Student's t-test was applied to analyze behavioral data between normal and diabetic control before treatment. P values less than 0.05 implied statistical difference.

3. Results

3.1 LC-MS analysis

Figure 2 and Table 2 summarize the major peaks in the chemical profile of the aqueous extract of SNP mixture. Twenty-two compounds were detected (Fig. 2a) among which, seven were isolated.

Table 1: Main signals exhibited in the LC-MS spectra of compounds detected in the aqueous extract of the combination of *Sclerocarya birrea* and *Nauclea latifolia* stem barks, and *Piper longum* fruits and proposed attribution.

Nº	Tr (min)	[M+H] +		error (ppm)	Molecular Formula	Name of Compound
		Experimental mass	Calculated mass.			
1	2.031	215.14467	214.13577	-7.61	$C_{15}H_{18}O$	7-Hydroxycadalene
2	2.139	279.2299	278.2245	6.91	$C_{18}H_{30}O_2$	Linolenic Acid
3	2.246	309.1887	308.1835	6.52	$C_{14}H_{28}O_7$	Not Identified
4	2.602	250.9822	249.9749	-0.01	$C_{10}H_2O_8$	Not Identified
5	2.709	271.2053	270.1983	1.07	$C_{19}H_{26}O$	Not Identified
6	2.842	279.0884	278.0790	-7.31	$C_{14}H_{14}O_6$	Not Identified
7	3.693	171.1016	170.0942	-0.5	$C_9H_{14}O_3$	Not Identified
8	4.115	313.0903	312.0845	4.79	$C_{14}H_{16}O_8$	Not Identified
9	4.239	281.1020	280.0946	-0.11	$C_{14}H_{16}O_6$	Euparvic Acid
10	4.437	423.0931	422.0849	-2.15	$C_{19}H_{18}O_{11}$	Mangiferin
11	4.719	277.1131	276.1056	-0.96	$C_8H_{20}O_{10}$	Not Identified
12	4.876	347.1076	346.0993	-2.87	$C_{25}H_{14}O_2$	Not Identified
13	5.140	333.0978	332.09	-1.86	$C_{12}H_{18}O_{10}$	Lepidimoic Acid
14	5.496	373.1209	372.1209	5.54	$C_{20}H_{20}O_7$	Quercetin Pentamethyl Ether
15	6.348	341.2472	340.2402	0.79	$C_{23}H_{32}O_2$	Not Identified
16	6.496	297.1119	296.1048	0.51	$C_{18}H_{16}O_4$	Not Identified
17	6.794	337.1797	336.1725	0.21	$C_{22}H_{24}O_3$	Not Identified
18	6.893	315.1740	314.1670	0.85	$C_{23}H_{22}O$	Not Identified
19	7.265	469.3162	468.3087	-0.48	$C_{26}H_{44}O_7$	Not Identified
20	7.646	227.1643	226.1568	-0.78	$C_{13}H_{22}O_3$	Not Identified
21	7.977	221.1535	220.1463	0.21	$C_{14}H_{20}O_2$	Not Identified
22	8.630	345.0963	345.0896	1.56	$C_{18}H_{16}O_7$	Xanthomicrol

3.2 Effects of the aqueous extract of SNP mixture on some parameters of carbohydrate metabolism in fructose/STZ-induced T2D rats

As shown in Table 2, Fructose-treated rats presented significantly high serum insulin levels and HOMA-IR index ($p < 0.05$), as well as decreased HOMA-B ($p < 0.001$) compared to normal control, 6 weeks after fructose treatment. There was significant increase in glucose levels ($p < 0.001$) and decrease in liver glycogen content ($p < 0.05$), serum insulin ($p < 0.01$), HOMA-IR and HOMA-B ($p < 0.001$) in untreated diabetic rats compared to normal control at the end of treatment period. The aqueous extract of *Sclerocarya birrea, Nauclea latifolia* and *Piper longum* mixture at 150 and 300 mg/kg significantly decreased glucose levels and HOMA-IR ($p < 0.001$) and increased hepatic glycogen ($p < 0.001$), serum insulin and HOMA-B ($p < 0.05$) compared to diabetic control. Likewise, metformin-treated rats presented significantly low blood glucose level ($p < 0.001$) and HOMA-IR ($p < 0.05$), and increased insulinemia ($p < 0.001$) and HOMA-B ($p < 0.05$) compared to diabetic control. Rats treated with the extract at 75 mg/kg presented no significant difference when compared to diabetic control.

Table 2: Effects of the aqueous extract of *Sclerocarya birrea, Nauclea latifolia* and *Piper longum* mixture on some parameters of glucose metabolism in fructose/STZ-induced T2D rats

Experimental groups	Blood glucose (mg/dL)	Hepatic glycogen (mg/g tissue)	Serum Insulin (µIU/mL)	HOMA-IR index	HOMA-B score
Before STZ (6th week)					
Normal control	65.30 ± 10.45	/	2.35 ± 0.53	0.42 ± 0.10	113. 00 ±19.70
Frcut.10	83.40 ± 9.45	/	3.23 ± 0.75●	0.72 ± 0.17●	52.20±20.00●●●
After STZ (16th week)					
Normal control	77.40 ± 18.20	72.80 ± 22.20	2.84 ± 0.68	0.53 ±0.10	108.00 ± 38.10
Diabetic control	362.00 ± 38.10***	40.20 ± 5.06*	1.65 ± 0.14**	1.56 ± 0.22***	1.86 ± 0.27***
Met 200	122.00 ± 30.90###	65.20 ± 10.60	3.25 ± 0.50###XXX	0.97 ± 0.31#	16.40 ± 3.30#X
SNP 75	384.00 ± 34.50***	44.10 ± 3.24	1.70 ± 0.15**	1.55 ± 0.15***	1.99 ± 0.20***
SNP 150	108.00 ± 25.10###XXX	86.30 ± 22.20##	2.26 ± 0.30#X	0.64 ±0.21###XXX	33.30 ±12.40#X
SNP 300	167.00 ± 20.70###XXX	94.50 ± 12.30###	2.20 ± 0.46#X	0.92 ± 0.43##X	32.00 ±16.50#X

*Data are shown as mean ± SD, before STZ (15 ≤ n ≥10); after STZ (n = 5);●p < 0.05, ●●●p < 0.001 as compared to normal control before STZ injection; *p < 0.05, **p < 0.01, ***p < 0.001 as compared to the normal control after STZ injection;#p < 0.05, ##p < 0.01, ###p < 0.001as compared to the diabetic control; Xp< 0.05,XXXp< 0.001 as compared to SNP 75.Met 200: diabetic animals receiving metformin at 200 mg/kg; SNP 75, 150, 300: diabetic animals receiving the mixture at 75, 150, 300 mg/kg*

3.3 Effects of the aqueous extract of SNP mixture on blood glucose levels and glucose tolerance in fructose/STZ-induced T2D rats

Figure 3A shows that blood glucose levels (BGL) were maintained stable throughout the six weeks following STZ administration. As of week 4, blood glucose remained significantly high ($p < 0.001$) compared to normal control with a marked increase in diabetic control (Fruct+STZ) after STZ administration (mean BGL between 265 and 296 mg/dl) from week 8 to week 12. Fructose-treated rats in figure 3C showed significantly elevated ($p < 0.05$) blood glucose peak 30 minutes after administration of

the oral glucose overload with BGL that remained significantly higher 2 hours ($p < 0.05$) and 3 hours ($p < 0.001$) later compared with those of normal animals. After treatment, diabetic control showed significantly elevated BGL ($p < 0.001$) between 273 and 311 mg/dL throughout the treatment period compared to the normal control (Figure 3B). Treatment with the extract at 150 and 300 mg/kg induced a progressive decrease in BGL. At 150 and 300 mg/kg dose levels, the extract induced a significant ($p < 0.001$) decrease in BGL from the first week and maintained BGL in normal ranges until the end of the treatment period (Day 21). The 75 mg/kg extract significantly decreased BGL ($p < 0.05$) on day 21 compared to the diabetic control, although BGL was still high compared to the 150 and 300 mg/kg extract dose levels. Metformin treatment resulted in a significant ($p < 0.001$) decrease in BGL on days 14 and 21 compared to the diabetic control. Following glucose overload, diabetic control had significantly elevated BGL ($p < 0.001$) compared to normal animals at all time points throughout the test. Untreated diabetic control presented a mean BGL higher than that obtained at the end of the test when compared to baseline (Figure 3D). A similar result was presented in rats treated with extract at 75 mg/kg. Conversely, Extract-treated rats at 150 mg/kg had significantly low blood glucose levels ($p < 0.001$) compared to diabetic control at all time points throughout the test. Besides, BGL returns to normal values 180 min following glucose overloading. Metformin-treated animals showed a similar result to that observed in extract-treated animals at 150 mg/kg. At 300 mg/kg dose extract level, BGL remained significantly low compared to diabetic control but remained slightly high when compared to baseline.

3.4 Effects of the aqueous extract of SNP mixture on working memory in fructose/STZ-induced T2D rats using Y-maze test

Working memory was investigated by performing the Y-maze test: Untreated diabetic animals exhibited a significantly reduced ($p < 0.05$) number of spontaneous alternations compared to normal control before treatment (Fig. 4A). This number was reduced further ($p < 0.01$) after treatment (Fig. 4B). The extract-treated group at 150 mg/kg showed a significantly higher percentage of spontaneous alternation ($p < 0.01$) compared to the diabetic control. Similarly, Metformin treatment also resulted in a significant increase ($p < 0.001$) in this number compared to diabetic control. These results are reflected in the plot showing the course of the animals (Fig. 4C) where rats treated with the extract (150 mg/kg) showed a more pronounced course in the different arms compared to diabetic control.

3.5 Effects of the aqueous extract of SNP mixture on short-term memory in fructose/STZ-induced T2D rats using novel object recognition test (NORT)

Untreated diabetic rats showed a significantly decreased recognition index ($p < 0.01$) before (Figure 5A) and after treatment (Figure 5B) compared to normal control. The time investigating both objects remained unchanged in diabetic control while normal control presented a higher time investigating the novel object ($p < 0.05$) (Fig. 5A and 5B). Treatment with the combination of plants at 150 and 300 mg/kg significantly increased ($p < 0.05$) recognition index compared to diabetic control with a more significant time spent investigating the novel object as seen in normal rats (Fig. 5B).

3.6 Effects of the aqueous extract of SNP mixture on long-term memory in fructose/STZ-induced T2D rats using the Morris water maze (MWM)test

The MWM test was employed to check the memory-improving ability of the aqueous extract. Untreated diabetic rats showed during the acquisition phase a significantly higher mean escape latencies from day 1 to day 4 before and after treatment (Figures6A and 6C). Diabetic control animals showed a significantly higher distance travelled compared to normal control (Figure 6C). Overall, there was a slight gradual decrease in the mean escape latency from the start to the end of the experiment, suggesting that these diabetic rats had impaired memory. In the probe test (Figures6B and 6D), diabetic animals spent significantly less time in target quadrant (South-west) as compared to normal control. These results were confirmed in the track map and the heat map showing that diabetic rats spent higher time in the North-east quadrant compared to normal rats. Animals treated with the extract at 150 and 300 mg/kg showed better memory during the acquisition phase (Figure 6C) with a significantly lower mean escape latency from day 1 to day 4 compared to diabetic control. Similarly, the extract at both doses (150 and 300 mg/kg) significantly decreased the distance animals covered to find the platform on different days of the acquisition phase. A similar result was observed in animals treated with metformin. In the probe trial, rats treated with the extract at 150 and 300 mg/kg showed good memory as normal control did, resulting in a significantly higher ($p < 0.01$) time spent in target quadrant (Figure 6D).

3.7 Effects of the aqueous extract of SNP mixture on serum proinflammatory markers and acetylcholinesterase activities in fructose/STZ-induced T2D rats

In untreated diabetic rats, serum TNF-α and INF-γ levels significantly increased ($p <0.05$), as did serum IL-1β levels ($p <0.01$). (Figure 7A). Similarly, when diabetic rats were compared to normal controls, brain AChE activity increased significantly in the prefrontal cortex ($p <0.05$) and the hippocampus ($p <0.001$). (Figure 7B). Both the extract and metformin treatment reduced serum TNF-α, INF-α, and IL-1β levels, as well as AChE activity in the prefrontal cortex and the hippocampus (Figures 7A and 7B). At 150 mg/kg, the extract had a more pronounced effect.

3.8 Effects of the aqueous extract of SNP mixture on some parameters of oxidative status in brain and pancreas in fructose/STZ-induced T2D rats

As shown in Figure 8A, malondialdehyde (MDA) levels were significantly increased in the prefrontal cortex ($p < 0.01$), the hippocampus ($p < 0.05$) and the pancreas ($p < 0.001$) of untreated diabetic rats as compared to normal rats. Both the extract and metformin treatment produced significant decreased in MDA levels in cortex ($p < 0.01$), hippocampus ($p < 0.01$) and pancreas ($p < 0.001$) compared to diabetic control.

Untreated diabetic rats exhibited a significantly increased nitrite levels in hippocampus ($p < 0.001$) and pancreas ($p < 0.01$) compared to normal rats (Figure 8B). The extract at 300 mg/kg produced a significant decrease in nitrite level in the hippocampus while in the pancreas, this level was significantly

decreased in both extract 150- and metformin-treated groups at $p < 0.001$ and $p < 0.05$, respectively (Figure 8B). No significant variation of this parameter was noted in the cortex.

Figure 8C showed that brain catalase activity was significantly reduced in the cortex ($p < 0.01$) and hippocampus ($p < 0.05$) in untreated diabetic rats compared to normal rats. In the pancreas, a non-significant decrease in catalase activity in diabetic control was noted compared to normal control. The extract at 150 mg/kg produced a significant increase in the cortex ($p < 0.05$) and hippocampus ($p < 0.01$), and a non-significant increase in the pancreas compared to diabetic control. At 300 mg/kg dose level, there was a significant increase in catalase activity in the cortex ($p < 0.001$) and hippocampus ($p < 0.05$) compared to untreated diabetic rats. Likewise, Metformin treatment produced significant increase in catalase activity in cortex ($p < 0.01$) and hippocampus ($p < 0.01$) compared to diabetic control (Figure 8C).

Reduced glutathione (GSH) levels were significantly reduced in the cortex ($p < 0.001$), hippocampus ($p < 0.05$) and pancreas ($p < 0.05$) in untreated diabetic rats compared to normal rats (Figure 8D). Rats treated with the extract at 75 mg/kg showed no significant difference as compared to diabetic control. On the contrary, rats treated with the extract at 150 and 300 mg/kg presented significantly elevated GSH levels in all investigated organs compared to diabetic control, with a marked effect at 150 mg/kg dose level ($p < 0.001$) (Figure 8D).

3.9 Effects of the aqueous extract of SNP mixture on pancreas and brain microarchitecture in fructose/STZ-induced T2D rats

3.9.1 Effects of the plant mixture on T2D-induced pancreatic alterations

In Figure 9A, untreated diabetic rats presented shrunken pancreatic islets whereas normal control rats had a healthy pancreas microstructure composed of acini (the exocrine pancreas) and islets of Langerhans (the endocrine pancreas). Islet hypotrophy observed in diabetic control was manifested by a significant decrease in pancreatic islet area by 161.15 % ($p < 0.001$) compared to normal rats (Figure 9B). When compared to the diabetic control, treatment with the extract at 150 and 300 mg/kg dose levels favored a significant rise ($p < 0.5$) in pancreatic islet size by 92.64 % and 101.71%, respectively. On the contrary, diabetic control rats and rats treated with the extract at 75 mg/kg did not show any significant differences in the pancreatic islet area.

3.9.2 Effects of the plant mixture on T2D-induced histopathological alterations and neuronal loss

Histological analysis revealed, in normal control animals, a normal histological feature in the hippocampus parenchyma and no histopathological alterations in all hippocampal regions (Figures 10A-1, 11A-1, 12-1). Additionally, Cresyl violet staining of the normal control group demonstrated neurons of intact appearance in the different layers (CA1, CA3, and DG regions). On the other hand, untreated diabetic rats exhibited a shrunken hippocampus and a severe loss of neuronal tissue integrity marked by the presence of neurons with pyknotic nuclei (hyperchromatic, Figures 10A-2), neuronal necrosis and

degeneration (Figures 11A-2), and neuronal vacuolation (Figure 12-2). Furthermore, significant pyknotic index (Figure 10B) and neuronal loss (Figure 11B) in the diabetic control group was detected by Cresyl violet staining. Interestingly, SNP 150 or 300 mg/kg revealed significant neuroprotection with intact neurons and minimal neurodegenerative changes, with SNP 150 providing the best protection as shown by the lowest pyknotic index (Figure 10B) and the maximum number of intact neurons (Figure 11B). Likewise, metformin ameliorated neuronal loss, vacuolation, and degeneration, as evidenced by the Cresyl violet staining (Figures 10A-3, 11A-3, 12-3). Moreover, metformin-treated rats showed a reduced pyknotic index and increased cell density (Figures 10B and 11B). However, SNP 75 mg demonstrated modest neuroprotective benefits, exhibiting almost the same histological alterations reported in the diabetic control group (Figures 10A-4, 11A-4, 12-4), with much lower cell density and a much higher pyknotic index than SNP 150 or 300 mg (Figure 10B and 11B).

4. Discussion

The current study evaluated the antidiabetic and memory-strengthening potential of the aqueous extract of the mixture of *Sclerocarya birrea* and *Nauclea latifolia* stem barks and *Piper longum* fruits in a type 2 diabetes rat model-induced memory impairment. Fructose/STZ-induced type 2 diabetes resulted in significant cognitive impairment, which was linked to hyperglycemia, insulin resistance, increased inflammation, increased AChE activity in brain tissues (cortex and hippocampus), and elevated oxidative stress markers in brain tissues and pancreas. In diabetic rats, treatment with the aqueous extract of *Sclerocarya birrea, Nauclea latifolia*, and *Piper longum* mixture significantly improved cognitive deficits, hyperglycemia, insulin resistance, inflammation, cholinergic dysfunction, and oxidative stress markers at 150 and 300 mg/kg dose levels. Furthermore, treatment with the extract significantly reversed fructose/STZ-induced histomorphological damage in brain tissues and pancreas.

T2D is a condition characterized by elevated blood glucose levels caused by increased glucose production by the liver, decreased insulin production by the pancreas, and "insulin resistance," which occurs when insulin responsiveness is reduced due to abnormal insulin receptor expression(Butterfield et al., 2014). Fructose/streptozotocin-induced type 2 diabetes is a well-studied experimental diabetes model. The combination of 10% fructose and STZ administration is an alternative model for inducing all major pathogeneses of T2D, insulin resistance, and partial pancreatic β-cell dysfunction in rats(Wilson and Islam, 2012), both of which are hallmarks of type 2 diabetes and have been heavily used in inducing T2D in experimental animals (Gheibi et al., 2017). This model is more similar to the later stages of T2D and has demonstrated a stable diabetic condition over an 11-week experimental period, making it useful for chronic research studies as well as routine pharmacological screening of anti-type 2 diabetic materials (Wilson and Islam, 2012). In the current study, fructose-treated rats had significantly higher serum insulin levels, as well as higher HOMA-IR (a measure of insulin resistance) and low pancreatic β-cell function, as measured by the HOMA-B score (Table 1). Furthermore, our findings revealed glycemic changes after a glucose load during the OGTT (Fig. 2C), confirming glucose intolerance, a factor strongly linked to insulin resistance(Miranda et al., 2019). One possible explanation for these findings is that drinking fructose-containing water has resulted in impaired β-cell function and decreased peripheral insulin use(Miranda et

al., 2019). These results indicate that rats given 10% fructose over a 6-week period developed significant insulin resistance. Type 2 diabetes was successfully maintained after streptozotocin administration, with mean blood glucose levels ranging between 265 and 296 mg/dl from week 8 to week 12. (Fig. 2A). Diabetic rats had significantly lower insulin levels, lower glycogen content and HOMA-B, and higher HOMA-IR indexes (Table 1). These findings support those of Obafemi and colleagues(Obafemi et al., 2019). In our study, the aqueous extract of SNP mixture significantly reduced blood glucose levels, improved deceased serum insulin levels, hepatic glycogen, HOMA-B, and increased HOMA-IR caused by diabetes induction. Interestingly, the extract's effects were more pronounced at the doses of 150 and 300 mg/kg when compared to the diabetic control, whereas no significant activity was observed at 75 mg/kg dose level. The metformin-treated group showed similar results to the 150 and 300 m/kg dose extract levels. This ability of the extract at these doses could be due to its active ingredients with the ability to restore progressive loss of pancreatic β-cells, increase insulin secretion by residual β-cells, or facilitate insulin action and hepatic glucose storage. This was confirmed by histopathological examination of pancreatic sections from extract-treated groups that revealed marked protection, as evidenced by enlarged pancreatic islet sizes compared to untreated diabetes controls.

Type 2 diabetes is a metabolic disease that can harm the structure and the function of many organs in the body including the eyes, kidneys, blood vessels and heart. Its long-term complications are not only affecting these tissues but also the central nervous system (Lotfy et al., 2017), which is linked to an increased risk of cognitive dysfunction, such as hippocampal-dependent cognitive impairment (Lang et al., 2009; Li et al., 2013), and also results in a significant loss of cortical neurons, resulting in cognitive impairment(Redish and Touretzky, 1997). It has been demonstrated that in people with type 2 diabetes, the speed of data processing, working memory, and some aspects of attention are impaired following chronic hyperglycemia (Cerasuolo and Izzo, 2017). Short-term memory is a function that lasts 20 seconds and temporarily retains stimuli that have just been perceived. Working memory is a short-term memory system that allows for concurrent retention and manipulation. It is used for reasoning about previously learned material and drawing conclusions from it (Sommerfield et al., 2003).This study used the Y-maze and NORT to test those abilities. Untreated diabetic rats demonstrated a significantly lower percentage of spontaneous alternations in the Y-maze (Figs. 3A and 3B) and a lower recognition index in the NORT (Figs. 4A and 4B). Additionally, impaired spatial learning and memory, as indicators of long-term memory impairment, were observed in untreated diabetic rats during the MWM experiment, with significantly higher escape latency on days 2, 3, and 4 before and after treatment (Figs. 5A and 5C), as well as significantly higher distance travelled from day 1 to 4 compared to normal control rats (Fig. 5C). Diabetic animals spent significantly less time in the target quadrant during the probe test (Figs. 5B and 5D).

This result is consistent with that of numerous other researchers who noted impaired spatial cognition in type 2 diabetic rats (Biessels et al., 1998; Njan et al., 2020).This study demonstrates that if diabetes is not treated, hyperglycemia rises, with disastrous consequences for cognitive memory. The results obtained before and after treatment in untreated diabetic rats demonstrated this. However, while insulin is best known for its role in regulating glucose metabolism in peripheral tissues, it also influences a variety of

brain functions such as cognition, memory, and synaptic plasticity via complex insulin/insulin receptor (IR) signaling pathways (Zhao *et al*., 2001). Given the importance of insulin in many aspects of neuronal function in both the peripheral and central nervous systems, it is possible that disruption of insulin signaling (hyperinsulinemia coupled with impaired β-cell function in T2D) is involved in the pathogenesis of neurological diseases and results in neurodegeneration, thereby deteriorating memory (Xu et al., 2004).Treatment with the aqueous extract of SNP mixture, particularly at the doses of 150 and 300 mg/kg, improved working memory as seen in the Y-maze test and the NORT with a greater percentage of spontaneous alternation (Figs. 3B and 3C),and an increased recognition index (Fig. 4B) in comparison to diabetic control. The extract at these 2 doses, during the MWM, reversed diabetes-induced long-term memory impairment as evidenced in the acquisition phase by decreased escape latency and distance traveled (Fig. 5C). On the probe trial day (day 5), when the platform was removed, rats given the extract (at 150 and 300 mg/kg) spent significantly more time in the target quadrant. The metformin-treated group had a similar outcome. These findings show that a combination of *Sclerocarya birrea*, *Nauclea latifolia* and *Piper longum* mixture aqueous extract improved memory in type 2 diabetic rats.

Overproduction of reactive oxygen species (ROS) and increased oxidative stress in pancreatic β-cells caused by STZ injection, as well as the resulting hyperglycemia, aggravate diabetes and its complications. (Barrett et al., 2017). It has been proposed that oxidative damage contributes to learning and memory impairment during hyperglycemia (Tamaddonfard et al., 2013), by damaging cholinergic neurons in the cerebral cortex and hippocampus, resulting in impaired cognitive functions(Fukui et al., 2001). Cellular damage caused by cytotoxic oxygen free radical species is thought to be an important possible mechanism for diabetes brain complications. These free radicals are produced in all types of cells via various metabolic pathways (Parinandi et al., 1990)but especially in central nervous system cells due to their high oxygen demand and weak antioxidant defensemechanism (Estévez, 2011). Malondialdehyde (MDA) is a representative product of lipid peroxidation that is commonly measured in experiments as an excellent oxidative stress endpoint(Arena et al., 2019). In the current study, MDA levels were found to be higher in the cortex, hippocampus, and pancreas of untreated diabetic rats compared to normal control rats (Fig. 7). Furthermore, diabetic rats had lower GSH levels (Fig. 9) and catalase activity (Fig. 10) in all organs studied. Under physiological conditions, the known antioxidants GSH and catalase team up with other antioxidants to scavenge pro-oxidants from the body(Arena et al., 2019).But this mechanism is impaired by persistently elevated hyperglycemia. A compromised antioxidant system and chronic hyperglycemia in diabetic rats led to oxidative stress, as shown by elevated cortical, hippocampal, and pancreatic MDA levels as well as decreased antioxidant defense markers. In contrast to diabetic control rats, rats given the extract at doses of 150 and 300 mg/kg showed a significant decrease in MDA levels and an increase in GSH levels and catalase activity in the cortex, hippocampus, and pancreas (Figs. 7, 9 and 10). A similar outcome was seen in the metformin group. The ability of the extract (SNP 150 and 300) or metformin to reverse this oxidative damage-prone condition may indicate an improvement in antioxidant status. As a result of the extract's high flavonoid content, our findings may support its antioxidant and antidiabetic capabilities. Indeed, Mangiferin, a flavonoid present in SNP mixture, was discovered to have neuroprotective effect by suppressing oxidative/nitrative stress,

increasing antioxidant mechanisms, and protecting against inflammation(Lum et al., 2021). Similarly, QuercetinPentamethyl Ether, a derivate of quercetin, is a flavonoid found in SNP mixture that has been extensively studied and has been shown to antagonize cell toxicity caused by oxidative stress in neurons, suppress neuroinflammatory processes by downregulating pro-inflammatory cytokines, and stimulate neuronal regeneration at low concentrations(Khan et al., 2019).

Nitrite levels are used as an indicator for nitrative stress, which is caused by excessive nitric oxide production and kills neurons and pancreatic cells equally(Tiwari et al., 2009). In order to determine whether nitrative stress may be a factor in T2D-related cognitive impairment, we measured the levels of nitrite in the cortical, hippocampal, and pancreatic tissues in the experimental animals. The hippocampus and pancreas of untreated diabetic animals had significantly higher nitrite levels than normal controls, indicating nitrative stress in this group. Treatment with the extract at 150 and 300 mg/kg reversed the pancreatic and hippocampal nitrative stress seen in diabetic rats (Fig. 8). The pancreatic nitrite level was lower in the metformin-treated group.

Numerous studies have demonstrated that type 2 diabetes patients have abnormally high levels of several cytokines (Herder et al., 2009). According to recent research, diabetes mellitus is no longer viewed as a metabolic disease but rather as an inflammatory condition(Kothari et al., 2016). Pro-inflammatory cytokines have been suggested to be a key factor in β-cell death(Kim et al., 2017). Furthermore, hyperglycemia-induced ROS increase the levels of pro-inflammatory proteins, primarily TNF-α, which can lead to local and systemic inflammation(Hacioglu et al., 2021). INF-γ production has also been linked to systemic inflammation in diabetics (Cozachenco et al., 2019). In recent years, it has become increasingly clear that systemic inflammation affects the brain in a variety of ways and is crucial in the development of neurodegenerative diseases such as Alzheimer's (Hacioglu et al., 2021).Preclinical and clinical studies have shown that systemic inflammatory mediators such as pro-inflammatory cytokines (IL-1β, TNF-α and INF-γ) can signal to the brain via alternative pathways, namely neural and humoral pathways (Harrison et al., 2009). During this process, blood-brain barrier dysfunction may occur, allowing these cytokines to enter the CNS. This can eventually result in neuroinflammation and microglial cell activation (Jiao et al., 2018). Unfortunately, when microglia remain activated for an extended period of time, as seen in T2D and Alzheimer's disease patients, they release cytokines and neurotoxic agents such as IL-1β, TNF-α, IL-6, NO, and ROS, increasing their accumulation and causing neuronal cell death (Hansen et al., 2018).This worsening of neuroinflammation as a result of prolonged activation of microglial cells has been linked to increased Aβ deposition (McManus et al., 2014) and AChE activity (Schmatz et al., 2009), which promotes the development of brain pathology and, eventually, cognitive impairments. In this study, we found significantly higher serum levels of TNF-α, INF-γ and IL-1β, as well as increased AChE activity in the prefrontal cortex and hippocampus of untreated diabetic rats (Fig. 6), indicating weakening cholinergic action as a result of prolonged systemic inflammation and uncontrolled neuroinflammation in some brain regions of diabetic rats. This finding supports the role of cerebral changes in the pathogenesis of T2D-related cognitive impairments caused by chronic systemic inflammation. Furthermore, histopathological examination of HE- and Cresyl violet-stained hippocampus sections revealed in untreated diabetic rats a shrunken hippocampus and a severe loss of neuronal tissue integrity marked by

the presence of neurons with pyknotic nuclei (hyperchromatic), neuronal necrosis and degeneration, as well as neuronal vacuolation.

When compared to diabetic controls, animals treated with the extract at 150 and 300 mg/kg had lower serum levels of TNF-α, INF-γ and IL-1β, as well as lower AChE activity (Fig. 6). Moreover, these extract-treated groups revealed significant neuroprotection with intact neurons and minimal neurodegenerative changes as shown by the lowest pyknotic index and the maximum number of intact neurons. Interestingly, the extract's effect was more pronounced at the dose of 150 mg/kg. We believe that the anti-inflammatory and cholinergic-enhancing activity, as well as neuroprotective properties of SNP mixture is due to the potential inhibitory activity of some naturally occurring compounds in SNP (primarily flavonoids) against pro-inflammatory cytokine release and AChE, or to an increase in Acetylcholine (Ach) levels by blocking its reuptake (Khan et al., 2019; Lum et al., 2021). Indeed, linoleic acid, a fatty acid found in SNP mixture, has been demonstrated to have neuroprotective and anti-inflammatory properties in both in vitro and in vivo neurodegenerative models (Alarcon-Gil et al., 2022). Furthermore, Xanthomicrol, a flavonoid identified in SNP combination, has shown inhibitory effects on pro-inflammatory cytokines such as TNF-α, protecting neurons from neuroinflammation-induced cell death (Nair et al., 2006).

Taking our findings together, we suggest the aqueous extract of SNP combination has antidiabetic and antiamnesic properties in this T2D model-induced memory impairment and that the extract can normalize blood glucose levels, thereby reducing diabetes-related brain complications as well as ameliorating cognitive and memory dysfunction caused by T2D by reducing oxidative stress and inflammation and subsequently modifying neuronal loss and degeneration. The effect of the extract may be attributed to its active metabolites, including linoleic acid, xanthomicrol, a quercetin derivative, and mangiferin.

5. Conclusion

In summary, this study indicates that the antidiabetic and antiamnesic properties of the combination of *Sclerocarya birrea and Nauclea latifolia* and *Piper longum* may be responsible for its protective action against fructose/STZ-induced memory impairment. The mixture's active metabolites, mainly flavonoids and fatty acids, act together to alleviate hyperglycemia, insulin resistance, inflammation, cognitive deficits, cholinergic dysfunction, and oxidative stress. Therefore, SNP mixture may have immense therapeutic and prophylactic potential for the treatment of diabetes-related neurodegenerative cognitive impairment. Nevertheless, future studies are needed to isolate the active ingredient(s) and to reveal their mechanism of action.

Abbreviations

SNP, *Sclerocarya birrea-Nauclea latifolia-Piper longum*; CNS, central nervous system; T2D, type 2 diabetes; STZ, streptozotocin; BGL, blood glucose level; MWM, Morris water maze; NORT, novel object recognition test; AChE, acetylcholinesterase; ROS, reactive oxygen species; MDA, malondialdehyde; GSH, reduced

glutathione; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; INF-γ, interferon-γ; HPLC-MS, high performance liquid chromatography-mass spectrometry; ELISA, enzyme-linked immunosorbent assay; *i.v.*, intravenously; *p.o.*, per os; HOMA, homeostasis model assessment; CV, Cresyl violet; H-E, Hematoxylin-eosin.

Declarations

Acknowledgements

The authors would like to acknowledge “Pathologie Cytologie Développement” for providing histological reagents. The authors are very thankful to the University of Yaoundé 1 (Cameroon) for their support in providing apparatus for behavioral testing.

Author Declarations

Funding

The authors did not receive support from any organization for the submitted work.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Availability of data and material

The figures and tables supporting the results of this study are included in the article and the original datasets are available from the first author or corresponding author upon request.

Code availability

Not applicable

Authors’ contributions

Conceptualization: Théophile Dimo, Florence Ngueguim Tsofack; Methodology: Jean Philippe Djientcheu Tientcheu, Florence Ngueguim Tsofack, Kandeda kavaye Antoine; Formal analysis and investigation: Jean Philippe Djientcheu Tientcheu, Rodrigue Fifen, Michel Arnaud Mbock; Writing – original draft preparation: Jean Philippe Djientcheu Tientcheu, Florence Ngueguim Tsofack; Writing – review and editing: Racelyne Kamkumo Gounoue, Théophile Dimo; Supervision: Théophile Dimo. All authors read and approved its final version.

Ethics declarations

All the experiments were conducted in accordance with the approval of the Cameroon National Ethical Committee (Ref No. FwIRb00001954, 04/09/2006), which adopted the guidelines of the National

Research Council's Guide for the Care and Use of Laboratory Animals. All animal experiments also comply with the ARRIVE guidelines 2.0.

Consent to participate

Not applicable

Consent for publication

Not applicable

References

1. Akash, M. S. H., Rehman, K., & Liaqat, A. (2018). Tumor Necrosis Factor-Alpha: Role in Development of Insulin Resistance and Pathogenesis of Type 2 Diabetes Mellitus. *J Cell Biochem*, *119*(1), 105-110. https://doi.org/10.1002/jcb.26174
2. Alarcon-Gil, J., Sierra-Magro, A., Morales-Garcia, J. A., Sanz-SanCristobal, M., Alonso-Gil, S., Cortes-Canteli, M., Niso-Santano, M., Martínez-Chacón, G., Fuentes, J. M., Santos, A., & Perez-Castillo, A. (2022). Neuroprotective and Anti-Inflammatory Effects of Linoleic Acid in Models of Parkinson's Disease: The Implication of Lipid Droplets and Lipophagy. *Cells*, *11*(15). https://doi.org/10.3390/cells11152297
3. Amouzoun, L.-L., Agbonon, A., Eklu-Gadegbeku, K., Aklikokou, K., & Gbéassor, M. (2008). Activités antipyrétique et anti-inflammatoire d'extraits hydro-alcooliques des racines et feuilles de Nauclea latifolia Smith (Rubiaceae) chez le rat Wistar. *Phytothérapie*, *6*(4), 228-231.
4. Arena, A., Zimmer, T. S., van Scheppingen, J., Korotkov, A., Anink, J. J., Mühlebner, A., Jansen, F. E., van Hecke, W., Spliet, W. G., & van Rijen, P. C. (2019). Oxidative stress and inflammation in a spectrum of epileptogenic cortical malformations: molecular insights into their interdependence. *Brain Pathology*, *29*(3), 351-365.
5. Barrett, E. J., Liu, Z., Khamaisi, M., King, G. L., Klein, R., Klein, B. E. K., Hughes, T. M., Craft, S., Freedman, B. I., Bowden, D. W., Vinik, A. I., & Casellini, C. M. (2017). Diabetic Microvascular Disease: An Endocrine Society Scientific Statement. *J Clin Endocrinol Metab*, *102*(12), 4343-4410. https://doi.org/10.1210/jc.2017-01922
6. Bhutada, P., Mundhada, Y., Bansod, K., Bhutada, C., Tawari, S., Dixit, P., & Mundhada, D. (2010). Ameliorative effect of quercetin on memory dysfunction in streptozotocin-induced diabetic rats. *Neurobiol Learn Mem*, *94*(3), 293-302. https://doi.org/10.1016/j.nlm.2010.06.008
7. Biessels, G. J., Kamal, A., Urban, I. J., Spruijt, B. M., Erkelens, D. W., & Gispen, W. H. (1998). Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. *Brain Res*, *800*(1), 125-135. https://doi.org/10.1016/s0006-8993(98)00510-1
8. Butterfield, D. A., Di Domenico, F., & Barone, E. (2014). Elevated risk of type 2 diabetes for development of Alzheimer disease: a key role for oxidative stress in brain. *Biochim Biophys Acta*,

1842(9), 1693-1706. https://doi.org/10.1016/j.bbadis.2014.06.010

9. Castro, M. F. V., Stefanello, N., Assmann, C. E., Baldissarelli, J., Bagatini, M. D., da Silva, A. D., da Costa, P., Borba, L., da Cruz, I. B. M., Morsch, V. M., & Schetinger, M. R. C. (2021). Modulatory effects of caffeic acid on purinergic and cholinergic systems and oxi-inflammatory parameters of streptozotocin-induced diabetic rats. *Life Sci*, *277*, 119421. https://doi.org/10.1016/j.lfs.2021.119421
10. Cerasuolo, J., & Izzo, A. (2017). Persistent impairment in working memory following severe hyperglycemia in newly diagnosed type 2 diabetes. *Endocrinol Diabetes Metab Case Rep*, *2017*. https://doi.org/10.1530/edm-17-0101
11. Chukwuma, C. I., Matsabisa, M. G., Ibrahim, M. A., Erukainure, O. L., Chabalala, M. H., & Islam, M. S. (2019). Medicinal plants with concomitant anti-diabetic and anti-hypertensive effects as potential sources of dual acting therapies against diabetes and hypertension: A review. *J Ethnopharmacol*, *235*, 329-360. https://doi.org/10.1016/j.jep.2019.02.024
12. Cozachenco, D., Selles, M. C., & Ribeiro, F. C. (2019). Interferon-γ as a potential link between diabetes mellitus and dementia. *Journal of Neuroscience*, *39*(24), 4632-4635.
13. Dimo, T., Rakotonirina, S. V., Tan, P. V., Azay, J., Dongo, E., Kamtchouing, P., & Cros, G. (2007). Effect of Sclerocarya birrea (Anacardiaceae) stem bark methylene chloride/methanol extract on streptozotocin-diabetic rats. *J Ethnopharmacol*, *110*(3), 434-438. https://doi.org/10.1016/j.jep.2006.10.020
14. Driscoll, I., Shumaker, S. A., Snively, B. M., Margolis, K. L., Manson, J. E., Vitolins, M. Z., Rossom, R. C., & Espeland, M. A. (2016). Relationships between caffeine intake and risk for probable dementia or global cognitive impairment: the Women's Health Initiative Memory Study. *J The Journals of Gerontology: Series A*, *71*(12), 1596-1602.
15. Duff, M., Demidova, O., Blackburn, S., & Shubrook, J. (2015). Cutaneous manifestations of diabetes mellitus. *Clinical Diabetes*, *33*(1), 40-48.
16. Ellman, G. L. (1959). Tissue sulfhydryl groups. *Arch Biochem Biophys*, *82*(1), 70-77. https://doi.org/10.1016/0003-9861(59)90090-6
17. Ellman, G. L., Courtney, K. D., Andres, V., Jr., & Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*, *7*, 88-95. https://doi.org/10.1016/0006-2952(61)90145-9
18. Estévez, M. (2011). Protein carbonyls in meat systems: a review. *Meat Sci*, *89*(3), 259-279. https://doi.org/10.1016/j.meatsci.2011.04.025
19. Fukui, K., Onodera, K., Shinkai, T., Suzuki, S., & Urano, S. (2001). Impairment of learning and memory in rats caused by oxidative stress and aging, and changes in antioxidative defense systems. *Annals of the New York Academy of Sciences*, *928*(1), 168-175.
20. Gheibi, S., Kashfi, K., & Ghasemi, A. (2017). A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. *Biomedicine Pharmacotherapy*, *95*, 605-613.

21. Gidado, A., Ameh, D. A., Atawodi, S. E., & Ibrahim, S. (2008). Hypoglycaemic activity of Nauclea latifolia Sm. (Rubiaceae) in experimental animals. *Afr J Tradit Complement Altern Med*, *5*(2), 201-208. https://doi.org/10.4314/ajtcam.v5i2.31274
22. Hacioglu, C., Kar, F., Kara, Y., Yucel, E., Donmez, D. B., Sentürk, H., & Kanbak, G. (2021). Comparative effects of metformin and Cistus laurifolius L. extract in streptozotocin-induced diabetic rat model: oxidative, inflammatory, apoptotic, and histopathological analyzes. *Environmental Science Pollution Research*, *28*(41), 57888-57901.
23. Hansen, D. V., Hanson, J. E., & Sheng, M. (2018). Microglia in Alzheimer's disease. *Journal of Cell Biology*, *217*(2), 459-472.
24. Harandi, S., Golchin, L., Ansari, M., Moradi, A., Shabani, M., & Sheibani, V. (2015). Antiamnesic Effects of Walnuts Consumption on Scopolamine-Induced Memory Impairments in Rats. *Basic Clin Neurosci*, *6*(2), 91-99.
25. Harrison, N. A., Brydon, L., Walker, C., Gray, M. A., Steptoe, A., Dolan, R. J., & Critchley, H. D. (2009). Neural origins of human sickness in interoceptive responses to inflammation. *Biological psychiatry*, *66*(5), 415-422.
26. Herder, C., Brunner, E. J., Rathmann, W., Strassburger, K., Tabák, A. G., Schloot, N. C., & Witte, D. R. (2009). Elevated levels of the anti-inflammatory interleukin-1 receptor antagonist precede the onset of type 2 diabetes: the Whitehall II study. *Diabetes Care*, *32*(3), 421-423.
27. Jiao, Z., Zhang, W., Chen, C., Zhu, X., Chen, X., Zhou, M., Peng, G., Liu, H., Qiu, J., & Lin, Y. (2018). Gene Dysfunction Mediates Immune Response to Dopaminergic Degeneration in Parkinson's Disease. *ACS Chemical Neuroscience*, *10*(2), 803-811.
28. Khan, H., Ullah, H., Aschner, M., Cheang, W. S., & Akkol, E. K. (2019). Neuroprotective Effects of Quercetin in Alzheimer's Disease. *Biomolecules*, *10*(1). https://doi.org/10.3390/biom10010059
29. Kilari, E. K., Rao, L. S. N., Sreemanthula, S., & Kola, P. K. (2015). Anti-stress and nootropic activity of aqueous extract of Piper longum fruit, estimated by noninvasive biomarkers and Y-maze test in rodents. *Environ Exp Biol*, *13*, 25-31.
30. Kim, Y. G., Baltabekova, A. Z., Zhiyenbay, E. E., Aksambayeva, A. S., Shagyrova, Z. S., Khannanov, R., Ramanculov, E. M., & Shustov, A. V. (2017). Recombinant Vaccinia virus-coded interferon inhibitor B18R: Expression, refolding and a use in a mammalian expression system with a RNA-vector. *PloS one*, *12*(12), e0189308.
31. Kothari, V., Galdo, J. A., & Mathews, S. T. (2016). Hypoglycemic agents and potential anti-inflammatory activity. *Journal of inflammation research*, *9*, 27.
32. Kouémou, N. E., Taiwe, G. S., Moto, F. C. O., Pale, S., Ngoupaye, G. T., Njapdounke, J. S. K., Nkantchoua, G. C. N., Pahaye, D. B., & Bum, E. N. (2017). Nootropic and Neuroprotective Effects of Dichrocephala integrifolia on Scopolamine Mouse Model of Alzheimer's Disease. *Front Pharmacol*, *8*, 847. https://doi.org/10.3389/fphar.2017.00847
33. Kraeuter, A. K., Guest, P. C., & Sarnyai, Z. (2019). The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice. *Methods Mol Biol*, *1916*, 105-111. https://doi.org/10.1007/978-1-4939-

8994-2_10

34. Kumar, S., Sharma, S., & Suman, J. (2011). In vivo anti-hyperglycemic and antioxidant potential of Piper longum fruit. *J Pharm Res*, *4*, 471-474.
35. Lang, B. T., Yan, Y., Dempsey, R. J., & Vemuganti, R. (2009). Impaired neurogenesis in adult type-2 diabetic rats. *Brain Res*, *1258*, 25-33. https://doi.org/10.1016/j.brainres.2008.12.026
36. Li, X. H., Xin, X., Wang, Y., Wu, J. Z., Jin, Z. D., Ma, L. N., Nie, C. J., Xiao, X., Hu, Y., & Jin, M. W. (2013). Pentamethylquercetin protects against diabetes-related cognitive deficits in diabetic Goto-Kakizaki rats. *J Alzheimers Dis*, *34*(3), 755-767. https://doi.org/10.3233/jad-122017
37. Liu, K. F., Niu, C. S., Tsai, J. C., Yang, C. L., Peng, W. H., & Niu, H. S. (2022). Comparison of area under the curve in various models of diabetic rats receiving chronic medication. *Arch Med Sci*, *18*(4), 1078-1087. https://doi.org/10.5114/aoms.2019.91471
38. Lotfy, M., Adeghate, J., Kalasz, H., Singh, J., & Adeghate, E. (2017). Chronic Complications of Diabetes Mellitus: A Mini Review. *Curr Diabetes Rev*, *13*(1), 3-10. https://doi.org/10.2174/1573399812666151016101622
39. Lum, P. T., Sekar, M., Gan, S. H., Pandy, V., & Bonam, S. R. (2021). Protective effect of mangiferin on memory impairment: A systematic review. *Saudi journal of biological sciences*, *28*(1), 917-927. https://doi.org/https://doi.org/10.1016/j.sjbs.2020.11.037
40. Mathiasen, J. R., & DiCamillo, A. (2010). Novel object recognition in the rat: a facile assay for cognitive function. *Curr Protoc Pharmacol*, *Chapter 5*, Unit 5.59. https://doi.org/10.1002/0471141755.ph0559s49
41. Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, *28*(7), 412-419. https://doi.org/10.1007/bf00280883
42. McManus, R. M., Higgins, S. C., Mills, K. H., & Lynch, M. A. (2014). Respiratory infection promotes T cell infiltration and amyloid-β deposition in APP/PS1 mice. *Neurobiology of aging*, *35*(1), 109-121.
43. Mehta, B. K., & Banerjee, S. (2017). Characterization of cognitive impairment in type 2 diabetic rats. *Indian Journal of Pharmaceutical Sciences*, *79*(5), 785-793.
44. Mendiola, A. S., & Cardona, A. E. (2018). The IL-1β phenomena in neuroinflammatory diseases. *J Neural Transm (Vienna)*, *125*(5), 781-795. https://doi.org/10.1007/s00702-017-1732-9
45. Miranda, C. A., Schönholzer, T. E., Klöppel, E., Sinzato, Y. K., Volpato, G. T., Damasceno, D. C., & Campos, K. E. (2019). Repercussions of low fructose-drinking water in male rats. *An Acad Bras Cienc*, *91*(1), e20170705. https://doi.org/10.1590/0001-3765201920170705
46. Moheet, A., Mangia, S., & Seaquist, E. R. (2015). Impact of diabetes on cognitive function and brain structure. *Annals of the New York Academy of Sciences*, *1353*(1), 60-71.
47. Nabi, S. A., Kasetti, R. B., Sirasanagandla, S., Tilak, T. K., Kumar, M. V., & Rao, C. A. (2013). Antidiabetic and antihyperlipidemic activity of Piper longum root aqueous extract in STZ induced diabetic rats. *BMC Complement Altern Med*, *13*, 37. https://doi.org/10.1186/1472-6882-13-37

48. Nair, M. P., Mahajan, S., Reynolds, J. L., Aalinkeel, R., Nair, H., Schwartz, S. A., & Kandaswami. (2006). The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-κβ system. *Clinical vaccine immunology*, *13*(3), 319-328.
49. Ngo Bum, E., Taiwe, G. S., Moto, F. C., Ngoupaye, G. T., Nkantchoua, G. C., Pelanken, M. M., Rakotonirina, S. V., & Rakotonirina, A. (2009). Anticonvulsant, anxiolytic, and sedative properties of the roots of Nauclea latifolia Smith in mice. *Epilepsy Behav*, *15*(4), 434-440. https://doi.org/10.1016/j.yebeh.2009.05.014
50. Njan, A. A., Adenuga, F. O., Ajayi, A. M., Sotunde, O., Ologe, M. O., Olaoye, S. O., Erdogan, O. N., & Iwalewa, O. E. (2020). Neuroprotective and memory-enhancing effects of methanolic leaf extract of Peristrophe bicalyculata in rat model of type 2 diabetes mellitus. *Heliyon*, *6*(5), e04011.
51. Nonmarmbaye, R., Kouemou, N., Neteydji, S., Njapdounke, J. S. K., & Bum, E. N. (2021). Mnemonic and neuroprotective properties of Sclerocarya birrea root bark decoction on mouse model of monosodium glutamate-induced neurotoxicity involve by its antioxidant activities. *GSC Biological Pharmaceutical Sciences*, *17*(1), 193-203.
52. Obafemi, T. O., Olaleye, M. T., & Akinmoladun, A. C. (2019). Antidiabetic property of miracle fruit plant (Synsepalum dulcificum Shumach. & Thonn. Daniell) leaf extracts in fructose-fed streptozotocin-injected rats via anti-inflammatory activity and inhibition of carbohydrate metabolizing enzymes. *J Ethnopharmacol*, *244*, 112124. https://doi.org/10.1016/j.jep.2019.112124
53. Papanas, N., & Ziegler, D. (2015). Risk factors and comorbidities in diabetic neuropathy: an update 2015. *he review of diabetic studies: RDS*, *12*(1-2), 48.
54. Parinandi, N. L., Thompson, E. W., & Schmid, H. H. (1990). Diabetic heart and kidney exhibit increased resistance to lipid peroxidation. *Biochimica et Biophysica Acta -Lipids Lipid Metabolism*, *1047*(1), 63-69.
55. Punthakee, Goldenberg R, & P., K. (2018). Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. *Can J Diabetes*, *42 Suppl 1*, S10-s15. https://doi.org/10.1016/j.jcjd.2017.10.003
56. Redish, A. D., & Touretzky, D. S. (1997). Cognitive maps beyond the hippocampus. *Hippocampus*, *7*(1), 15-35. https://doi.org/10.1002/(sici)1098-1063(1997)7:1<15::Aid-hipo3>3.0.Co;2-6
57. Ryan, R. M., & Deci, E. L. (2006). Self-regulation and the problem of human autonomy: does psychology need choice, self-determination, and will? *J Pers*, *74*(6), 1557-1585. https://doi.org/10.1111/j.1467-6494.2006.00420.x
58. Sadeghi, A., Beigy, M., Alizadeh, S., Mazloom, H., Vakili, S., Ahmadi, S., & Meshkani, R. (2017). Synergistic Effects of Ad-Libitum Low-Dose Fructose Drinking and Low-Dose Streptozotocin Treatment in Wistar Rats: A Mild Model of Type 2 Diabetes. *Acta Med Iran*, *55*(5), 304-310.
59. Schmatz, R., Mazzanti, C. M., Spanevello, R., Stefanello, N., Gutierres, J., Corrêa, M., da Rosa, M. M., Rubin, M. A., Schetinger, M. R. C., & Morsch, V. M. (2009). Resveratrol prevents memory deficits and

the increase in acetylcholinesterase activity in streptozotocin-induced diabetic rats. *European journal of pharmacology*, *610*(1-3), 42-48.

60. Sinha, A. K. (1972). Colorimetric assay of catalase. *Anal Biochem*, *47*(2), 389-394. https://doi.org/10.1016/0003-2697(72)90132-7
61. Sommerfield, A. J., Deary, I. J., McAulay, V., & Frier, B. M. (2003). Short-term, delayed, and working memory are impaired during hypoglycemia in individuals with type 1 diabetes. *Diabetes Care*, *26*(2), 390-396. https://doi.org/10.2337/diacare.26.2.390
62. Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., Stein, C., Basit, A., Chan, J. C., & Mbanya, J. C. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes research clinical practice*, *183*, 109119.
63. Tamaddonfard, E., Farshid, A. A., Asri-Rezaee, S., Javadi, S., Khosravi, V., Rahman, B., & Mirfakhraee, Z. (2013). Crocin improved learning and memory impairments in streptozotocin-induced diabetic rats. *Iranian journal of basic medical sciences*, *16*(1), 91.
64. Tang, L.-J., Li, C., Hu, S.-Q., Wu, Y.-P., Zong, Y.-Y., Sun, C.-C., Zhang, F., & Zhang, G.-Y. (2012). S-nitrosylation of c-Src via NMDAR-nNOS module promotes c-Src activation and NR2A phosphorylation in cerebral ischemia/reperfusion. *J Molecular cellular biochemistry*, *365*(1), 363-377.
65. Tiwari, V., Kuhad, A., Bishnoi, M., & Chopra, K. (2009). Chronic treatment with tocotrienol, an isoform of vitamin E, prevents intracerebroventricular streptozotocin-induced cognitive impairment and oxidative–nitrosative stress in rats. *Pharmacology Biochemistry Behavior*, *93*(2), 183-189.
66. Tsikas, D. (2007). Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. *J Chromatogr B Analyt Technol Biomed Life Sci*, *851*(1-2), 51-70. https://doi.org/10.1016/j.jchromb.2006.07.054
67. Umegaki, H. (2014). Type 2 diabetes as a risk factor for cognitive impairment: current insights. *Clin Interv Aging*, *9*, 1011-1019. https://doi.org/10.2147/cia.S48926
68. Van Handel, E. (1965). Estimation of glycogen in small amounts of tissue. *Anal Biochem*, *11*(2), 256-265. https://doi.org/10.1016/0003-2697(65)90013-8
69. Welsh, B., & Wecker, L. (1991). Effects of streptozotocin-induced diabetes on acetylcholine metabolism in rat brain. *Neurochemical research*, *16*(4), 453-460.
70. Wilbur, K. M., Bernheim, F., & Shapiro, O. W. (1949). The thiobarbituric acid reagent as a test for the oxidation of unsaturated fatty acids by various agents. *Arch Biochem*, *24*(2), 305-313.
71. Wilson, R. D., & Islam, M. S. J. P. R. (2012). Fructose-fed streptozotocin-injected rat: an alternative model for type 2 diabetes. *64*(1), 129-139.
72. Xu, Q.-G., Li, X.-Q., Kotecha, S., Cheng, C., Sun, H., & Zochodne, D. (2004). Insulin as an in vivo growth factor. *Experimental neurology*, *188*(1), 43-51.
73. Zhao, W.-Q., Alkon, D. L. J. M., & endocrinology, c. (2001). Role of insulin and insulin receptor in learning and memory. *Molecular cellular endocrinology*, *177*(1-2), 125-134.

74. Zhong, Y., Zhu, Y., He, T., Li, W., Yan, H., & Miao, Y. J. N. l. (2016). Rolipram-induced improvement of cognitive function correlates with changes in hippocampal CREB phosphorylation, BDNF and Arc protein levels. *610*, 171-176.
75. Zilliox, L. A., Chadrasekaran, K., Kwan, J. Y., & Russell, J. W. J. C. d. r. (2016). Diabetes and cognitive impairment. *16*(9), 1-11.

Figures

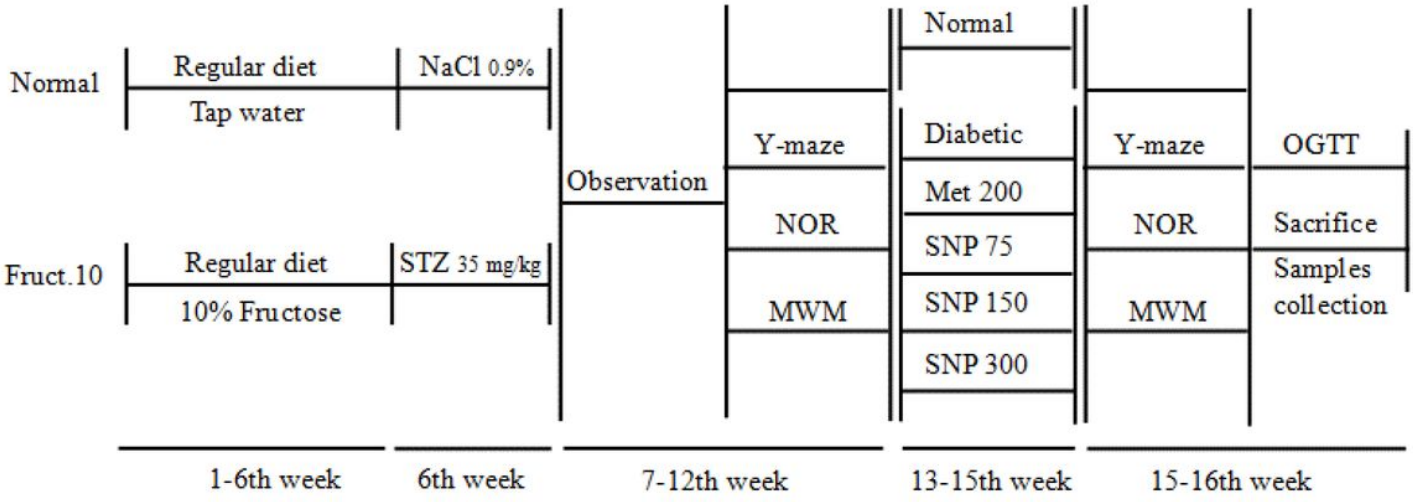

Figure 1

Outline diagram of the experimental protocol

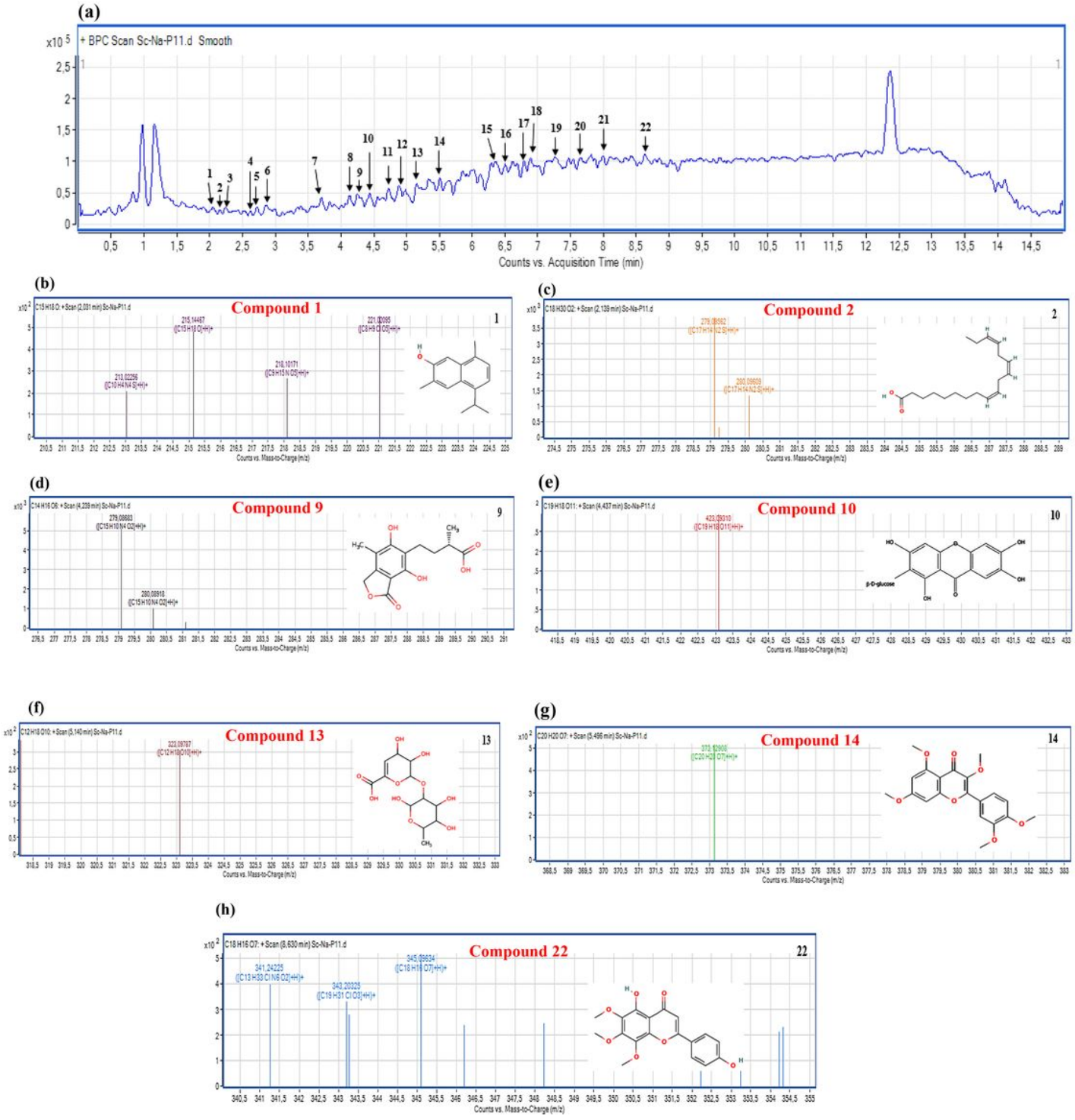

Figure 2

HPLC-MS profile of the aqueous extract of the combination of *Sclerocarya birrea* and *Nauclea latifolia* stem barks, and *Piper longum* fruits (a) and mass spectra of each detected compounds (b to h)

summarizes the major peaks in the chemical profile of the aqueous extract of SNP mixture. Twenty-two compounds were detected (Fig. 2a) among which, seven were isolated.

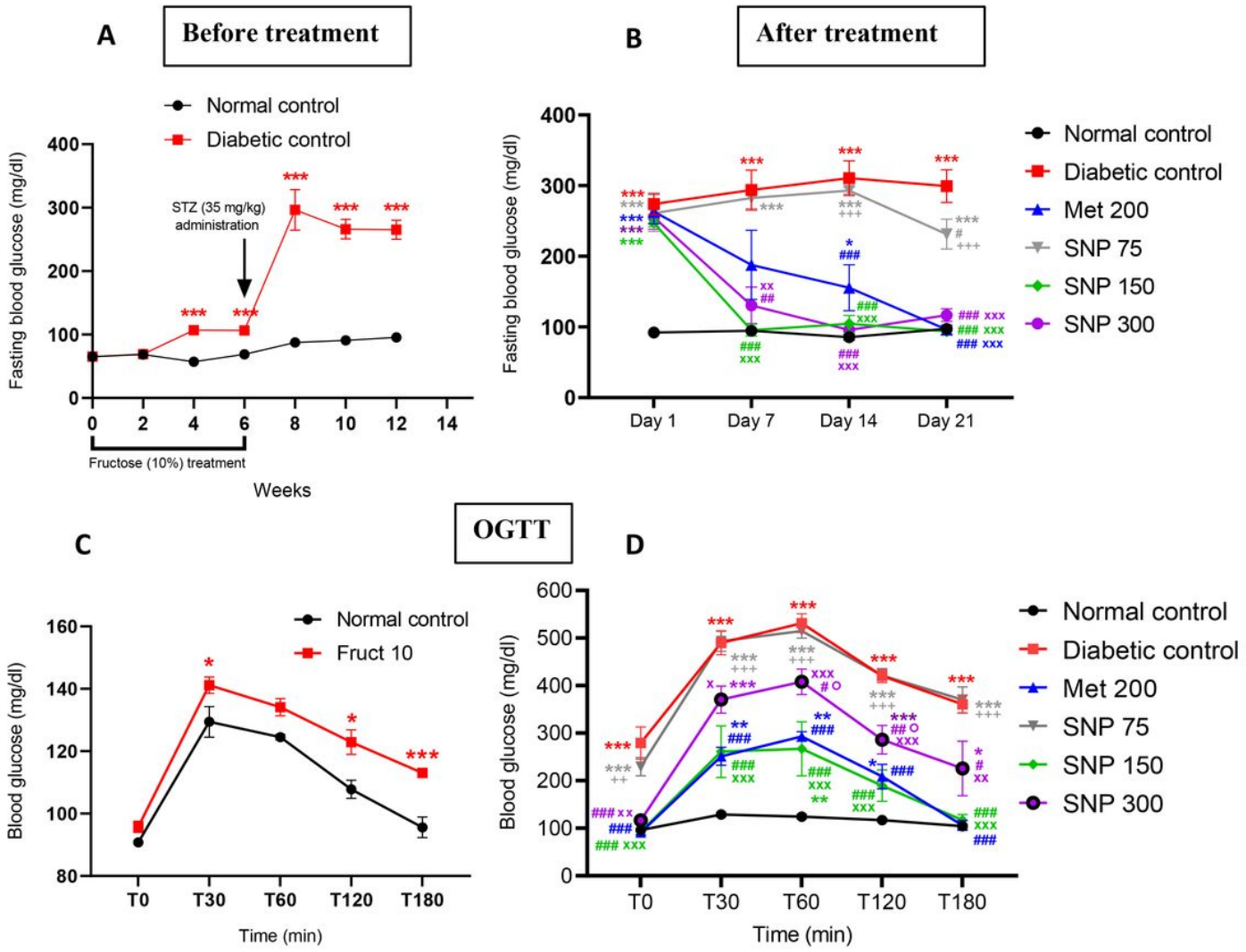

Figure 3

A: Impact of chronic hyperglycemia on blood glucose level before treatment; C: Impact of fructose regimen on glucose tolerance before STZ administration. B, D: Effects of SNP mixture on blood glucose levels (B) and glucose tolerance (D) in fructose/STZ-induced T2D rats. OGTT: Oral glucose tolerance test

Data are shown as mean ± SEM, before STZ ($15 \leq n \geq 10$); after STZ ($n = 5$);*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as compared to the normal control ;#$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ as compared to the diabetic control ; ++$p < 0.01$, +++$p < 0.001$ as compared to the positive control (Met 200) ; Xp< 0,05, XXp< 0,01, XXXp< 0,001 as compared to SNP 75 ; °p< 0,05, °°p< 0.01as compared toSNP150. Met 200: diabetic animals receiving metformin at 200 mg/kg; SNP 75, 150, 300: diabetic animals receiving the mixture at 75, 150, 300 mg/kg

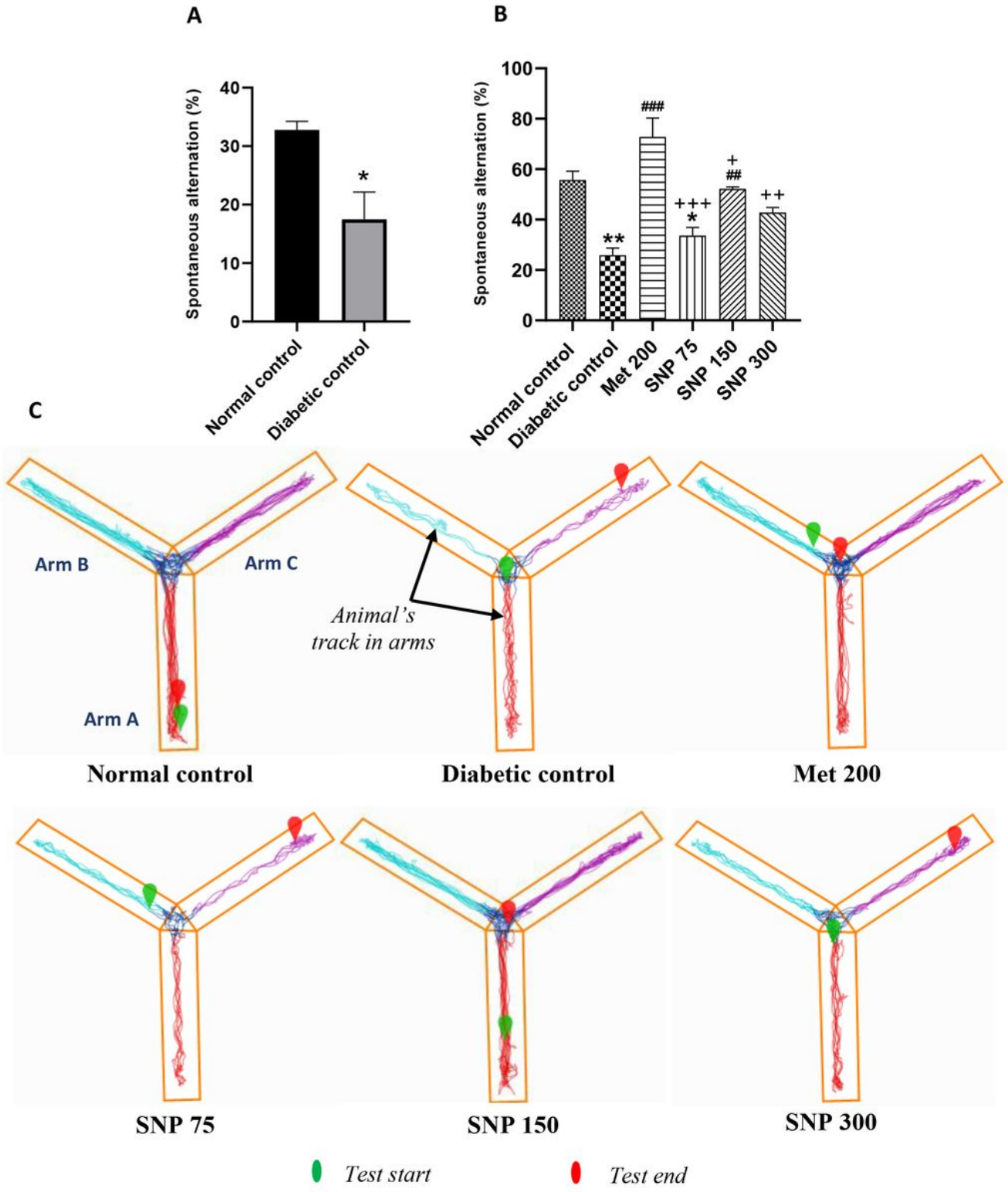

Figure 4

A: Impact of chronic hyperglycemia on working memory before treatment during Y-maze; B, C: Effects of SNP mixture on working memory during Y-maze test infructose/STZ-induced T2D rats

Data are shown as mean ± SEM, A: before treatment (20 ≤ n ≥10); B, C: after treatment (n = 5); *p < 0.05, **p < 0.01 as compared to the normal control; #p < 0,05, ##p < 0,01, ###p < 0,001 as compared to the

diabetic control;+p < 0,05, ++p < 0,01, +++p < 0,001as compared to the positive control (Met 200). Met 200: diabetic animals receiving metformin at 200 mg/kg; SNP 75, 150, 300: diabetic animals receiving the mixture at 75, 150, 300 mg/kg

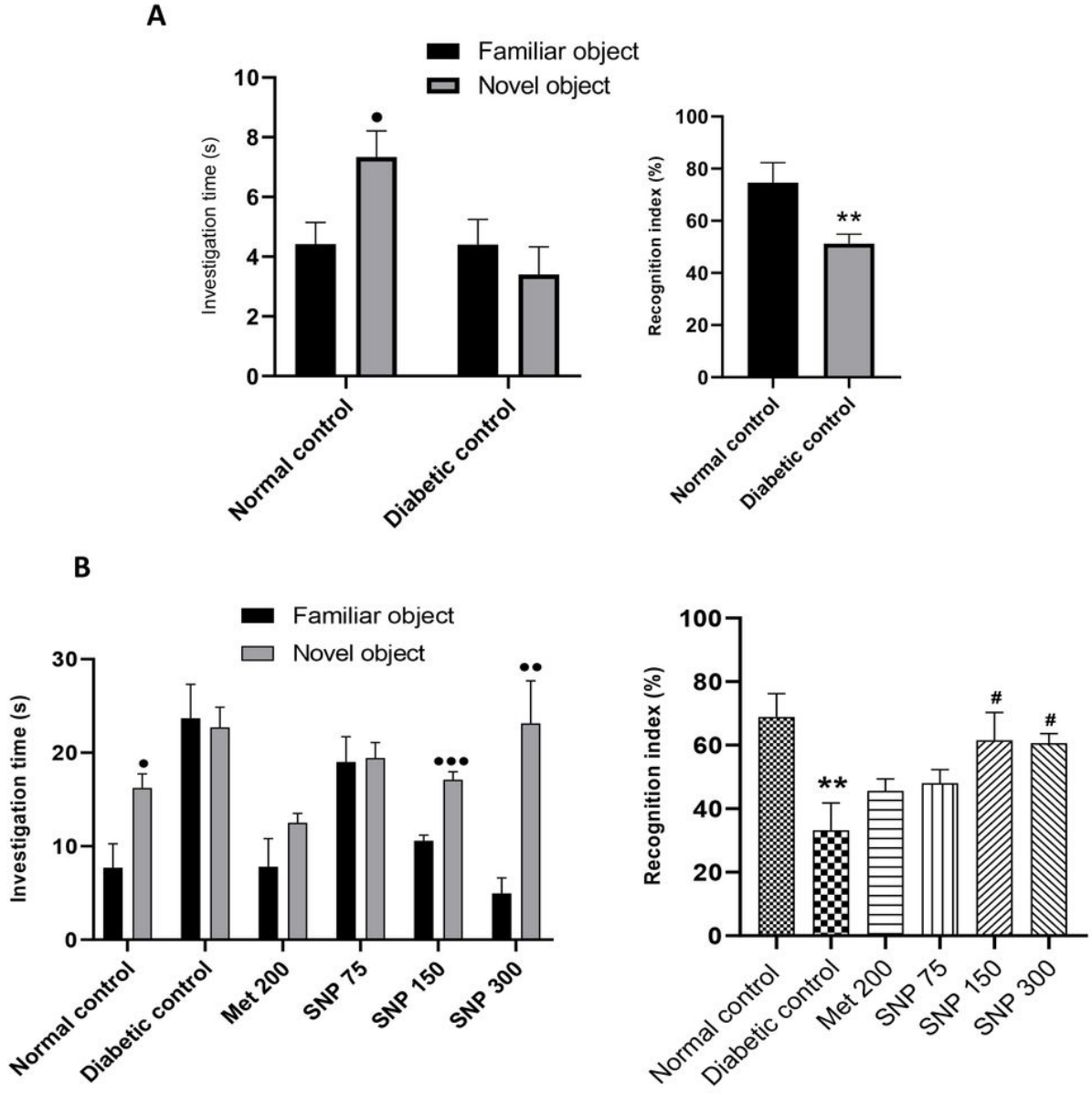

Figure 5

Impact of chronic hyperglycemia on short-term memory before treatment during NORT (A) and effects of the aqueous extract of SNP mixture on short-term memory during NORT in fructose/STZ-induced T2D rats (B)

Data are shown as mean ± SEM, A: before treatment (20 ≤ n ≥10); B: after treatment (n = 5); ●p < 0.05,

●●p < 0.01, ●●●p < 0,001 as compared to the familiar object; **p < 0.01 as compared to the normal control; #p < 0,05 as compared to the diabetic control. Met 200: diabetic animals receiving metformin at

200 mg/kg; SNP 75, 150, 300: diabetic animals receiving the mixture at 75, 150, 300 mg/kg

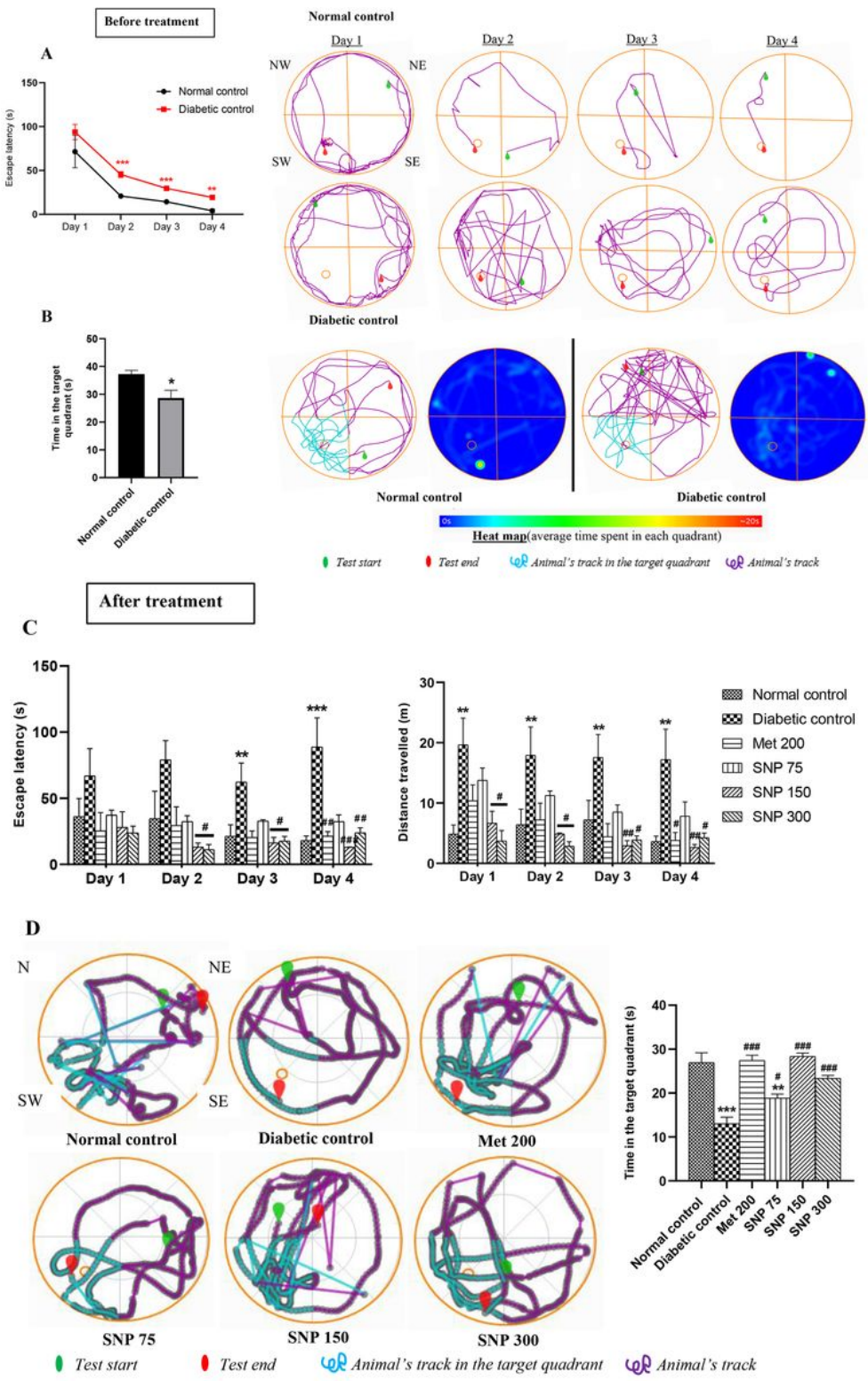

Figure 6

A, B: Impact of chronic hyperglycemia on learning and memory before treatment [acquisition phase (A); retention phase (B)] during MWM test. C, D: Effects of the aqueous extract of SNP mixture on learning

and memory after treatment [acquisition phase (C); retention phase (D)] during MWM test in fructose/STZ-induced T2D rats

Data are shown as mean ± SEM, A, B: before treatment (20 ≤ n ≥10); C, D: after treatment (n = 5); **p < 0.01, ***p < 0.001 as compared to the normal control; #p < 0.05, ##p < 0.01, ###p < 0.001 as compared to the diabetic control; Met 200: diabetic animals receiving metformin at 200 mg/kg; SNP 75, 150, 300: diabetic animals receiving the mixture at 75, 150, 300 mg/kg. NW: North-west quadrant, SW: South-west quadrant, NE: North-east quadrant, SE: South-east quadrant.

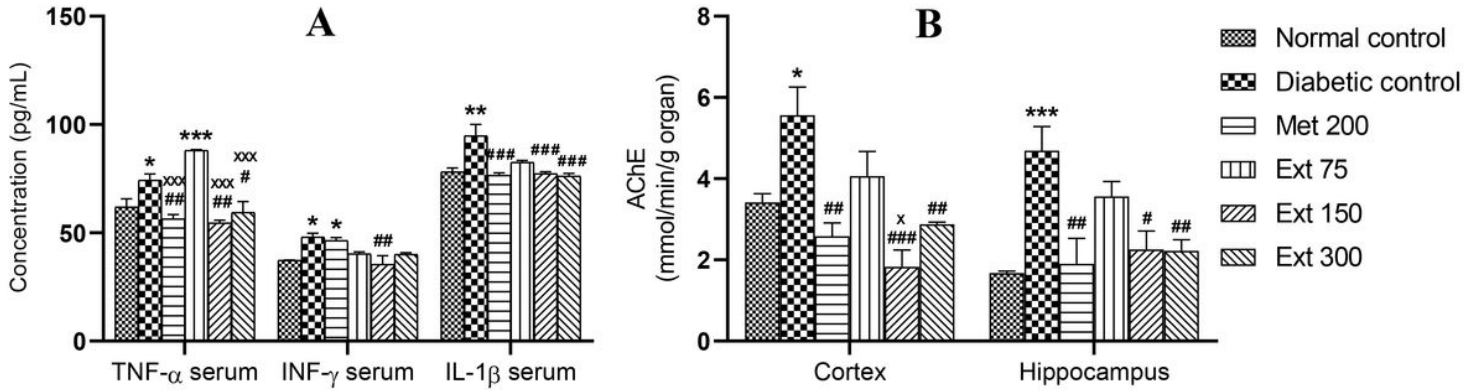

Figure 7

Effects of the aqueous extract of SNP mixture on TNF-α, INF-γ, IL-1β levels and AChE activities infructose/STZ-induced T2D rats

Data are shown as mean ± SEM, (n = 5); *p < 0.05, ***p < 0.001 as compared to the normal control;#p < 0.05; ##p < 0.01, ###p < 0.001as compared to the diabetic control; Xp< 0.05,XXXp< 0.001 as compared to Ext 75.Met 200: diabetic animals receiving metformin at 200 mg/kg; SNP 75, 150, 300: diabetic animals receiving the mixture at 75, 150, 300 mg/kg.

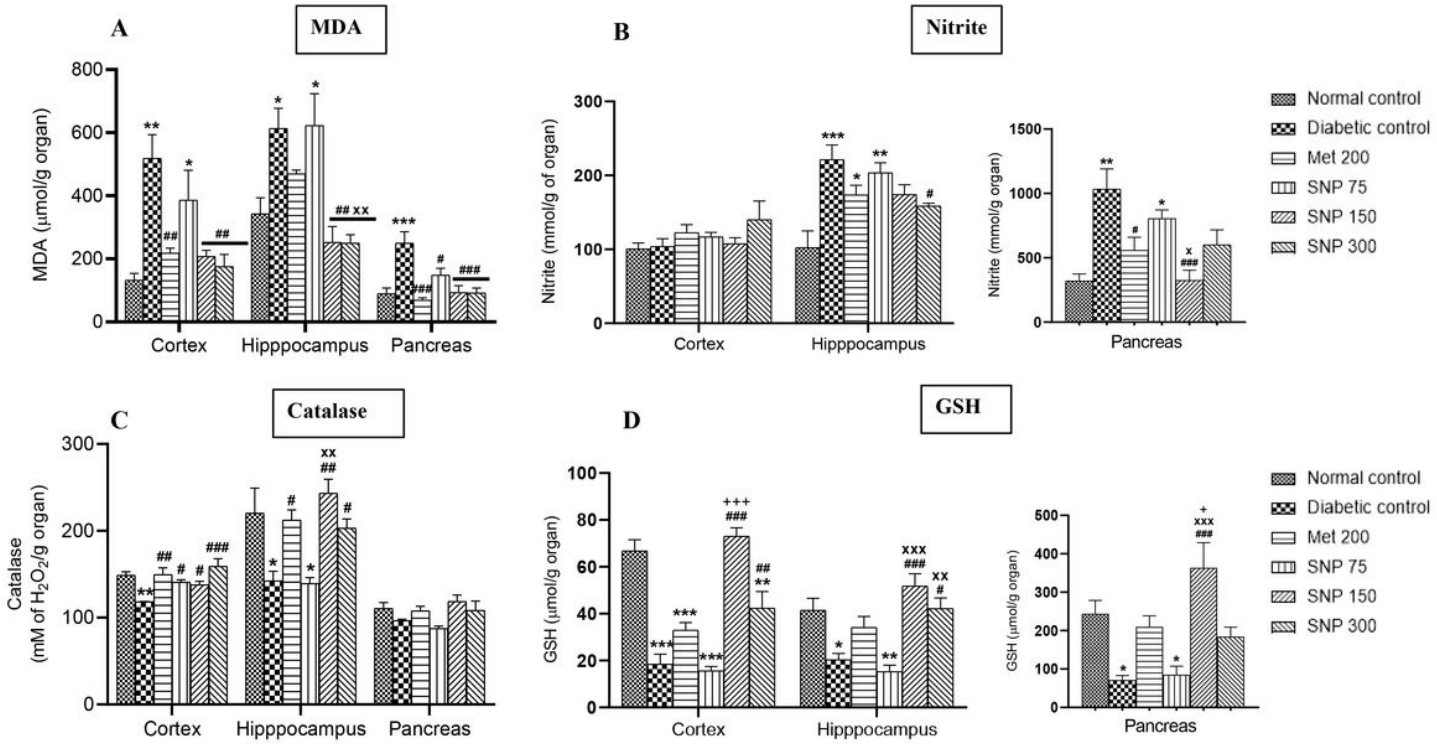

Figure 8

Effects of SNP mixture on parameters of oxidative stress in some brain regions and pancreas in fructose/STZ-induced T2D rats.

Data are shown as mean ± SEM, (n = 5); *p < 0.05, **p < 0.01, ***p < 0.001 as compared to the normal control;#p < 0.05, ##p < 0.01, ###p < 0.001as compared to the diabetic control; Xp< 0.05, XXp< 0.01, XXXp< 0.001as compared to SNP 75;+p < 0.05, +++p < 0.001as compared to the positive control (Met 200).Met 200: diabetic animals receiving metformin at 200 mg/kg; SNP 75, 150, 300: diabetic animals receiving the mixture at 75, 150, 300 mg/kg.

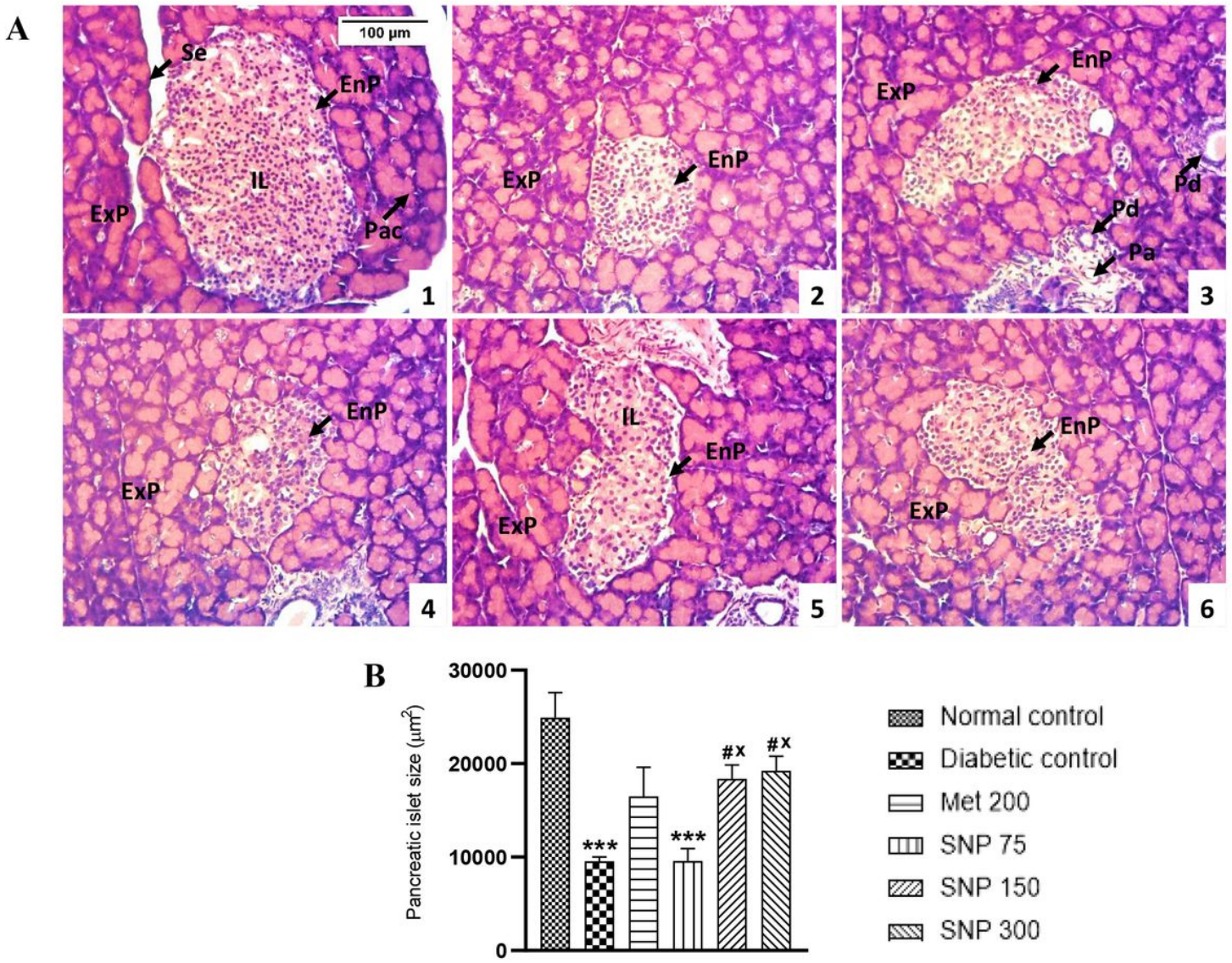

Figure 9

Effects of SNP mixture on pancreas microarchitecture. (A): photomicrographs of HE-stained sections from the pancreas(200X) (B): Histomorphometry of pancreatic islets.; *Hematoxylin-Eosin staining (HE). (B): Data are shown as mean ± SEM, ***p < 0.001 as compared to the normal control; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as compared to the diabetic control; $^{X}p < 0.05$, as compared to SNP 75. Met 200: diabetic animals receiving metformin at 200 mg/kg; SNP 75, 150, 300: diabetic animals receiving the mixture at the doses of 75, 150, 300 mg/kg, respectively.(B): 1 = normal control; 2 = diabetic control; 3 = Met 200; 4, 5, 6 = diabetic rats treated with SNP mixture at doses of 75, 150 and 300 mg/kg;* ***IL*** *= islet of Langerhans;* ***Pac*** *= pancreatic acini;* ***ExP*** *= exocrine pancreas;* ***EnP*** *= endocrine pancreas;* ***Pd*** *= pancreatic duct;* ***Pa*** *= pancreatic artery;* ***Se*** *= Septa.*

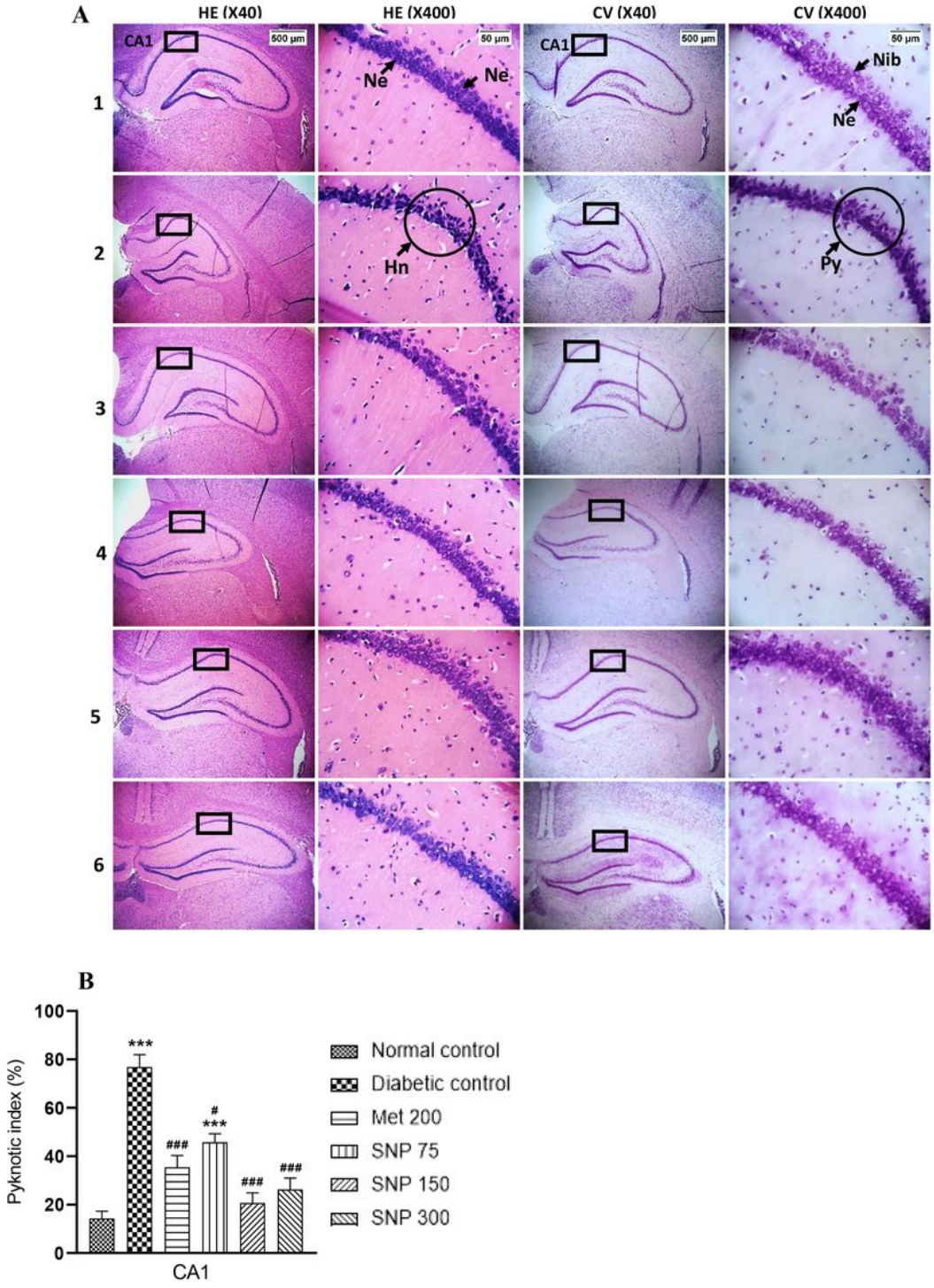

Figure 10

Effects of SNP mixture on hippocampal neurons from CA1 subfield in diabetic rats (A). Changes in the pyknotic index (%) (B). ***HE*** *= Hematoxylin-Eosin;* ***CV*** *= Cresyl violet;* ***1*** *= normal control;* ***2*** *= diabetic control;* ***3*** *= Met 200;* ***4****,* ***5, 6*** *= diabetic rats treated with SNP mixture at the doses of 75, 150 and 300 mg/kg, respectively;* ***Ne*** *= Neurons;* ***Hn*** *= Hyperchromatic neurons;* ***Py*** *= Pyknosis;* ***Nib*** *= Nissl bodies. The boxed areas in the first and third column were revealed at higher magnification in the second and forth column*

*respectively. Scale bar in the first and third column is 500 µm (magnification X40); Scale bar in the second and forth column is 50 µm (magnification X400).***p < 0.001 as compared to the normal control; #p < 0.05, ###p < 0.001 as compared to the diabetic control.*

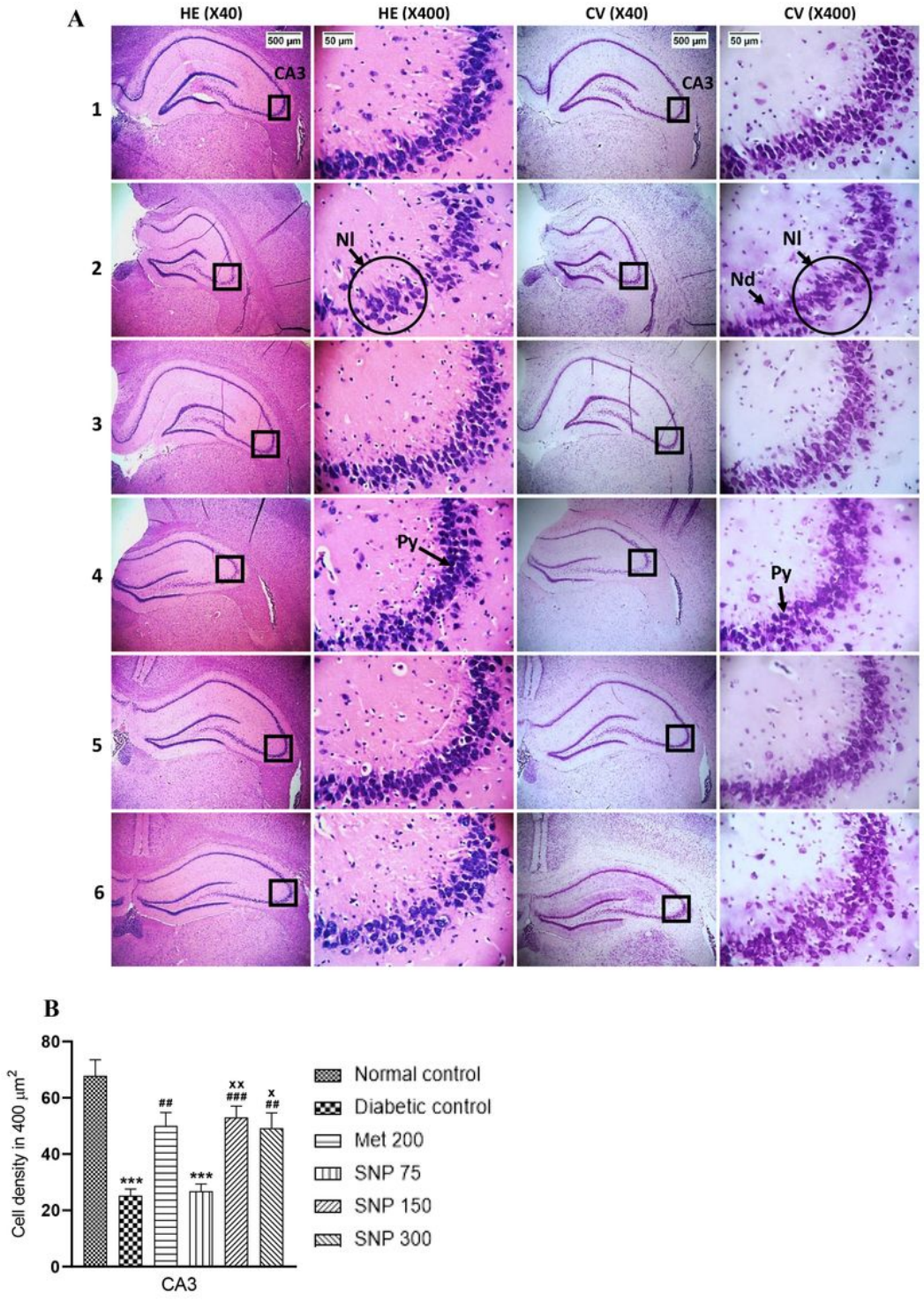

Figure 11

Effects of SNP mixture on hippocampal neurons from CA3 subfield in diabetic rats (A). Cell density in CA3 (B) ***HE*** *= Hematoxylin-Eosin;* ***CV*** *= Cresyl violet;* ***1*** *= normal control;* ***2*** *= diabetic control;* ***3*** *= Met 200;* ***4****,* ***5, 6*** *= diabetic rats treated with SNP mixture at the doses of 75, 150 and 300 mg/kg, respectively;* ***Nl*** *= Neuronal loss;* ***Nd*** *= Neuronal degeneration. The boxed areas in the first and third column were revealed at higher magnification in the second and forth column respectively. Scale bar in the first and third column is 500 µm (magnification X40); Scale bar in the second and forth column is 50 µm (magnification X400). ***p < 0.001 as compared to the normal control;* $^{\#}$*p < 0.05,* $^{\#\#}$*p < 0.01,* $^{\#\#\#}$*p < 0.001 as compared to the diabetic control;* ^{X}*p < 0.05,* ^{XX}*p < 0.01 as compared to SNP 75.*

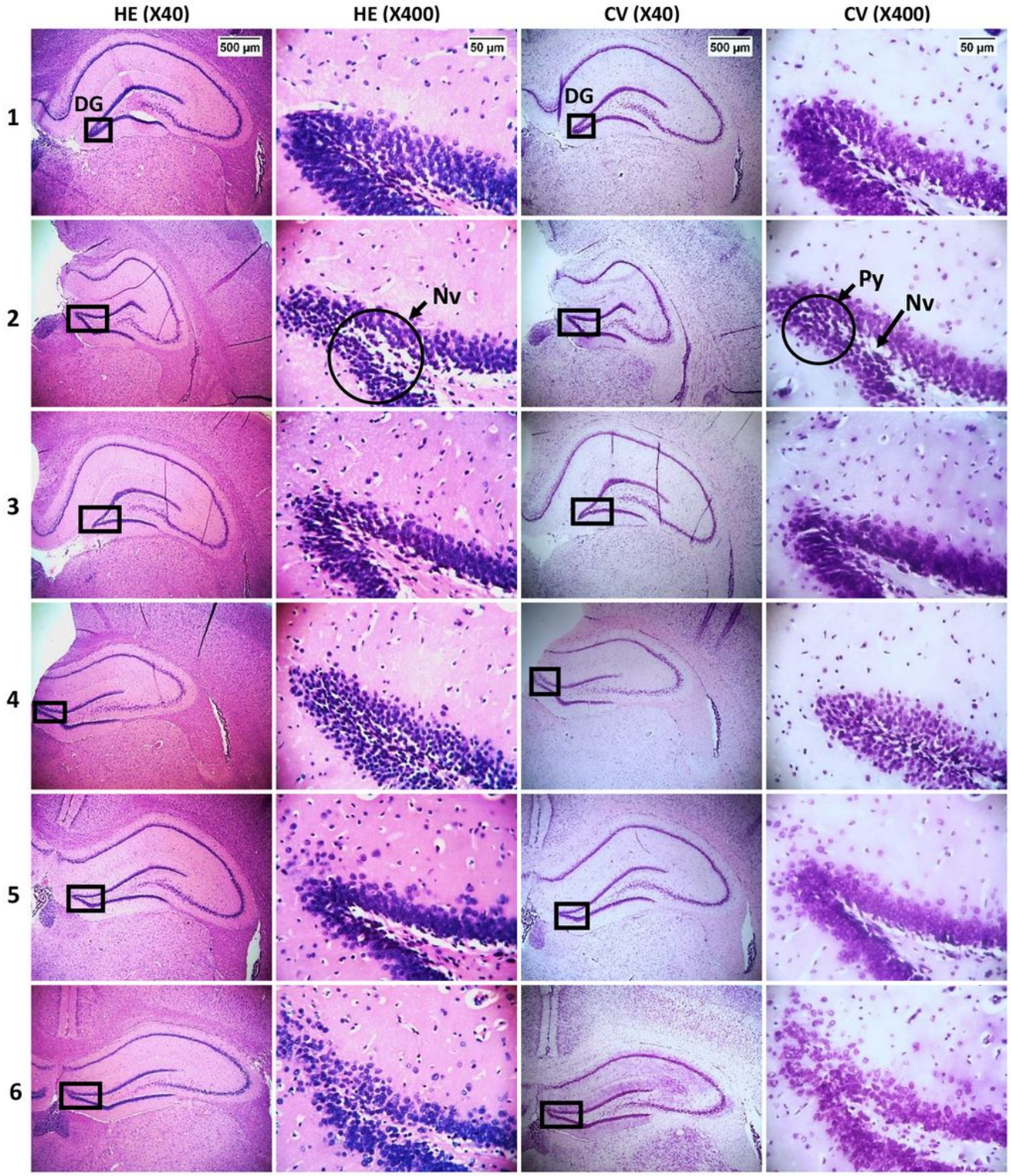

Figure 12

Effects of SNP mixture on hippocampal neurons from Dentate gyrus (DG) subfield in diabetic rats. ***HE*** *= Hematoxylin-Eosin;* ***CV*** *= Cresyl violet;* ***1*** *= normal control;* ***2*** *= diabetic control;* ***3*** *= Met 200;* ***4, 5, 6*** *= diabetic rats treated with SNP mixture at the doses of 75, 150 and 300 mg/kg, respectively;* ***Nv****= Neuronal vacuolation;* ***Py*** *= Pyknosis. The boxed areas in the first and third column were revealed at higher*

magnification in the second and forth column respectively. Scale bar in the first and third column is 500 µm (magnification X40); Scale bar in the second and forth column is 50 µm (magnification X400)