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Research article

Neuroprotective and memory-enhancing effects of methanolic leaf extract of *Peristrophe bicalyculata* in rat model of type 2 diabetes mellitus.

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ABSTRACT

This study investigated the effect of methanolic leaf extract of *Peristrophe Bicalyculata* (MEPb) on type 2 diabetes mellitus (T2DM) associated cognitive decline in Wistar rats.

36 male rats weighing 130–200 g were assigned into 6 groups (n = 6) as follows: normal control, diabetic control, pioglitazone-treated diabetic and three MEPb-treated diabetic groups, type 2 diabetes mellitus was induced with low dose streptozocin (STZ) injection following 3 weeks of high fat diet (HFD) intake. Thirty days after diabetes induction, rats exhibited marked and persistent hyperglycemia, animals were treated with MEPb (50, 100 and 200 mg/kg) and pioglitazone (10 mg/kg) as standard. Morris water maze (MWM) test and Novel object recognition test (NORT) were used to assess learning and memory. Blood glucose level, oxidative stress makers, pro-inflammatory marker and acetylcholinestarase activities were analysed.

Both MEPb and pioglitazone significantly ($P < 0.05$) reduced escape latency in treated animals compared to the diabetic control group in the MWM test. Methanolic leaf extract of *Peristrophe bicalyculata* and pioglitazone also significantly ($P < 0.05$) increased discrimination index in treated animals compared to the diabetic control group in the novel object recognition test. Serum, brain and liver MDA levels were significantly ($P < 0.05$) decreased in MEPb and pioglitazone treated rats compared to diabetic control. Serum and liver GSH as well as CAT levels were significantly ($P < 0.05$) increased while brain GSH and CAT levels shows apparent increase in MEPb and pioglitazone treated rats compared with diabetic control. Treatment with MEPb caused a significant ($P < 0.05$) decrease in brain nitrite level, interleukin 6 and acetylcholinesterase activity compared to diabetic control group.

We conclude that Methanolic leaf extract of *Peristrophe bicalyculata* enhanced antioxidant capacity and prevented neuroinflammation, consequently improving brain neuronal cholinergic function in experimental animals.

1. Introduction

Diabetes Mellitus (DM) results from the body's inability to produce insulin as a result of either total knockdown of the insulin producing mechanisms (type 1) or insufficient insulin production and/or inefficient use of insulin by the body (type 2) [1]. Type 1 diabetes mellitus (T1DM) is a consequence of autoimmune damage of insulin-producing ß-cells of Langerhans in the pancreas while Type 2 diabetes mellitus results from progressive decline in insulin action [2].

In the year 2000, about 284 million people (2.8% of the world population) were estimated to be suffering from DM [3]. This figure is projected to reach 439 million (4.4% of the world population) by the year 2030 as a result of the rising incidence of DM in the world [3]. The diseases possess great public health challenge and this is particularly worsened by other complicating clinical conditions that increase morbidity and mortality of the illness [4]. These complications affects mainly the central and peripheral nervous systems as well as organs such as the kidneys and eyes leading to cognitive decline, peripheral neuropathy, diabetic nephropathy and diabetic retinopathy respectively [5].

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Cognitive decline is one of the complications of type 2 diabetes mellitus, it is associated with atrophy of hippocampal formation (a locus specifically involved in learning and memory processing in mammals [6]) resulting from chronic hyperglycemia, oxidative stress, and cholinergic dysfunction [7]. Though some antidiabetics, insulin sensitizing agents, anti-oxidants and acetylcholinesterase inhibitors have been reported to have beneficial effects in animal models [7, 8], there is yet no specific medications designed for the prevention and/or management of this DM associated morbidity.

Peristrophe bicalyculata, an erect hispid undershrub is 60–180 cm high flowering plant usually found in forest undergrowth hedges and wasteland [9] native to tropical African regions, Thailand and India. It belongs to the Acanthaceae family [10] and is known as chotiharjori in India, moto in Senegal and tubanin-dawaki amongst the Hausa speaking ethnic group in Nigeria [11]. Its leaf is used traditionally in the management of eye and ear diseases, bacterial infections and as an antidote to venomous insect's stings and bites [10]. It is also employed in the traditional management of tuberculosis, snakebites, hysteria and psychomotor disorders. Its crude aqueous extract has been reported to possess antihypertensive, antibacterial and anti-cancer activities [12, 13, 14], and the methanolic leaf extract was also recently reported to possess anticonvulsant [15] and antidiabetic activities [11]. Since exaggerated neuronal discharges characterising epilepsy promotes hippocampal scarring and dysfunction [16], we hypothesis that *P. bicalyculata* may protect the hippocampus by promoting neurogenesis, stabilising neurons and subsequently, enhancing hippocampal function based on its reported anticonvulsant property.

Thus, the present study was designed to investigate the possible effect of methanolic leaf extract of *Peristrophe bicalyculata* on learning and memory in high fat diet/low dose streptozocin induced type 2 diabetes mellitus in rat model.

2. Materials and methods

2.1. Drugs and reagents

All drugs and reagents used in this study were of analytical grade and purchased either from Sigma Aldrich, USA or British Drug House, UK.

2.2. Plant material and preparation

Fresh plants of *Peristrophe bicalyculata* were obtained from the Staff Quarters of Obafemi Awolowo University, Ile Ife, Osun State, Nigeria and identified by a botanist at the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Leaves were shredded off from the plants' stems, washed in clean running water to remove debris and air-dried at room temperature to a constant weight. The dried leaves were milled into coarse powder form using pestle and mortar. 213g of the powdered leaves was extracted by soxhlet extraction with 1 L of 80% v/v methanol (80% Methanol: 20% distilled water) for 48 h. The extract was evaporated to dryness in a thermostat oven at 50 °C. The dried extract was weighed and stored in a desiccator until needed for the main experiments.

2.3. Preparation of high fat diet

Rat food used was 20% fat, 60 % carbohydrates and 20 % proteins (Kesmak Animal Feed Centre, Ibadan) were compacted into pellets, mixed carefully with liquefied animal fat and allowed to harden to yield a solid homogeneous mixture of lard/food pellet (40/60 w/w). The liquefied animal fat was acquired from bovine fat obtained from local butchers in Ibadan, Nigeria. The fat was melted to eliminate debris and other non-fat components before use. The 40/60 mixture of lad and animal grub gives a 52% fat diet. Having in mind that carbohydrates and fats produce 4.2 and 9 kcal/g respectively, the high fat food prepared was made up of 70% fat, 23% carbohydrate and 7 % protein calories respectively [17].

2.4. Experimental animals

36 Adult male Wistar albino rats used for this study were selected from a pool of 41 animals weighing between150 and 200g obtained from the Central Animal House, University of Ibadan. The animals were housed in groups of six in standard plastic rodent cages and acclimatise to laboratory conditions for 2 weeks before the commencement of the experiments. Animals had unhindered access to feed and tap water. The experimental groups were fed with high fat diet while the normal control group remained on normal rat chow throughout the experimental period.

2.5. Induction of diabetes mellitus

Diabetes was induced in the animals following the method described by Umathe *et al* [18]. After 3 weeks of being on HFD, the experimental animals were administered intraperitoneal injection of streptozotocin (STZ) dissolved in 0.1M citrate buffer, pH 4.4 at 40 mg/kg dose after 13 h of food and water fast. The Streptozotocin-treated rats subsequently had 10% glucose solution for 3 days in order to prevent death from Streptozotocin-induced hypoglycemic shock. Blood samples obtained from the tail vein of rats were evaluated for fasting blood glucose (FBG) 10 days post induction [19] and only rats with fasting blood glucose levels greater than 200 mg/dl were considered diabetic and used for the study.

2.6. Animal grouping and treatment schedule

2.6.1. Animal grouping

Rats were divided into six groups of six animals each as follow: group 1: vehicle (received distilled water and served as normal control), group 2: HFD/STZ + vehicle (served as diabetic control), group 3: HFD/STZ+ 50 mg/kg *Peristrophebicalyculata* extract (Test group I), group 4: HFD/STZ +100 mg/kg *Peristrophebicalyculata* extract (Test group II), group 5: HFD/STZ +200 mg/kg *Peristrophebicalyculata* extract (test group III), group 6: HFD/STZ + pioglitazone (10 mg) (served as cognitive decline treatment control).

2.6.2. Treatment schedule

Treatment was given to animals for 30 days beginning from the day of confirmation of diabetes in induced animals. Group 1 received distilled water and served as normal control, Group 2 received HFD/STZ +0.5 ml of distilled water served as diabetic control; Group 3, 4 and 5 had 50, 100 and 200 mg/kg *P. bicalyculata* respectively while group 6 had 10 mg/kg pioglitazone for 30 days. FBG was evaluated in the animals on days 0, 15 and 30 of treatment.

2.7. Assessment of cognitive function

Learning and memory functions were evaluated on days 31–36 for Morris water maze test and day 32–33 for Novel Object Recognition test.

2.7.1. Morris water maze test

Cognitive function of rats was assessed using Morris Water Maze test as described earlier [20]. The test apparatus was a circular water tank (180 cm in diameter and 60 cm high) made up of dark grey plastic that was partially filled with water. Full cream milk (liquid) was used to render the water opaque. The pool was divided virtually into four equal quadrants, labelled A– B–C–D. A platform (12.5 cm in diameter and 38 cm high) was placed in one of the four maze quadrants (the target quadrant) and submerged 2.0 cm below the water surface. The platform remained in the same quadrant for the entire experimental duration. The rats were required to find the platform using only distal spatial extra-maze cues available in the testing room. The cues were maintained constant throughout the testing period. The rats received four consecutive daily training trials for 6 days following completion of treatment, with each trial having a ceiling time of 60s and a trial interval of

approximately 90s. Each rat had to swim until it climbed onto the platform submerged underneath the water. After climbing onto the platform, each rat remained there for 30s before the commencement of the next trial. The escape platform was kept in the same position relative to the distal cues. If any rat fails to reach the escape platform within the maximally allowed time of 60s, it was gently guided to the platform and allowed to remain there for 30s before the next trial. The time to climb the platform (latency in seconds) was measured. A probe trial was conducted on the 6^{th} day. The platform was removed from the maze and rats were expected to spend more time in the quadrants initially housing the platform. The total time spent in quadrants other than that for the platform was measured and recorded for each rat as a measure of memory decline.

2.7.2. Novel object recognition test (NORT)

The object recognition test for assessing non spatial memory in rats was deployed. The test environment was an open field (80cm long × 50cm high × 60cm wide). 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 south east pole of the test field while the rats were allowed to explore the test area. On day 3, one of the identical object was replaced with a novel object and day two experiment was repeated for each rat. During the inter trial interval, 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 novel object ratio (NOR) as follows: [21]

$$NOR = (T_{novel} - T_{familiar})/(T_{novel} + T_{familiar}), \quad (1)$$

Where T_{novel} = time spent to explore the novel object and $T_{familiar}$ = time spent to explore the familiar object.

2.8. Preparation of blood and organs for biochemical assay

Animals were sacrificed on day 36 by cervical dislocation. Blood was collected by cardiac puncture into plain sample bottles and centrifuged at room temperature for 15 min at 3000 rpm. Serum was aliquoted into different sets of plain bottles for determination of serum anti-oxidant and anti-inflammatory parameters. Organs such as brain, heart, kidney and liver were isolated and weighed. Homogenate of liver and brain were prepared in 50 volume of 0.1M phosphate buffered saline using auto-homogeniser for the evaluation of organ oxidative and nitrosative stress markers and brain acetylcholinesterase (AChE) activity.

2.9. Determination of lipid peroxidation

Malondialdehyde (MDA) was assayed as an indicator of lipid peroxidation following the method described by Okhawa *et al* [22]. This assay relies on the principle that lipid peroxidation produces unstable lipid peroxides that decompose to form complex series of compounds including reactive carbonyl compounds. Unstable peroxides of polyunsaturated fatty acid decomposes to malondialdehyde which is a 1:2 adduct with thiobarbituric acid (TBA) that generates a pink colour product when heated in acidic pH, with a maximum absorbance at 532nm. Briefly, an aliquot of 0.4ml of each sample was mixed with 1.6ml of Tris-potassium chloride (Tris-KCl) buffer to which 0.5ml of 30% trichloroacetic acid (TCA) was added. Then 0.5ml of 0.75% TBA was be added and placed in a water bath for 45 min at 80 °C. This was then cooled in ice and centrifuged at 3000g for 15 min. The clear supernatant was harvested and absorbance measured against a reference blank of distilled water at 532nm. MDA concentration was calculated using a Molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ CM^{-1}$ and the value was expressed as nmol formed per milligram protein [22]

$$MDA\ (nmol/mg\ protein) = Absorbance\ x\ volume\ of\ mixture/E_{532nm}\ x\ volume\ of\ sample\ x\ mg\ protein, \quad (2)$$

2.10. Estimation of reduced glutathione (GSH) level

GSH was assayed following the method described by Jollow *et al* [23]. This method is based upon production of a relatively stable yellow colour when 5′, 5′–dithiobis-(2-nitrobenzoic acid) (DTNB) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of DTNB with the reduced glutathione, 2 – nitro-5-thiobenzoic acid is maximally absorbed at 412nm and the amount of reduced glutathione in the sample was proportional to the absorbance at the wavelength. Briefly, 0.4 ml of each sample was added to 0.4ml of 20% trichloroacetic acid (TCA) and mixed by gentle swirling motion and centrifuged at 10, 000 rpm for 10 min at 4 °C (in cool centrifuge). 0.25ml of the supernatant was withdrawn and added to 2ml of 0.6 mM DTNB and the final volume of the solution was made up to 3 ml with (0.75 ml) phosphate buffer (0.2M, pH 8.0). Absorbance was read at 412nm against blank reagent [2ml of 0.6 mM DTNB +1 ml phosphate buffer (0.2M, pH 8.0)] using a spectrophotometer. The concentration of reduced GSH in the brain and tissue is expressed as micromoles per gram of protein (μmol/mg).

2.11. Determination of catalase activity

Catalase level was determined by the method described by Sinha [24] which is based on the principle of Hydrogen peroxide (H_2O_2) degradation in the presence of catalase enzyme. 0.5ml of supernatant fraction of the brain homogenate was mixed with 9.5ml distilled water to give a 1: 20 dilution. The assay mixture contained 2.5ml of phosphate buffer, pH 7.0 and 2ml of H_2O_2 solution (800μmoles) in a 5ml flat bottom flask. 0.5ml of properly diluted sample was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. 1ml portion of the reaction mixture was withdrawn and added into 2ml dichromate/-acetic acid reagent [5% solution of $K_2Cr_2O_7$ with glacial acetic acid (1:3 by volume)]. The absorbance was measured using two cuvettes at 60 and 180 s intervals respectively at 570nm using a spectrophotometer. Catalase activity was calculated by extrapolating the remaining H_2O_2 from the standard curve. H_2O_2 consumed = 800μmoles – H_2O_2 remaining [24].

$$Catalase\ activity = H_2O_2\ consumed\ (mean) \div mg\ protein, \quad (3)$$

mg protein was obtained from the protein estimation for each respective sample.

2.12. Determination of brain nitrite level

Nitrite, a measure of nitric oxide production, was assayed as an indicator of nitrosative stress in the brain using Griess reagent which was prepared by mixing 50ml of 1% sulfanilamide with 50ml of 0.1% naphthylethylenediamine and stored at 4 °C. 1% sulfanilamide was prepared by dissolving 0.5g of sulfanilamide in 50ml of 5% phosphoric acid (which was itself prepared by mixing 2.5 ml of phosphoric acid with 50 ml of distilled water). 0.1% naphthylethylenediamine was also prepared by dissolving 0.05g of naphthylethylenediamine in 50ml of distilled water. 0.1ml of Griess reagent was added to 0.3ml of brain homogenate followed by the addition of 2.9ml of distilled water. The blank was prepared by mixing 0.1ml Griess reagent with 2.9ml of distilled water. The absorbance was read at 548nm and nitrite concentration was determined using a standard sodium nitrite curve.

2.13. Determination of IL-6

IL-6 production was quantified from rats' brain homogenate by using standard ELISA kits Endogen (Woburn, MA, USA). Each brain section was mixed with 10 volumes of ice-cold buffer (20 mMTris–HCl, pH 7.4) containing 0.5 mM PMSF, 0.5 mMbenzamidine, 1.0 mM DTT and 1.0 mM EDTA. Total protein was mechanically dissociated from tissue using an ultrasonic cell disrupter. The sonicated samples were immediately

centrifuged at 30,000 × g for 30 min at 4 °C and the supernatants were removed and stored at 28 °C until an ELISA was performed. Total protein concentrations of sonicated brain samples were determined by using a Bio-Rad assay kit using bovine serum albumin as the standard.

2.14. Evaluation of acetylcholinestarase activities on brain

AChE levels where evaluated by colorimetric method to mimic the cholinergic function in rats' brain. A reaction mixture of 200 μl of 0.1mM sodium phosphate buffer (pH 8.0), 10 μl of 0.2M DTNB (5,5′-dithio-bis-(2-nitrobenzoic acid)), and 20 μl of the sample solution was incubated for five minutes, and the absorbance at 415nm was recorded via microplate reader (iMark Microplate Absorbance Reader). We then added 10 μl of acetylcholine thiochloride (ACTI) was added, incubated for 5 min, and recorded the absorbance at 415 nm [25].

$$\text{AChE activity} = \Delta A/1.36 \times 10^4 \times 1/\ 20/230\ C, \quad (4)$$

where ΔA = the difference of absorbance/minute and C = protein concentration of brain homogenate.

2.15. Ethical considerations

The experimental protocols for this study were approved by the institutional Animal Care and Use Research Ethics Committee of the University of Ibadan and performed in conformation with the "Guide to the care and use of laboratory animals in research and teaching" (NIH publications volume 25 No.28 revised in 1996).

2.16. Statistical analysis

Data are expressed as mean ± SEM, presented by scatter graph and bar charts and were analysed using kruskal-wallis test and one–way analysis of variance (ANOVA) followed by the Dunnett's post-hoc test for multiple comparisons where appropriate with the aid of Graphpad Software. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of methanolic leaf extract of Peristrophe bicalyculata (MEPb) on blood glucose levels in HFD/STZ-induced diabetic rats

Blood glucose levels remained significantly high ($P < 0.05$) in diabetic groups (treated or untreated) compared to normal control. Figure 1.

3.2. Effect of methanolic leaf extract of Peristrophe bicalyculata (MEPb) on learning and memory in HFD/STZ-induced diabetic rats using Morris water maze (MWM) test

There was significant increase ($P < 0.05$) in escape latency in untreated diabetic rats compared to normal control. The extract significantly decreased ($P < 0.05$) escape latency similar to pioglitazone treatment in treated diabetic rats compared to the diabetic control (Figure 2A). Total time spent in non-targeted quadrants significantly increased ($P < 0.05$) in untreated diabetic rats compared to normal control. The extract at 100 and 200 mg/kg, and pioglitazone however, significantly decreased this time in treated rats compared to diabetic control (Figure 2B).

3.3. Effect of methanolic leaf extract of Peristrophe bicalyculata (MEPb) on learning and memory dysfunction in HFD/STZ-induced diabetic rats using novel object recognition test (NORT)

Untreated diabetic rats had significant decreased ($P < 0.05$) discrimination index compared to normal control. Treatment with MEPb significantly increased discrimination index similar to pioglitazone treatment in treated rats compared to diabetic control (Figure 2C).

3.4. Antioxidative effects of MEPb in HFD/STZ-induced diabetic rats

3.4.1. Effect of MEPb on plasma malondialdehyde (MDA), reduced glutathione (GSH) and catalase (CAT) levels

Methanolic leaf extract of *Peristrophe bicalyculata* reduced oxidative stress and improved systemic anti-oxidative capacity in diabetic rats. Plasma malondialdehyde (MDA) levels increased significantly ($P < 0.05$) in untreated diabetic rats compared to normal control. The extract produced dose-dependent decreased in plasma MDA levels in treated rats compared to diabetic control. Figure 3A Plasma reduced glutathione (GSH) and catalase (CAT) levels significantly ($P < 0.05$) decreased in untreated diabetic rats compared to normal control. The extract dose-dependently increased plasma reduced glutathione (GSH) and catalase (CAT) levels in treated rats compared to the diabetic control (Figure 3 B & C).

3.4.2. Effect of methanolic leaf extract of Peristrophe bicalyculata (MEPb) on organs' malondialdehyde (MDA) levels in HFD/STZ-induced diabetic rats

Brain and liver malondialdehyde (MDA) levels significantly ($P < 0.05$) increased in untreated diabetic rats compared to normal control. Both the extract and pioglitazone treatment produced significant ($P <$

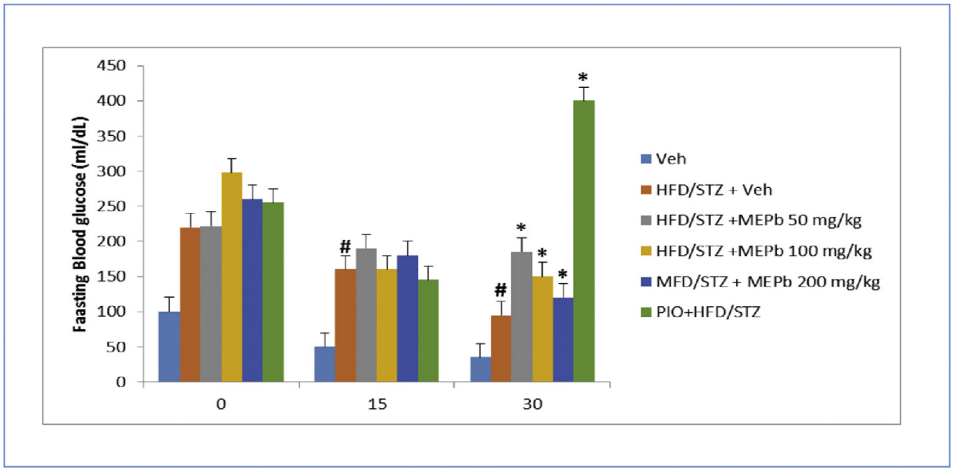

Figure 1. Effect of methanolic leaf extract of *Peristrophe bicalyculata* (MEPb) on blood glucose level in HFD/STZ-induced diabetic rats. Data presented as mean ± SEM. n = 6, #P < 0.05 Diabetic control vs Normal control, *P < 0.05 treatment group vs Diabetic control.

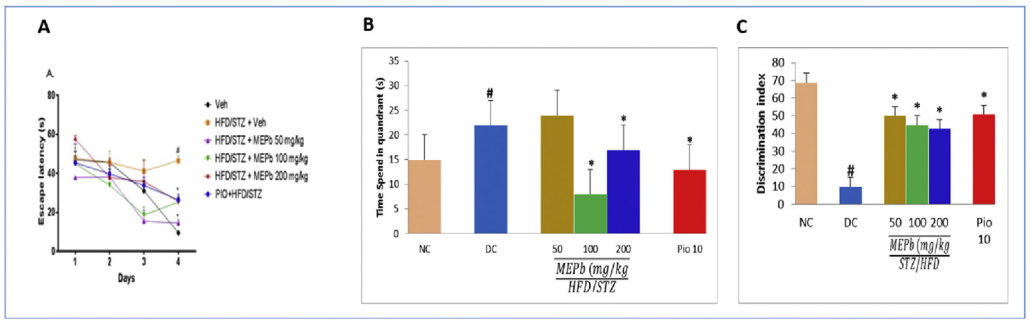

Figure 2. (A) Effect of MEPb on escape latency during training trials (4 days), (B) time spent in target quadrant during probe trial on the performance of spatial memory acquisition phase in Morris water maze and (C) memory performance (Novel Object Recognition Test) in HFD/STZ-induced diabetic rats. Data presented as mean ± SEM. n = 6, #P < 0.05 Diabetic control vs Normal control, *P < 0.05 treatment group vs Diabetic control. NC = normal control; DC = diabetes control.

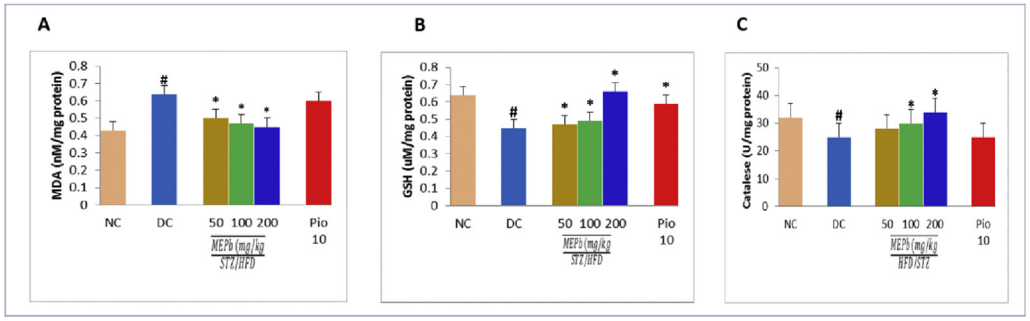

Figure 3. Effect of MEPb on plasma antioxidant status in HFD/STZ-induced diabetic rats. (A) Malondialdehyde (MDA) level (B) Reduced glutathione (GSH) level (C) Catalase level. Data presented as mean ± SEM. n = 6, #P < 0.05 Diabetic control vs Normal control, *P < 0.05 treatment group vs Diabetic control. NC = normal control; DC = diabetes control.

0.05) decrease in brain and liver MDA levels in treated rats compared to diabetic control (Figure 4A & B).

3.4.3. *Effect of methanolic leaf extract of Peristrophe bicalyculata (MEPb) on organs' reduced glutathione (GSH) levels in HFD/STZ-induced diabetic rats*

Methanolic leaf extract of *Peristrophe bicalyculata* improved antioxidative capacity of brain and liver in diabetic rats. Brain and liver reduced glutathione (GSH) levels significantly (P < 0.05) decreased in untreated diabetic rats compared to normal control. The extract at 100 and 200 mg/kg, similar to pioglitazone treatment showed non-significant and significant increase in brain and liver reduced glutathione (GSH) levels respectively compared to diabetic control (Figure 5A & B).

3.4.4. *Effect of methanolic leaf extract of Peristrophe bicalyculata (MEPb) on organs catalase (CAT) activity in HFD/STZ-induced diabetic rats*

Methanolic leaf extract of *Peristrophe bicalyculata* improved antioxidative capacity of brain and liver in diabetic rats. Brain and liver catalase (CAT) levels significantly (P < 0.05) decreased in untreated diabetic rats compared to normal control. The extract and pioglitazone treatment showed non-significant and significant increase in brain and liver catalase (CAT) levels respectively compared to diabetic control (Figure 6A & B).

3.5. *Effect of methanolic leaf extract of Peristrophe bicalyculata (MEPb) on brain nitrite level in HFD/STZ-induced diabetic rats*

Methanolic leaf extract of *Peristrophe bicalyculata* decreased nitrosative stress in brain of diabetic rats. Brain nitrite levels significantly (P <

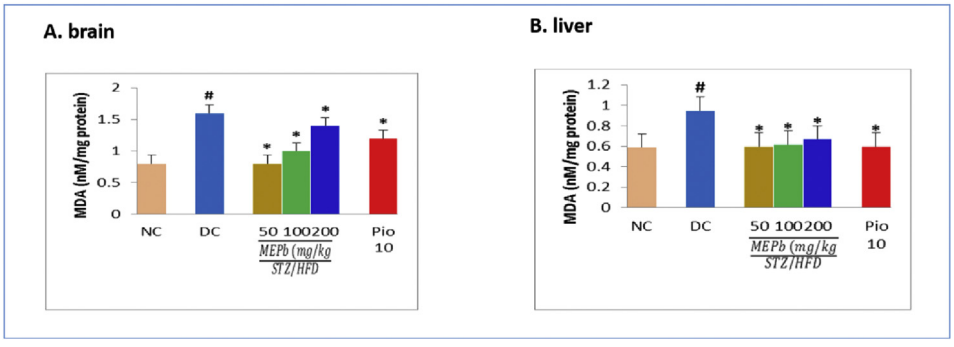

Figure 4. Effect of MEPb on malondialdehyde (MDA) in tissues of HFD/STZ-induced diabetic rats. (A) Brain, (B) Liver. Data presented as mean ± SEM. n = 6, #P < 0.05 Diabetic control vs Normal control, *P < 0.05 treatment group vs Diabetic control. NC = normal control; DC = diabetes control.

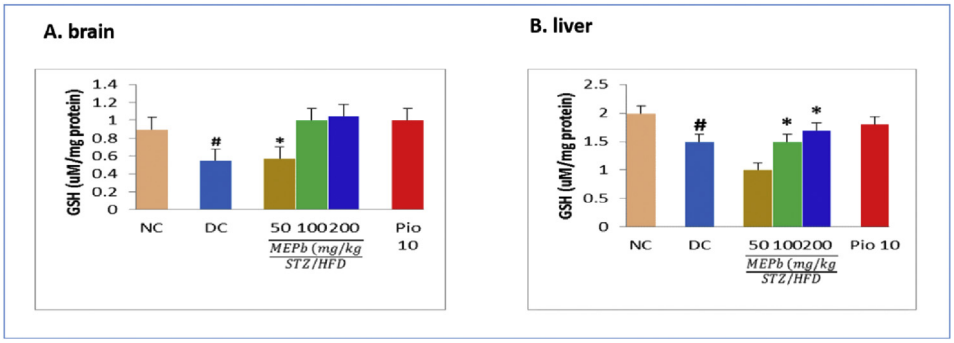

Figure 5. Effect of MEPb on reduced glutathione (GSH) in tissues of HFD/STZ-induced diabetic rats, (A) Brain, (B) Liver. Data presented as mean ± SEM. n = 6, #P < 0.05 Diabetic control vs Normal control, *P < 0.05 treatment group vs Diabetic control. NC = normal control; DC = diabetes control.

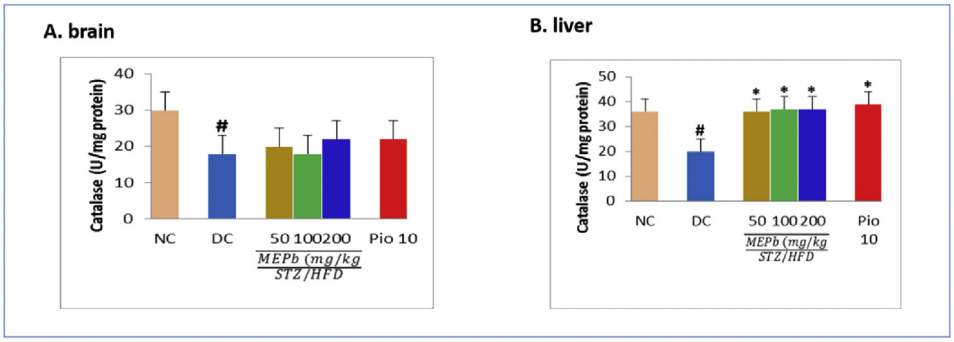

Figure 6. Effect of MEPb on catalase (CAT) in tissues of HFD/STZ-induced diabetic rats, (A) Brain, (B) Liver. Data presented as mean ± SEM. n = 6, #P < 0.05 Diabetic control vs Normal control, *P < 0.05 treatment group vs Diabetic control. NC = normal control; DC = diabetes control.

0.05) increased in untreated diabetic rats compared to normal control. The extract and pioglitazone treatment produced significant (P < 0.05) dose-dependent decreased in brain nitrite levels in treated rats compared to diabetic control (Figure 7A).

3.6. Effect of methanolic leaf extract of Peristrophe bicalyculata (MEPb) on brain Interleukin-6 (IL-6) level in HFD/STZ-induced diabetic rats

Brain IL-6 levels significantly (P < 0.05) increased in untreated diabetic rats compared to normal control. Both the extract and pioglitazone treatment produced significant (P < 0.05) decreased in brain IL-6 levels in treated rats compared to diabetic control (Figure 7B).

3.7. Effect of methanolic leaf extract of Peristrophe bicalyculata (MEPb) on brain acetylcholinesterase (AchE) activity in HFD/STZ-induced diabetic rats

Brain AchE levels significantly (P < 0.05) increased in untreated diabetic rats compared to normal control. Both the extract and pioglitazone treatment produced significant (P < 0.05) decreased of these levels in treated rats compared to diabetic control (Figure 7C).

4. Discussion

Cognitive impairment, a complication of diabetes mellitus, remains a global public health burden on the care and management of the disease condition [26]. Epidemiological and biological evidences particularly provide links between type 2 diabetes mellitus and memory loss [27]. Diabetes mellitus associated cognitive decline is attributed to the production of advanced glycated end products which promotes oxidative stress following deposition of such moieties in the brain [28]. The resulting oxidative state causes neuro-inflammation, damaging neuronal connections involving cholinergic neural pathways within the hippocampal formation and consequently causes cognitive decline in diabetics [7]. In essence, oxidative stress and inflammatory markers are particularly increased in patients with memory impairment [29].

Though studies on anti-diabetics e.g. pioglitazone, anti-oxidants, and acetylcholinesterase (AChE) inhibitors have been found to be beneficial in animal models of T2DM associated cognitive impairment [7], there are yet no drugs specifically designed for the prevention and/or treatment of this complication. Leaf extract of *Peristrophe bicalyculata* (MEPd) has been used by locals in northern Nigeria to enhance cognition in humans for management of convulsions [15] and there are no studies to support its use. We therefore evaluated the potentials of its methanolic leaf extract on learning and memory in high fat diet/streptozocin induced type 2 diabetes mellitus rat model. Combination of High fat diet and low dose streptozotocin (HFD/STZ) induce T2DM in animal models [30] by initiating gradual inflammation-mediated destruction of β-cells of the Islet of Langerhans as against sudden, rapid and complete β-cells death occasioned by administering single high dose STZ injection [31]. The HFD/STZ model therefore closely reflects the natural history and metabolic characteristics of human type 2 diabetes mellitus and was thus employed in this study.

Methanolic leaf extract of *Peristrophe bicalyculata* treated animals as well as the diabetic controls had considerably high blood glucose levels on days 0, 15 and 30 of treatment following diabetes induction compared to normal control (Figure 1). This is in variance with the observation by Duniya *et al* [11] who observed significant decrease in blood glucose levels in diabetic rats treated with *Peristrophe bicalyculata* powder at 15 and 20g doses respectively.

Experimental induction of Type 2 Diabetes Mellitus (T2DM) by HFD followed by low dose STZ in this study impaired spatial learning and memory as shown in the Morris Water Maze experiment, untreated diabetic rats exhibited significant higher escape latency on day 2, 3 and 4

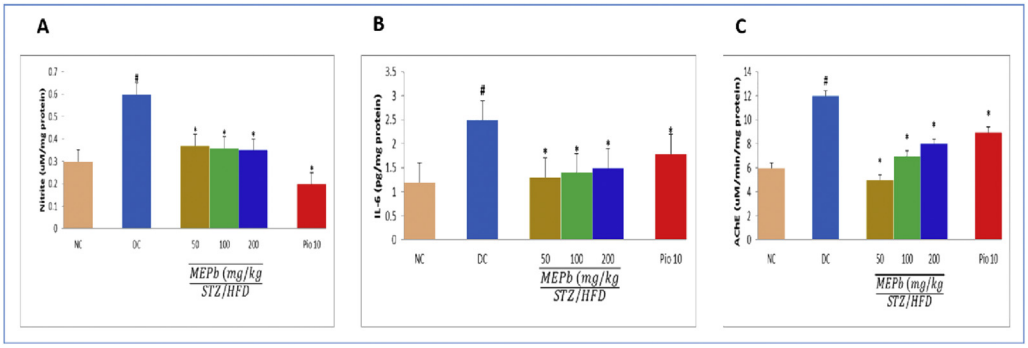

Figure 7. (A) Effect of MEPb on the brain level of Nitrite (B) brain level of IL-6 and (C) brain level of Acetylcholinesterase in HFD/STZ-induced diabetic rats. Data presented as mean ± SEM. n = 6, #P < 0.05 Diabetic control vs Normal control, *P < 0.05 treatment group vs Diabetic control. NC = normal control; DC = diabetes control.

compared with normal control. This finding agrees with that of Biessels *et al* [32] who observed spatial cognition impairment in diabetic rats. Treatment with MEPb significantly reversed this impairment, thus reducing escape latency in the treated animals, this effect was also observed in the pioglitazone group (Figure 2A). On the probe trial day (day 6) when the platform was removed, untreated diabetic rats failed to locate the target quadrant on time, thereby spending more time in other quadrants compared to the normal control. This impaired memory in the untreated diabetic group was also reversed in MEPb and pioglitazone treated diabetic rats compared to diabetic control (Figure 2B).

Similarly, in the novel object recognition test (NORT) the untreated diabetic rats exhibited significant reduced discrimination index compared to normal control. This was reversed in the MEPb and pioglitazone treated rats, implying cognition enhancement in these groups (Figure 2C).

Gradual build-up of oxidative stress is associated with hyperglycaemia following STZ induced diabetes mellitus [33] and this has produced nerve damage in many experimental and human diabetes studies [34]. The central nervous system is highly sensitive to oxidative stress because of its high oxygen demand and weak antioxidant defence mechanism [35]. Oxidative stress results from an imbalance in body's redox homeostasis favouring pro-oxidants build-up as well as antioxidant pool depletion [36]. Overproduction of pro-oxidants like oxygen radicals causes damages to cell structures such as lipids, carbohydrate, proteins and nucleic acids [37]. Such damages to cholinergic neurons of the cerebal cortex and hippocampus produces impairment in cognitive functions [38]. Oxidative stress causes lipid peroxidation, and malondialdehyde (MDA) is the representative product of lipid peroxidation which is typically quantified in experiments as an excellent endpoint of oxidative stress [39].

In this study, induction of diabetes resulted in significant build-up of this marker in brain and liver of the experimental animals. This is demonstrated by the increase MDA levels in the serum and organ lysates of untreated diabetic rats compared to control. This observation was reversed with MEPb treatment as treated animals at all doses showed significant decrease in serum, brain and liver MDA levels compared to the diabetic control and the pioglitazone treated groups (Figures 3A, 4A, and B).

Under physiologic conditions, enzymatic antioxidants such as glutathione peroxide (GPX), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) as well as non-enzymatic antioxidants such as reduced glutathione (GSH), Vitamin C and Vitamin E mops up pro-oxidants in the body [39]. These systems are however, overwhelmed during oxidative stress conditions leading to their gradual depletion. In this study, induction of diabetes resulted in significant decrease in brain, serum and liver levels of catalase (a representative enzymatic antioxidant) and reduced glutathione (a representative non-enzymatic antioxidant) in the untreated diabetic rats compared to control. Treatment with MEPb caused a substantial increases in serum, brain and liver catalase and reduced gluthathione levels in treated animals compared to the untreated diabetic group (Figures 3B, C, 5 and 6). This reduction in oxidative stress markers particularly in the brain could be a factor responsible for the reversal of the DM-associated cognitive dysfunction in treated rats.

Nitrosative stress precipitated by overproduction of nitric oxide is equally lethal to neurons and nitrite level is measured as its indicator [40]. We assayed brain nitrite level in the experimental animals to ascertain possibility of nitrosative stress as a contributor to cognitive impairment in DM. Animals in the diabetic control group shows significant increase in brain nitrite level compared to control implying nitrosative stress in this group. This effect was reversed with MEPb treatment as treated animals showed dose dependent decrease in brain nitrite levels compared to the untreated diabetic groups. This was also observed in the pioglitazone treated group (Figure 7A).

Stressful condition of the CNS promotes neuronal inflammation leading to cognitive impairment such as seen in Alzeihmer's disease [41]. In central neuro-inflammation, cytokines such as interleukin 6 (IL-6) and TNFα are produced in significant levels and are particularly known to aggravate neuronal disease progressions and subsequent death [42]. Higher levels of these interleukins precipitates hippocampal neurodegeneration leading to memory impairment [43]. In this study, induction of diabetes in rats significantly increased the brain level of IL-6 in the untreated diabetic group, signifying neuroinflammatory activity in the rats. Treatment with MEPb significantly and dose dependently decreased brain levels of IL-6 in the treated rats compared to the diabetic control group, similar observation was seen in the pioglitazone treated group (Figure 7B).

Cholinergic neuro-activities are markedly expressed in the hippocampus and the cerebral cortex which are specific regions for the control of learning and memory in the brain [44]. Normal cholinergic function mediated by continuous presence of acetylcholine either by sustained production or decrease acetylcholinesterase (AChE) activity plays significant role in normal cognitive function, however, a surge in AChE could influence impairment of neuronal functions [45]. Several studies have demonstrated relationship between increase AChE activity in the brain and cognitive impairment [46]. In this study we detected an increased in acetylcholinesterase activity in the untreated diabetic group, indicating weakening cholinergic action as a result of depletion of

acetylcholine in the brain of the diabetic rats. Animals in the MEPb treated groups showed diminishing acetylcholinesterase activity (Figure 7C), interestingly the effect was more prominent at the 50 mg/kg dose compared with the diabetic control. Similar finding was also noted in the pioglitazone treated group.

We conclude that Methanolic leaf extract of *Peristrophe bicalyculata* enhanced antioxidant capacity and prevented neuroinflammation, consequently improving brain neuronal cholinergic function in experimental animals.

Declarations

Author contribution statement

A.A. Njan: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

A.F. Opeyemi, A. Mayowa and S. Olasubomi: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

S.O. Olaoye and E.O. Iwalewa: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M. Ologe: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

O.N. Erdogan: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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