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Effect of *Solanum macrocarpon* Linn leaf aqueous extract on the brain of an alloxan-induced rat model of diabetes

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Abstract

Objective: This study was designed to evaluate the protective effect of aqueous extract of *Solanum macrocarpon* Linn leaf in the brain of an alloxan-induced rat model of diabetes.

Methods: The experimental model of diabetes was induced by a single intraperitoneal injection of freshly prepared alloxan. Rats were then divided into six groups: normal control, diabetes control, diabetes group treated with metformin, and three diabetes groups treated with different concentrations of *S. macrocarpon*. Rats were sacrificed on day 14 of the experiment and different brain biochemical parameters were assessed and compared between groups.

Results: Administration of different doses of *S. macrocarpon* leaf aqueous extract was associated with significantly reduced levels of fasting blood glucose, lipid peroxidation, neurotransmitters, cholinesterases, cyclooxygenase-2 and nitric oxide compared with diabetes control rats. In addition, antioxidant enzyme activities were significantly increased in diabetes rats administered 12.45, 24.9 and 49.8 mg/kg body weight of *S. macrocarpon* versus diabetes control rats.

Conclusion: Aqueous extract of *S. macrocarpon* Linn leaf may be useful in the management of diabetic neuropathy.

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Introduction

Diabetes mellitus is a common metabolic disorder linked to different complications in various organs, such as the heart, eyes, lower-limb blood vessels, lungs, and brain.[1] Persistent hyperglycaemia in patients with diabetes mellitus always promotes increased oxidative stress that is characterized by cognitive impairment and memory loss.[2] This may be linked to the fact that brain tissues are extremely vulnerable to oxidative damage, possibly due to the high oxygen consumption rate of 20%, the presence of abundant polyunsaturated fatty acids in cell membranes, and the high iron (Fe) content, as well as low enzymatic activity of antioxidants.[2] Therefore, diabetes mellitus is a crucial risk factor for cognitive dysfunction.

Several drugs are useful in the management of diabetes mellitus and its related complications, but they are known to be associated with various side effects.[3] As a result, a number of herbs are used to manage diabetes mellitus and its associated complications, particularly neuropathy, including an African eggplant (*Solanum macrocarpon*) that is normally consumed as a vegetable, especially in Nigeria.[4] Few studies have been performed with this plant, and there is currently little or no information regarding the effect of *S. macrocarpon* on the brain of rats with alloxan-induced diabetes. Thus, the aim of the present study was to evaluate the protective effect of *S. macrocarpon* Linn leaf aqueous extract in the brain of an alloxan-induced rat model of diabetes.

Materials and methods

Plant collection and identification

The *S. macrocarpon* leaves were purchased from the Oleh market in Delta State, Nigeria, and were then authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria (voucher number FHI: 111316).

Extract preparation

The *S. macrocarpon* leaves were dried at room temperature for 4 weeks and processed to powder form using an electric blender. Thereafter, a known weight of the powdered sample was soaked in distilled water (1: 10 w/v) for 72 h. The solution was then filtered and the obtained filtrate was freeze-dried. In order to use an ethnobotanical dose in this study, an equivalent of the cup normally used in the home was freeze-dried separately to obtain the yield, which was then used to calculate three different doses.

Experimental animals and induction of diabetes mellitus

A total of 36 Wistar albino rats, age between 7 and 8 weeks (weight range, 130–140 g) were obtained from the Animal House of Afe Babalola University, Ado-Ekiti, Ekiti, Nigeria. The animals were acclimatised for 2 weeks at room temperature with free access to feed and water, with a 12-h light/12-h dark cycle. This work was approved by the Animal Ethical Committee of Afe Babalola University, Ado-Ekiti,

Ekiti State, Nigeria (Ethics approval number ABUAD/SCI/19/016), and was performed according to the Committee's ethical standards.

Diabetes mellitus was induced in 30 of the experimental animals by a single intraperitoneal injection of 150 mg/kg body weight of alloxan monohydrate (Sigma-Aldrich; St Louis, MO, USA). At 48 h following alloxan-induction, the fasting blood glucose level of each animal was checked using an Accu-check glucometer (OneTouch Glucometer, supplied by Central Diagnostic Laboratory, Ilorin, Kwara State, Nigeria) to confirm that fasting blood glucose levels were $\geq$250 mg/dl, as previously described.[5]

Experimental design

The animals were divided into 6 groups ($n = 6$) as follows:

- Group 1: normal control
- Group 2: diabetes control
- Group 3: rats with diabetes administered 5 mg/kg metformin (Sigma-Aldrich)
- Group 4: rats with diabetes administered 12.45 mg/kg body weight of *S. macrocarpon* leaf aqueous extract
- Group 5: rats with diabetes administered 24.9 mg/kg body weight of *S. macrocarpon* leaf aqueous extract
- Group 6: rats with diabetes administered 49.8 mg/kg body weight of *S.* macrocarpon *leaf aqueous extract*

The animals were sacrificed on day 14 by cervical dislocation and the brain of each rat was quickly excised, homogenized using Tris-HCl buffer (Sigma-Aldrich) and centrifuged at 4 000 *g* for 15 min at 24°C to obtain a clear supernatant for use in different biochemical analyses, as previously described.[6] Samples were then deep frozen for storage prior to analysis.

Fasting blood glucose

Fasting blood glucose levels were determined at baseline, at 48 h following alloxan induction of diabetes, and at day 14 of treatment, using an Accu-chek® glucometer, by placing a drop of tail vein blood onto the glucose strip, as previously described.[7]

Oxidative stress biomarkers

The level of malondialdehyde (MDA) and activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in brain homogenates were determined using an RX Daytona automated analyser (Randox; County Antrim, UK) according to the manufacturer's instructions.

Neurotransmitter levels

Levels of epinephrine, norepinephrine, dopamine, and serotonin in rat brain homogenates were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Cusabio; Houston, TX, USA) according to the manufacturer's instructions.

Determination of cholinesterase activity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity levels in the rat brain on experimental day 14 were measured as follows. Briefly, 50 ml of brain homogenate, 50 ml of 2-nitrobenzene acid (Sigma-Aldrich) and 175 ml of 0.1 mol/l phosphate buffered saline (pH 8.0; Sigma-Aldrich) were mixed together and incubated for 20 min at 25°C. Thereafter, 25 ml of both acetylthiocholine iodide and butyrylthiocholine iodide solution was added to the solution. The absorbance was measured

at 412 nm using a Randox microplate reader, as previously described.[8]

Determination of other biochemical parameters

Cyclooxygenase (COX)-2 and nitric oxide (NO) levels in the rat brain on experimental day 14 were determined using commercial ELISA kits (Merck, Darmstadt, Germany), according to the manufacturer's instructions.

Statistical analyses

Data are reported as mean ± SD of six replicates, and were statistically analysed using GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA). Between-group and within-group differences were assessed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Statistical significance was set at $P < 0.05$.

Results

At the end of the experimental period (day 14), fasting blood glucose levels were significantly higher in diabetes control rats compared with normal controls ($P < 0.05$). However, a statistically significant ($P < 0.05$) reduction in fasting blood glucose levels at day 14 was observed in rats with diabetes administered different doses of *S. macrocarpon* leaf aqueous extract, as well as 5 mg/kg body weight of metformin ($P < 0.05$ versus diabetes control rats at day 14 and versus own group at 48 h of diabetes induction; Figure 1)

At experimental day 14, levels of MDA in control diabetes rats were significantly higher compared with all other groups ($P < 0.05$; Figure 2). There were no statistically significant differences in levels of MDA between normal control rats, and rats with diabetes administered 12.45, 24.9 or 49.8 mg/kg body weight of *S. macrocarpon* leaf aqueous extract or 5 mg/kg body weight of metformin ($P > 0.05$; Figure 2).

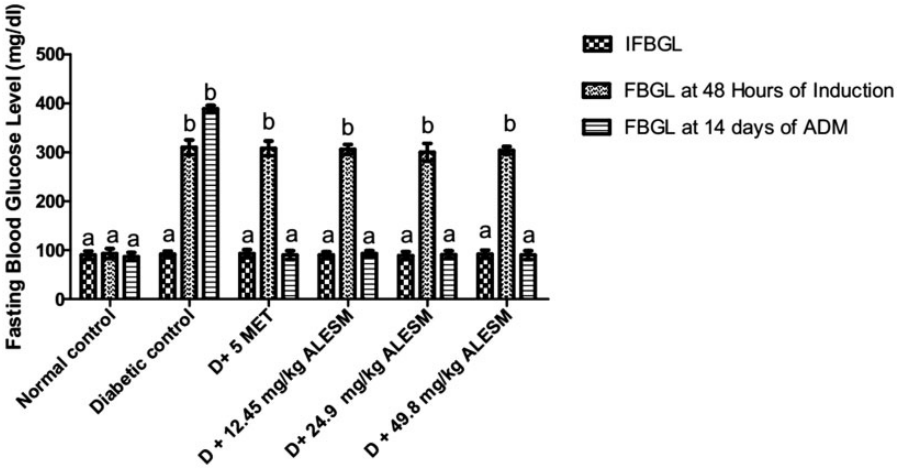

Figure 1. Fasting blood glucose level in rats with alloxan-induced diabetes administered aqueous extract of *Solanum macrocarpon* leaf. D, diabetes; 5 MET, 5 mg/kg metformin; ALESM, aqueous leaf extract of *S. macrocarpon*; IFBGL, initial fasting blood glucose level; FBGL, fasting blood glucose level; ADM, administration of treatment. Data presented as mean ± SD of six determinations; different letters represent statistically significant difference ($P < 0.05$; analysis of variance).

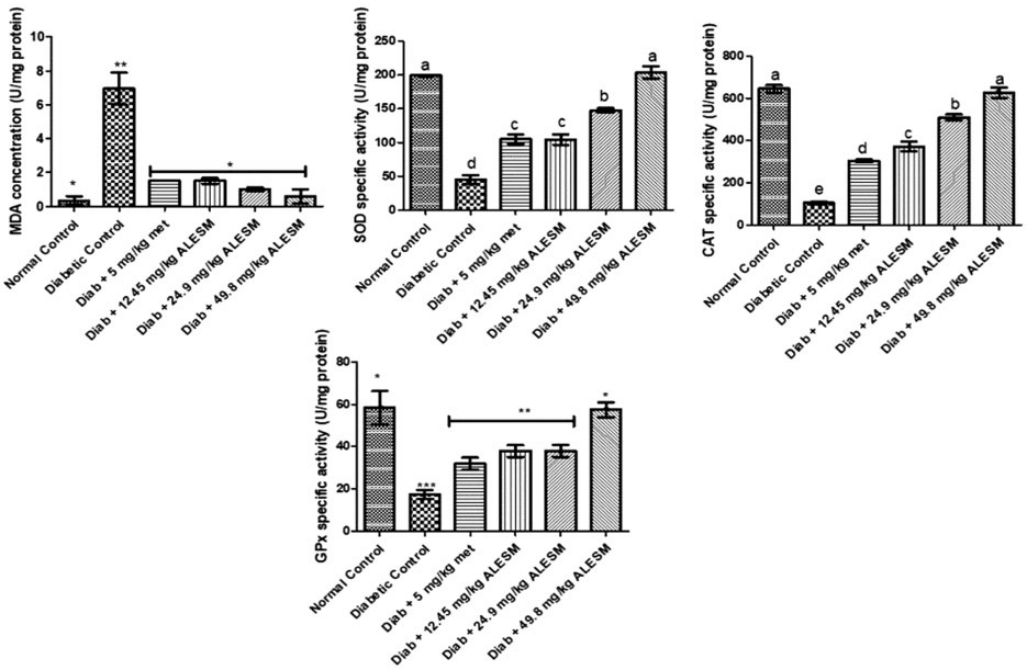

Figure 2. Oxidative stress biomarkers in brain tissue of rats with alloxan-induced diabetes administered aqueous extract of *Solanum macrocarpon* leaf. Diab, diabetes; met, metformin; ALESM, aqueous leaf extract of *S. macrocarpon*. Data presented as mean ± SD of six determinations; different letters or number of asterisks represent statistically significant difference ($P < 0.05$; analysis of variance).

Rats with diabetes administered 12.45, 24.9 and 49.8 mg/kg body weight of *S. macrocarpon* leaf aqueous extract revealed significantly increased SOD activity in brain tissue at experimental day 14 compared with control diabetes rats ($P < 0.05$). In addition, SOD activity in rats with diabetes administered 49.8 mg/kg body weight of *S. macrocarpon* leaf aqueous extract was not significantly different from normal control rats ($P > 0.05$). SOD activity in rats with diabetes administered 12.45 mg/kg body weight of *S. macrocarpon* leaf aqueous extract showed no significant difference compared with diabetes rats administered 5 mg/kg body weight of metformin ($P > 0.05$; Figure 2).

On experimental day 14, brain tissue in diabetes control rats showed significantly lower CAT and GPx activity versus normal control rats ($P < 05$), but there were no statistically significant differences in the activities of CAT and GPx in the brains of rats with diabetes administered 49.8 mg/kg body weight of *S. macrocarpon* leaf aqueous extract compared with normal controls ($P > 0.05$). In addition, diabetes control rats showed significantly decreased CAT and GPx activity compared with all of the treated groups ($P < 0.05$; Figure 2). There was no significant difference in GPx activity in the brain tissue of diabetes rats administered 12.45 and 24.9 mg/kg body weight of *S. macrocarpon* leaf aqueous extract compared with 5 mg/kg body weight of metformin ($P > 0.05$; Figure 2).

Brain tissue levels of epinephrine, norepinephrine, dopamine, and serotonin were significantly increased in diabetes control rats compared with diabetes rats

administered 12.45, 24.9 and 49.8 mg/kg body weight of *S. macrocarpon* leaf aqueous extract, as well as those administered 5 mg/kg body weight of metformin ($P < 0.05$; Figure 3). In addition, diabetes rats administered different doses of *S. macrocarpon* leaf aqueous extract exhibited no significant difference in levels of these neurotransmitters compared with normal control rats ($P > 0.05$). There was no significant difference in epinephrine and norepinephrine levels in diabetes rats administered different doses of *S. macrocarpon* leaf aqueous extract and diabetes rats administered 5 mg/kg body weight of metformin ($P > 0.05$). But levels of dopamine and serotonin were significantly different between diabetes rats administered different doses of *S. macrocarpon* leaf aqueous extract and diabetes rats administered 5 mg/kg body weight of metformin ($P < 0.05$; Figure 3).

Levels of AChE and BChE were significantly increased in the brain of diabetes control rats compared with other groups ($P < 0.05$; Figure 4). However, on day 14 of the experiment, diabetes rats administered different doses of *S. macrocarpon* leaf aqueous extract revealed significantly decreased AChE levels compared with diabetes controls ($P < 0.05$). Also, there was no significant difference in AChE levels between diabetes rats administered 24.9 and 49.8 mg/kg body weight of *S. macrocarpon* leaf aqueous extract and normal control rats ($P > 0.05$). Diabetes rats administered 12.45 mg/kg body weight of *S. macrocarpon* leaf aqueous extract showed significantly lower AChE levels versus diabetes rats administered 5 mg/kg body weight of metformin ($P < 0.05$; Figure 4). In addition, there were no significant differences in BChE levels between

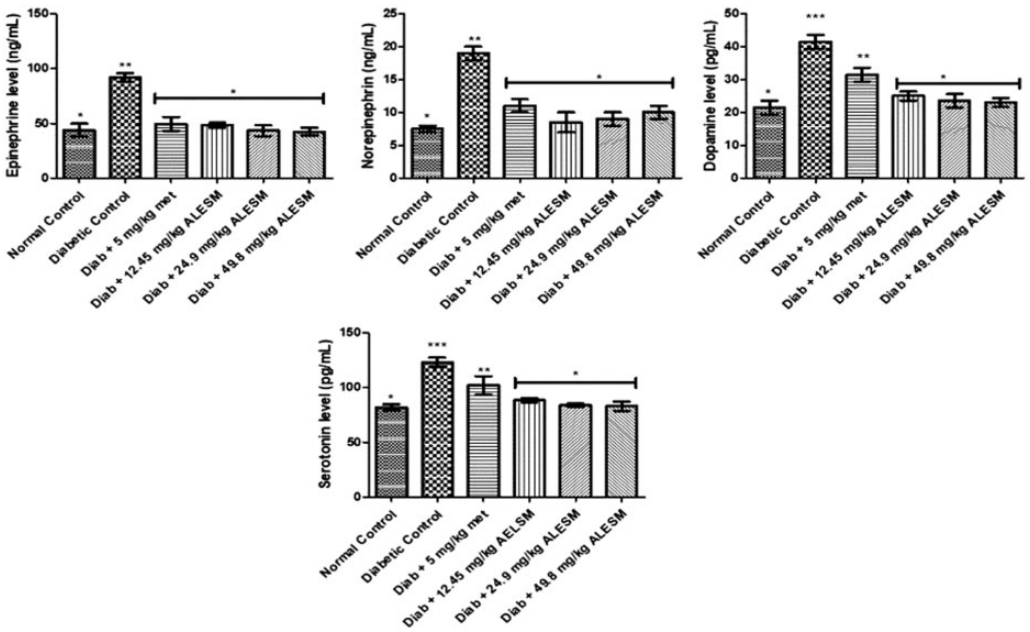

Figure 3. Neurotransmitters biomarker in brain tissue of rats with alloxan-induced diabetes administered aqueous extract of *Solanum macrocarpon* leaf. Diab, diabetes; met, metformin; ALESM, aqueous leaf extract of *S. macrocarpon*. Data presented as mean ± SD of six determinations; different number of asterisks represent statistically significant difference ($P < 0.05$; analysis of variance).

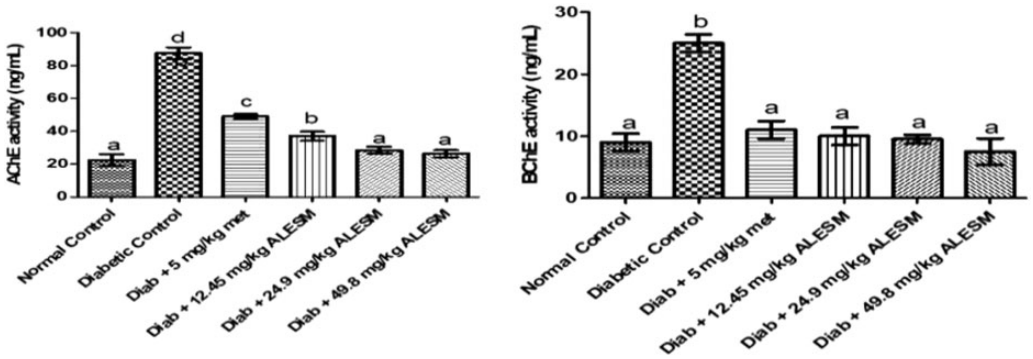

Figure 4. Cholinesterase parameters in brain tissue of rats with alloxan-induced diabetes administered aqueous extract of *Solanum macrocarpon* leaf. AChE, acetylcholinesterase; BChE, butyrylcholinesterase; Diab, diabetes; met, metformin; ALESM, aqueous leaf extract of *S. macrocarpon*. Data presented as mean ± SD of six determinations; different letters represent statistically significant difference ($P < 0.05$; analysis of variance).

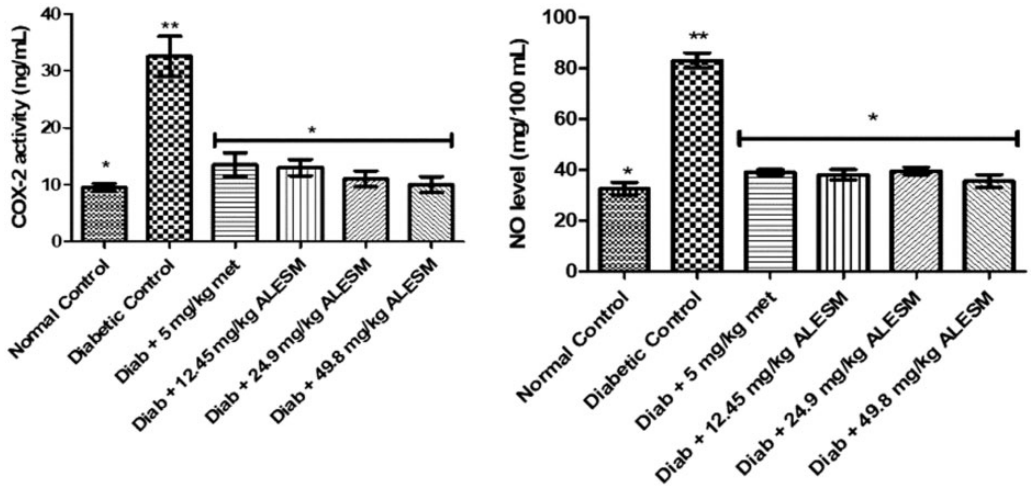

Figure 5. Cyclooxygenase (COX)-2 activities and nitric oxide (NO) level in brain tissue of rats with alloxan-induced diabetes administered aqueous extract of *Solanum macrocarpon* leaf. Diab, diabetes; met, metformin; ALESM, aqueous leaf extract of *S. macrocarpon*. Data presented as mean ± SD of six determinations; different number of asterisks represent statistically significant difference ($P < 0.05$; analysis of variance).

normal control rats and diabetes rats administered 12.45, 24.9 and 49.8 mg/kg body weight of *S. macrocarpon* leaf aqueous extract, or diabetes rats administered 5 mg/kg body weight of metformin ($P > 0.05$; Figure 4).

Finally, diabetes control rat brain tissue demonstrated significantly increased COX-2 activity and NO level compared with normal controls and all of the treated diabetes groups ($P < 0.05$; Figure 5). On experimental day 14, diabetes rats administered

12.45, 24.9 and 49.8 mg/kg body weight of *S. macrocarpon* leaf aqueous extract, as well as those administered 5 mg/kg body weight of metformin, exhibited no significant difference versus normal control rats in COX-2 activity and NO level.

Discussion

Diabetes mellitus is associated with hyperglycaemia,[3] the persistence of which can affect different organelles in the body system, including the brain, leading to neuropathy. Hyperglycaemia was also observed in the present rat model of diabetes, and the normoglycaemia results observed in the diabetes rats administered different doses of *S. macrocarpon* leaf aqueous extract (Figure 1) support the local usage of this plant in the management of diabetes mellitus, and may be attributed to phenolic compounds present in the plant as reported previously.[5,9]

Oxidative stress plays an important role in cellular injury due to persistent hyperglycaemia, stimulating free radical production, and thereby, weakening the immune system of such individuals, who become unable to counteract the enriched reactive oxygen species (ROS) generation leading to oxidative stress.[10] Lipids are reported as one of the primary targets of ROS, especially in the brain due to its high level, and this is probably responsible for the high level of lipid peroxidation in the brain of rats with induced diabetes. The increased MDA level in the present rat model of diabetes may be linked to a decline in defence mechanisms of antioxidant enzymes (Figure 2).[11] This may be responsible for the reduction in activities of SOD, CAT, and GPx in the brain of diabetes rats, as shown in the current study. SOD is an important enzyme against cellular damage produced by ROS, and it promotes the conversion of superoxide radicals into hydrogen peroxide. CAT encourages the conversion of hydrogen peroxide into water,[12] and this is supported by the cytosolic enzyme GPx. The rapid decrease in activities of these antioxidant enzymes could be attributed to their combatting effect on free radicals.[13] The ability of aqueous extract of *S. macrocarpon* leaf to boost the activities of SOD, CAT, and GPx with a correspondent decrease in MDA (Figure 2) may be attributed to the antioxidant nature of the extract supporting the anti-neuropathy effects. This may be the main mechanism of action of aqueous extract of *S. macrocarpon* leaf as an anti-neuropathy agent.

Neurotransmitters are endogenous substances that encourage neurotransmission, transmitting signals across a chemical synapse, such as a neuromuscular junction, from one neuron to another target neuron, muscle cell, or gland cell.[14] The persistent hyperglycaemia in a diabetes mellitus state, which triggers an increase in ROS production, may be responsible for the abnormal increase in levels of all neurotransmitters in the present study (epinephrine, norepinephrine, dopamine, and serotonin; Figure 3). The abnormal increase in levels of epinephrine, norepinephrine, dopamine, and serotonin supports the neuropathy complication of diabetes mellitus, and the aqueous extract of *S. macrocarpon* leaf was able to inhibit levels of these neurotransmitters in the present study, supporting its anti-neuropathy effects, probably due to its ability to boost antioxidant enzymes booster.

Cholinesterases (AChE and BChE) are important enzymes related to memory and cognitive functions, and persistent hyperglycaemia may trigger memory loss, particularly in type II diabetes mellitus.[2] Also, AChE and BChE are crucial enzymes in the management of neurodegenerative diseases, such as Alzheimer's disease,[15] another secondary complication of diabetes mellitus. Increased activities of both AChE and BChE in the present study (Figure 4) suggest that memory loss and

neurodegenerative diseases may be present in diabetes control rats. It is noteworthy that rats with diabetes administered different doses of *S. macrocarpon* leaf aqueous extract showed inhibited AChE and BChE activity, suggesting a reduction in the hydrolysis of acetylcholine, as well as amelioration of neuronal damage, which may be associated with correction of memory loss.

The inflammatory enzyme COX-2 plays an important role in the pathogenesis of diabetes mellitus.[16] Inhibition of this enzyme is crucial role in protecting patients with diabetes mellitus from inflammation. An increase in COX-2 activity in diabetes control rats in the present study (Figure 5) suggests a high level of inflammation in their brain. Hence, the ability of *S. macrocarpon* leaf aqueous extract to inhibit the activity of COX-2 further supports its anti-neuropathy effects, and this may be linked to different bioactive compounds present in the extract, as reported previously.[9] In addition, increased NO level due to diabetic pathophysiology may be responsible for both cell apoptosis and necrosis, as the reaction of NO with superoxide anion produces peroxynitrite,[17] as noticed in the present study (Figure 5), and this may also be associated with increased lipid peroxidation also observed (Figure 2). The aqueous extract of *S. macrocarpon* leaf administered at different doses exhibited potential in inhibiting the level of NO in the brain of animals with diabetes, also supporting the anti-neuropathy effects linked to the antioxidant activity of the extract, as well as possible synergistic reactions of different bioactive compounds reported in the extract.[9]

In conclusion, the aqueous extract of *S. macrocarpon* leaf demonstrated the ability to reduce lipid peroxidation, and boost the brain tissue activities of CAT, SOD, and GPx. The anti-neuropathy effects of the extract were also substantiated by the amelioration of epinephrine, norepinephrine, dopamine and serotonin levels, cholinesterase activities, and COX-2 and NO levels.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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