Journal of Applied Pharmaceutical Science Vol. 14(10), pp 141-151, October, 2024
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2024.174900
ISSN 2231-3354

Ameliorative effect of *Vitis gracilis* leaf decoction against sensory and motoric disorders, oxidative stress, and cerebellar degeneration in diabetic mice

Putra Santoso[1]*, Alfi Yuniarti[1], Rita Maliza[1], Alimuddin Tofrizal[2], Dinda Fadhila Belahusna[1]

[1]Biology Department Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia.
[2]Department of Anatomical Pathology Faculty of Medicine Andalas University, Padang, Indonesia.

ARTICLE HISTORY

Received on: 30/04/2024
Accepted on: 04/09/2024
Available Online: 05/10/2024

Key words:
Diabetes mellitus, malondialdehyde, neuropathy, oxidative stress, Purkinje cells.

ABSTRACT

Vitis gracilis (Vitaceae) is a potent medicinal plant, but its effectiveness in counteracting neuronal issues due to diabetes is uncertain. This study aimed to investigate whether the leaf decoction of *V. gracilis* could manage sensory and motoric disorders, oxidative stress, and cerebellar degeneration caused by diabetes mellitus. Twenty-five adult male mice were divided into five treatment groups: non-diabetic (non-DM), diabetic (alloxan-induced diabetes mellitus; DM), and DM treated with *V. gracilis* leaf decoction at doses of 25 g/l (DM + 25 g/l VgD), 50 g/l (DM + 50 g/l VgD), or 100 g/l (DM + 100 g/l VgD). The decoction was administered orally for 30 days. The results showed that *V. gracilis* leaf decoction did not significantly lower blood glucose levels or improve insulin tolerance in diabetic mice ($p > 0.05$). However, it substantially maintained sensory response and motoric balance and reduced malondialdehyde levels in brain tissue, especially at a 100 g/l dose ($p < 0.05$). Moreover, higher decoction doses alleviated histopathological alterations in the cerebellum by significantly preserving molecular and granular layer thickness while reducing degenerated Purkinje cells ($p < 0.05$). In conclusion, despite its inability to manage hyperglycemia, the decoction effectively improved sensory and motoric function, reduced oxidative stress, and mitigated cerebellar histopathological changes. Therefore, *V. gracilis* could be an alternative treatment for diabetes-related neurological disorders.

INTRODUCTION

Among various life-threatening metabolic diseases, diabetes mellitus is taken into account as one of the major causes of substantial increment of health issues worldwide [1]. The result of the International Diabetes Federation (IDF) survey in 2019 revealed that 463 million adult people are suffering from diabetes mellitus globally [2]. Without any proper efforts to explore the ways of managing diabetes progression and its related detrimental outcomes, it is estimated that by 2045 more than 700 million people will live with diabetes [2]. Importantly, the progression of chronic hyperglycemia in people with diabetes has been shown to be closely associated with diverse subsequent deleterious effects including kidney failure, liver diseases, cardiovascular problems, and neurological issues [3–5].

Nowadays many synthetic drugs have been widely used to treat people with diabetic neuropathy and balance disorder in common medical practices [6,7]. Unfortunately, diverse unwanted side effects are also frequently observed. For instance, the neuropathy drugs in the groups of anticonvulsants and antidepressants have been reported to cause water retention, body weight gain, and psychological issues including mood and behavior changes [8]. Other drugs also promote drowsiness, dizziness, and failure to concentrate [9]. Moreover, it is also indicated that diabetic neuropathy drugs cause gastrointestinal problems such as constipation, nausea, diarrhea, and vomiting

*Corresponding Author
Putra Santoso, Biology Department Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia.
E-mail: putrasantoso @ sci.unand.ac.id

[7,10] while increasing the risk of having cardiovascular diseases [11]. In addition, the cost of the medications has recently soared [12]. Therefore, the explorative efforts to find and formulate natural-based alternative medicines, with lesser unwanted outcomes while highly effective and affordable, are urgently needed.

Among diverse potent bioresources for natural-based medicines, plants in the family of Vitaceae namely wild vine (*Vitis gracilis*) are well known for their benefits against diseases [13]. Local people in north Sumatra (Karo tribe, Indonesia) usually consume its leaves in the form of decoction as a vitality enhancer [14]. Previous investigations also revealed that *V. gracilis* leaf extract contained terpenoids, flavonoids, alkaloids, glycosides, tannins, and saponins [13,14]. Another study in animal models also found that *V. gracilis* leaves could prevent inflammation and apoptosis in the gastrocnemius muscle cells [13]. A previous study showed that *V. gracilis* is capable of counteracting apoptosis in the alveolar cells [15]. Moreover, *V. gracilis* leaf extracts have been shown to protect hippocampal cells from apoptosis and inflammation caused by intense swimming activity [16]. In addition, it has been indicated that the nano herbal prepared from *V. gracilis* leaf extract was capable of protecting lung cells against apoptosis caused by maximum swimming exercise [17]. However, to date, the scientific evidence supporting the beneficial effects of *V. gracilis* in counteracting sensory and motoric disorders, oxidative stress, and brain degeneration due to diabetes mellitus remains limited. Thus, this present study was conducted to provide proof regarding the effects of *V. gracilis*, in the form of leaf decoction, in managing hyperglycemia and the impairments of sensory response and motoric balance as well as degeneration of cerebellar tissue and accumulation of oxidative stress in alloxan-induced diabetic mice as animal models.

MATERIALS AND METHODS

Materials

High-grade reagents and chemicals namely phosphate-buffered saline (PBS, pH 7.4), neutral-buffered formalin (NBF), xylene, ethanol, and alloxan monohydrate were purchased from Sigma-Aldrich (Merck KGaA, Germany). Physiological saline (NaCl 0.9%) was sourced from Otsuka (Japan). The hematoxylin-eosin staining kit and paraffin were purchased from Vector Laboratories (USA). The lipid peroxidation assay kit was obtained from Abcam (Abcam Inc., UK). The plant sample (*V. gracilis*) was collected from a tropical forest area in Langkat (North Sumatra, Indonesia). Male mice of the BALB/c strain were purchased from the Baso Veterinary Center (Bukittinggi, West Sumatra, Indonesia). The rodent-specific chow diet (RATBIO) was acquired from Citra InaFeed (Jakarta, Indonesia).

Sample collection and preparation of *V. gracilis* leaf decoction

The fresh leaves of *V. gracilis* (Fig. 1) were collected from the forest area in Langkat, North Sumatra Province, Indonesia (during September 2022). The species identity of the plant was verified by a botanist in Biology Department Faculty of Mathematics and Natural Sciences Andalas University and a voucher specimen was deposited in the Herbarium Andalas (Andalas University). The procedures of decoction preparation were performed as previously described [18] with several modifications. Briefly, 1 kg of fresh samples were first washed using distilled water five times before being sliced and subsequently shade dried at room temperature (25°C–26°C) for 5 days to achieve a constant weight. Thereafter, the samples were assigned to three different portions (25 g, 50 g, and 100 g) before being soaked into 1,000 ml distilled water to achieve the concentration of decoction to be 25 g/l, 50 g/l, and 100 g/l, respectively. Afterward, the samples were boiled at 100°C for 25 minutes and filtered using Whatman filter paper. Next, the decoction was cooled and kept in a dark bottle before being used for the treatments.

Figure 1. The leaves of wild vine (*V. gracilis*).

Provision of animal models

All procedures for animal use and experimental treatments in this study have been approved by the Research Ethics Committee of Andalas University (No.5210/2022). This study used adult BALB/c strain male mice (n = 25; 2 months old; body weight 25–26 g) purchased from the Baso Veterinary Center (Bukittinggi West Sumatra). The acclimatization of the mice was conducted for a week in the animal room at the Biology Department of Andalas University with regulated environmental conditions (temperature 25°C–26°C; humidity 67%–68%; illumination 12 hours dark and 12 hours light). Each individual mouse was reared in a single cage and fed with a chow diet special for rodents (RATBIO, Citra InaFeed Jakarta) *ad libitum* while having free access to a water bottle filled with tap water. After the acclimatization period, 20 mice were randomly chosen to be alloxan-induced diabetic models, while other five mice were assigned as a control group (healthy; non-diabetic model). The alloxan-induced diabetic models were prepared by injecting alloxan monohydrate (Sigma Aldrich, Germany; a single dose of 150 mg/kg body weight) intraperitoneally [19]. Five days post injection, blood glucose levels were measured using a glucometer (AGM Dr Glucose 2100) by collecting blood samples from the tail vein. Mice with blood glucose levels of 250 mg/dl or higher were considered as diabetic. Accordingly, all 20 mice assigned for alloxan-induced diabetic models met the criteria of diabetes melltius, thus, eligible to be used in the

experiments. Eventually, the mice were randomly divided into five different groups as follows:
Group 1: non-diabetic mice (healthy; non-DM)
Group 2: diabetic mice without any treatment (DM)
Group 3: diabetic mice treated with 25 g/l of *V. gracilis* decoction (DM + 25 g/l VgD)
Group 4: diabetic mice treated with 25 g/l of *V. gracilis* decoction (DM + 50 g/l VgD)
Group 5: diabetic mice treated with 25 g/l of *V. gracilis* decoction (DM + 100 g/l VgD).

In this experimental study, the non-diabetic group (healthy; non-DM) was assigned as the standard (control) group. Consequently, the justification of the decoction's efficacy depended exclusively on comparisons with healthy mice, and not with diabetic mice treated with standard drug.

The animal treatments with decoction were immediately carried out upon confirmation of diabetic status. The decoction was orally given to the mice (0.5 ml/individual for each dose) on a daily basis in the morning (08.00–09.00) continuously for 30 days. The non-diabetic mice were also given distilled water (0.5 ml/individual) orally. The dose levels and the administration procedures of decoction were decided based on the previous study [20].

Blood glucose, body weight, water, and food intake measurements

The levels of random blood glucose were monitored weekly (08.00–09.00 a.m.) during the treatment by collecting blood samples from the vein tails before being measured using a glucometer. In addition, the fasting blood glucose levels were examined at the end of treatment under an 18-hour fasting condition. Moreover, the body weights of mice were monitored every 2 days, and food and water intakes were measured on a daily basis.

Assessment of sensory response by a hot plate test

The sensory response of mice was evaluated using a hot plate test performed at the end of treatment by following the procedure as described previously elsewhere [21]. Shortly, immediately after the treatments were ceased, each mouse was tested by putting its limbs on a hot plate (with a sustained temperature of 50°C). The latency time of the mouse responding to the hot sensation was recorded using a timer.

Assessment of motoric function by a balance beam test

A day after the hot plate test, mice were subsequently prepared for a balance beam test. The procedures of the test were as described previously [22]. Shortly, each mouse was positioned on a balance beam with a 40 cm length. The time spent by the mouse to completely cross the beam from end to end was recorded using a camera (Olympus TG6 Tough).

Determination of malondialdehyde (MDA) level in the brain tissue

Immediately after completing the other tests, mice were euthanized by cervical vertebrae dislocation. Subsequently, the head was dissected, and the brain was collected. For the purpose of measuring MDA levels, 0.3 g of brain tissue was sampled and homogenized in a PBS solution using a tissue homogenizer (Omni International). The homogenate was then used for MDA measurement, employing a lipid peroxidation assay kit (Abcam Ab118970, Abcam). The measurement procedures were carried out following the manufacturer's instructions. Sample absorbance was determined using a UV-Vis Spectrophotometer (Biorad Lab) at a wavelength of 532 nm.

Histopathological examination of the cerebellum

Histopathological observations of cerebellum tissue were conducted using histological slides stained with hematoxylin-eosin (HE). First, the cerebellum was collected, immediately washed with physiological saline, and then fixed in 10% NBF overnight. The tissue was subsequently dehydrated using graded ethanol (70%–100%) and cleared with xylene. The sample was then embedded in paraffin and sectioned using a rotary microtome to a thickness of 5 µm. Afterward, the tissue was stained with HE and mounted under a cover glass. Microscopic observations were conducted using a light microscope equipped with an Olympus CX-33 camera. Five distinct slices of cerebellar tissue were prepared for each mouse, and the examinations involved five different view fields for each slice. Counting of the Purkinje cells and measurements of the molecular and granular layers of the cerebellar tissues were undertaken using the ImageJ software (NIH, USA).

Statistical analysis

The data were first subjected to tests for homogeneity and distribution. Since our data were homogeneous and normally distributed, they were subsequently analyzed using an analysis of variance, followed by Duncan's New Multiple Range Test. A p-value of < 0.05 was considered significant. The analysis was performed using IBM SPSS Statistics Base version 22.0 for Windows.

RESULTS AND DISCUSSION

Effect of *V. gracilis* leaf decoction on blood glucose profile

To assess the blood glucose profile, the random blood glucose, fasting blood glucose levels, and insulin tolerance were determined. As shown in Figure 2A, 5 days after alloxan injection (0 week), blood glucose levels were markedly soared to the hyperglycemic state. This state was sustained until the end of the experiment (30 days). In contrast, non-diabetic mice (non-DM group) exhibited normal blood glucose levels during all the time points of measurements. There were no statistical differences in blood glucose levels of mice in the DM group as compared with those treated with various doses of *V. gracilis* leaf decoction ($p > 0.05$). However, the blood glucose values in the non-DM group were substantially lower at every time point of measurement as compared with all other groups ($p < 0.01$). Furthermore, fasting blood glucose levels (Fig. 2B) showed a noticeable increment in the DM group that was significantly different from the non-DM group ($p < 0.05$). There was a tendency for lower fasting blood glucose levels in decoction-treated groups as compared with the DM group. However, statistical analysis revealed that there were

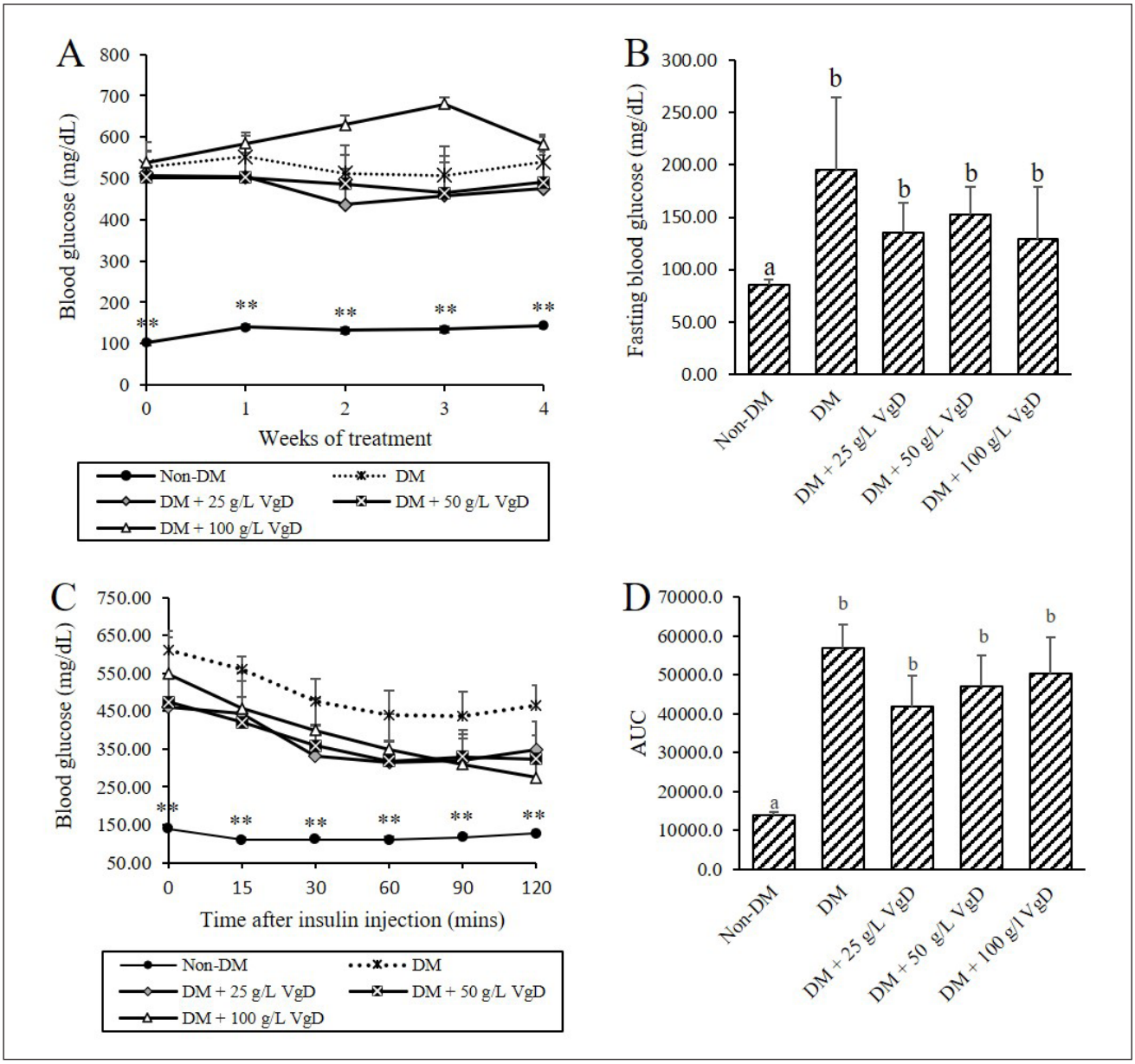

Figure 2. Effect of *V. gracilis* leaf decoction on blood glucose profile in alloxan-induced diabetic mice. (A) Random blood glucose levels measured weekly, (B) fasting blood glucose measured at the end of treatment, (C) blood glucose levels in insulin tolerance test (ITT), and (D) area under the curve of blood glucose levels in ITT. **) in A and C indicate statistical significance between the non-DM group compared to all other groups ($p < 0.01$); different lower case letters above the bars in B and D indicate statistically significant differences (a *vs.* b indicates significant difference at $p < 0.05$). Non-DM (non-diabetes mellitus), DM (Diabetes mellitus), VgD (*V. gracilis* decoction). $n = 5$ for each group.

no significant differences in fasting blood glucose values in the DM group as compared with decoction-treated groups ($p > 0.05$). The fasting blood glucose levels were substantially higher in mice treated with *V. gracilis* decoction (at all doses) as compared with the non-DM group ($p > 0.05$). In addition, ITT conducted at the end of treatment (Fig. 2C) indicated that upon insulin injection, blood glucose levels in the DM group remained higher and became substantially different as compared with the non-DM group ($p < 0.01$). Similarly, blood glucose values of mice treated with *V. gracilis* decoction at all doses were also sustained higher than non-DM group ($p < 0.05$). The AUC values (Fig. 2D) also showed that those in the DM group and decoction-treated groups had significantly higher AUC of ITT values as compared with the non-DM group ($p < 0.05$).

Effect of *V. gracilis* leaf decoction on body weight, food, and water intake

The results of monitoring on body weight (Fig. 3A) indicated that mice in the DM group exhibited lesser body weight increment from the beginning until the end of the experiment. Otherwise, mice in a non-DM group showed

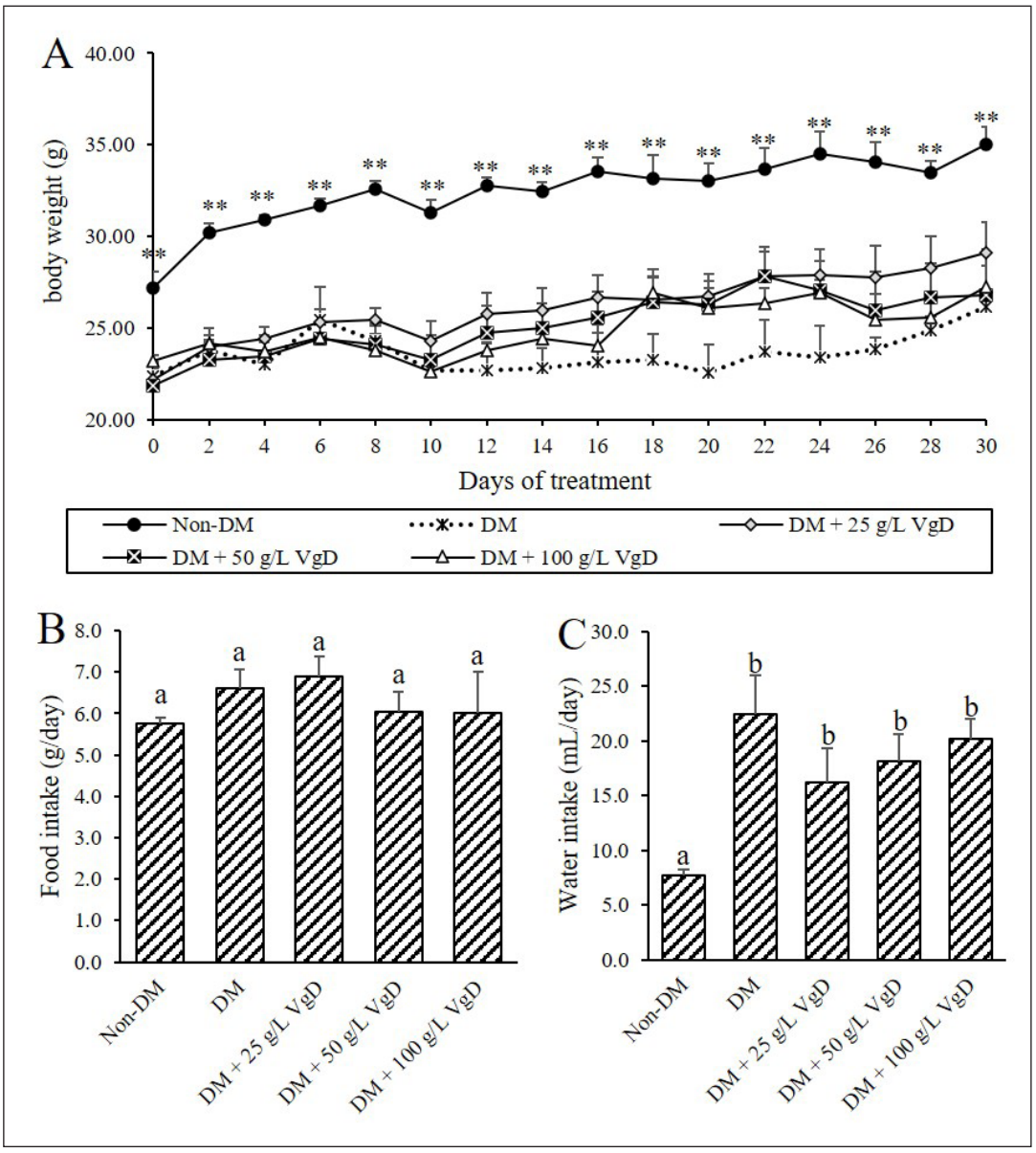

Figure 3. Effect of *V. gracilis* leaf decoction on body weight evolution, food and water intakes in alloxan-induced diabetic mice. (A) Body weight as measured every 2 days, (B) daily food intake, and (C) daily water intake. **) in A indicates statistical significance between the non-DM group compared to all other groups ($p < 0.01$); different lower-case letters in A and C indicate statistical significance (a *vs.* b indicates a significant difference at $p < 0.05$).

a significant increase in their body weight and became significantly higher at any time points of measurement as compared with the DM group ($p < 0.01$). Moreover, mice treated with *V. gracilis* leaf decoction at all doses of treatment exhibited comparable body weight with the DM group ($p > 0.05$). In addition, measurements of daily food intake (Fig. 3B) found that there was no significant difference among all groups of treatment ($p > 0.05$). However, daily water intake measurements (Fig. 3C) revealed that mice in the DM group had a higher water intake as compared with non-DM ($p < 0.01$). Similarly, mice treated with *V. gracilis* leaf decoction exhibited a substantial increment of water intake as compared with those in the non-DM group ($p < 0.01$).

Effect of *V. gracilis* decoction on sensory response and motoric balance

To evaluate sensory response and motoric balance, mice were tested using a hot plate test and a balance beam test. In a hot plate test, those in the DM group exhibited delayed latency response to hot sensation that was significantly longer as compared with the non-DM group ($p < 0.05$; Fig. 4A). Otherwise, mice treated with *V. gracilis* leaf decoction at all doses of treatment had substantial decrement in latency response against hot stimulation as compared with DM group ($p < 0.05$). Moreover, response latency in decoction-treated groups was comparable with those in non-DM group ($p > 0.05$). Furthermore, the result of the balance beam test (Fig. 4B)

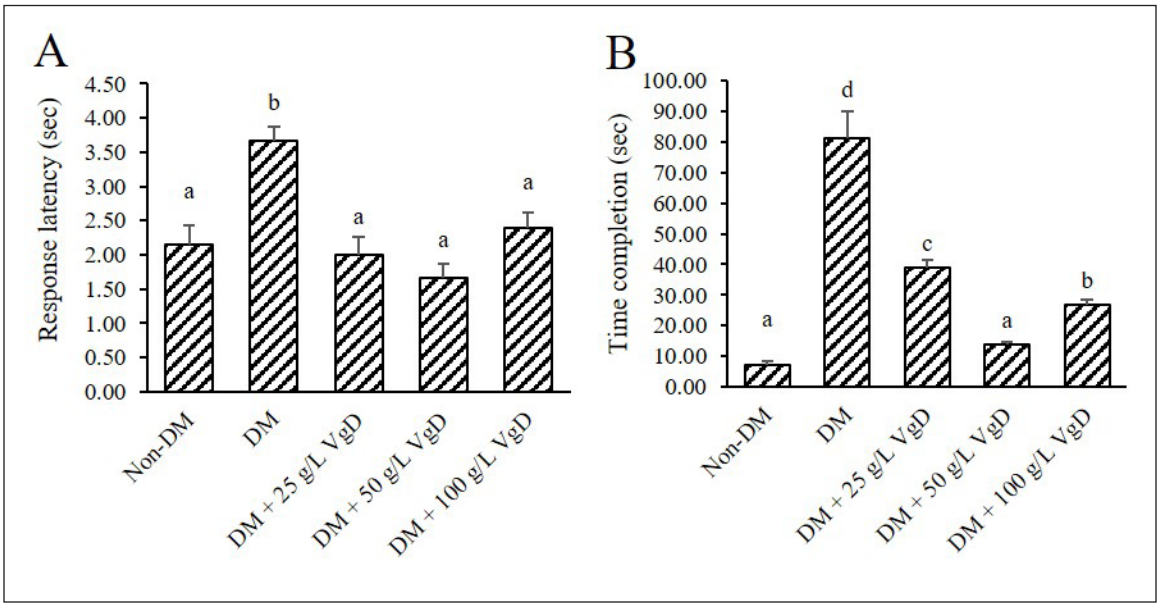

Figure 4. Effect of *V. gracilis* leaf decoction on sensory and motoric disorders in alloxan-induced diabetic mice. (A) Response latency of mice in hot plate test. (B) Time completion of mice in balance beam test. Different lower case letters above the bars represent statistical significance ($p < 0.05$).

demonstrated that mice in the DM group had a markedly slower time in completing the balance beam test (8 times slower) than those in the non-DM group ($p < 0.01$). However, mice treated with *V. gracilis* decoction showed improvement as indicated by a significantly faster time of balance beam completion as compared with the DM group ($p < 0.01$). Those treated with decoction at a dose of 50 g/l had comparable time completion with non-DM, while mice received 25 and 100 g/l decoction had statistically longer time of balance completion than non-DM ($p < 0.05$).

Effect of *V. gracilis* decoction on the oxidative stress and histopathology of the cerebellum

An examination of oxidative stress in the brain tissue (Fig. 5) found that the DM group had substantially higher MDA (a marker of oxidative stress) levels than the non-DM group ($p < 0.05$). However, treatment with *V. gracilis* leaf decoction at all doses caused a significant reduction of MDA ($p < 0.05$). Moreover, those treated with 100 g/l decoction had an apparent reduction of MDA to be substantially lower than non-DM mice ($p < 0.05$). A microscopic examination revealed the apparent alterations in the cerebellum of mice in the DM group (Fig. 6). It was found that in the non-DM group, the cerebellar tissue exhibited intact cerebellum with the linearly arranged Purkinje cells. Moreover, the molecular and granular layers were thicker. Otherwise, mice in the DM group had a reduction in the molecular and granular layers' thickness with many areas exhibiting irregular thinning while other areas showed edema. The populations of Purkinje cells were also lesser with many cells exhibiting hydropic degeneration and lysed nuclei (pycnotic). Furthermore, those treated with *V. gracilis* decoction depicted thicker granular and molecular layers and denser Purkinje cell populations. In addition, the degenerated cells were also lesser in decoction-treated mice. The measurements of molecular

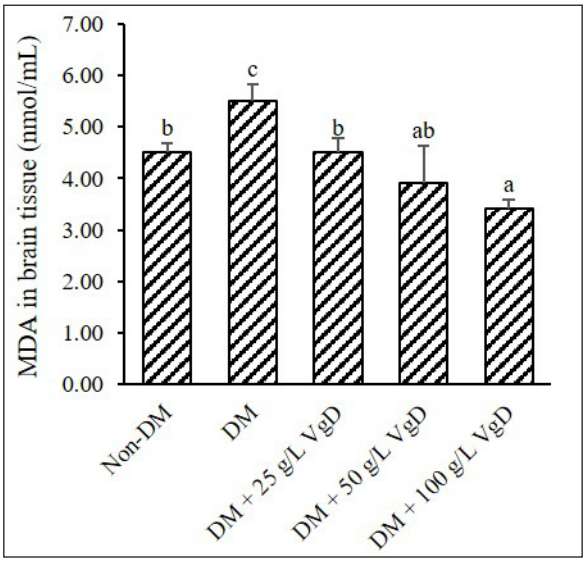

Figure 5. Effect of *V. gracilis* leaf decoction on oxidative stress in the brain of alloxan-induced diabetic mice. MDA (malondialdehyde). Different lower case letters above the bars represent statistical significance ($p < 0.05$).

layer thickness (Fig. 7A) revealed a significant decrement as compared with the non-DM group ($p < 0.05$). In contrast, mice treated with decoction had substantially thicker molecular layers than the DM group, particularly at the higher doses (50 and 100 g/l). Next, the measurements on the thickness of the granular layer (Fig. 7B) found that mice in the DM group also had significantly thinner granular layers as compared with the non-DM group ($p < 0.05$). Moreover, mice treated with higher doses of decoction (50 and 100 g/l) tended to have thicker granular layers as compared with the DM group, but it was apparently non-significant ($p > 0.05$). The counting on Purkinje

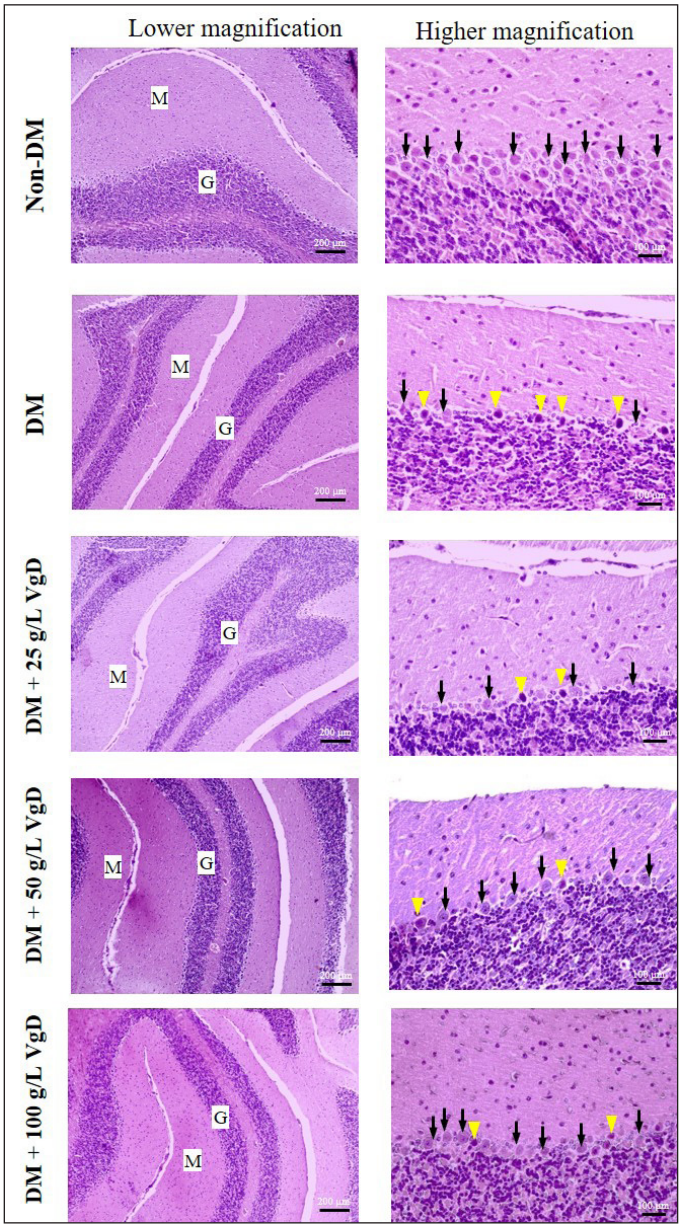

Figure 6. Representative photomicrographs of mice cerebellum. (M) Molecular layer, (G) granular layer, black arrow in right panels indicated normal Purkinje cells, and yellow head arrows indicated degenerated Purkinje cells. Tissue was stained with HE. Scale bar in left panels = 200 µm, right panel = 100 µm.

cells (Fig. 7C) revealed that DM caused a substantial reduction in total Purkinje cells as compared with non-DM ($p < 0.05$) while those given with *V. gracilis* decoction at higher doses (50 and 100 g/l), but not at a lower dose (25 g/l), had significantly higher number of Purkinje cells in the cerebellum. Importantly, mice in the group treated with decoction at a dose of 100 g/l had a comparable Purkinje cell number as non-DM mice ($p > 0.05$). In addition, the DM also exhibited a marked increase of degenerated Purkinje cells (Fig. 7D) as compared with non-DM ($p < 0.001$). Otherwise, mice treated with decoction exhibited significantly lower degenerated Purkinje cell numbers than DM mice ($p < 0.01$) in a dose-dependent manner.

The findings of this present study indicated that *V. gracilis* leaf decoction could effectively ameliorate sensory and motoric disorders while reducing MDA levels in brain tissue, particularly at higher doses, in alloxan-induced diabetic mice. Moreover, *V. gracilis* leaf decoction was capable of alleviating histopathological alterations in the cerebellum, a brain area involved in regulating motoric balance and sensory response. However, the decoction did not exert substantial beneficial effects against hyperglycemia and insulin intolerance, as well as body weight reduction. Moreover, the decoction did not affect food intake while failing to improve elevated water intake under diabetic conditions.

Oxidative stress and the accumulation of its by-products play a pivotal role in the pathological development of neurological disorders caused by hyperglycemia in diabetes [23,24]. Likewise, our present findings indicate that mice with diabetic conditions without any treatment exhibited a noticeable elevation of MDA in brain tissue and an apparent reduction in sensory response and motoric balance. Otherwise, daily administrations of *V. gracilis* leaf decoction appeared to be effective in sustaining high performance of sensory response and motoric balance alongside a marked depletion of MDA in brain tissue. This finding suggests that the *V. gracilis* decoction exerted a protective effect on neurological functions via an antioxidative mechanism. In previous studies, it was found that *V. gracilis* leaves contained some potent compounds that function as antioxidants [13,14,20]. Another report also suggests that *V. gracilis* leaf extract could suppress the MDA level while increasing superoxide dismutase (an endogenous antioxidant) in the blood plasma of rats [16]. Taken together, despite failing to manage blood glucose elevation, *V. gracilis* leaf decoction could effectively inhibit the elevation of oxidative stress, thereby alleviating the detrimental effect of hyperglycemia on neurological functions.

The cerebellum plays a crucial role in controlling motor function, and its impairment is significantly implicated in motoric function disorders [25]. Neuronal circuits involving the granular layer, molecular layer, and Purkinje cell layer in the cerebellum are essential for motor learning activities [26]. Furthermore, the cerebellum has been found to have functional connectivity with somatosensory areas, thereby participating in sensory response mechanisms [27]. As demonstrated in a previous report, chronic exposure of cerebellar tissue to hyperglycemia promotes the degeneration of Purkinje cells and granular cells in the cerebellum [28,29]. Similarly, in our current study, the hyperglycemic state in alloxan-induced diabetic mice resulted in a substantial reduction in Purkinje cells, the molecular layer, and the granular layer in the cerebellum. Interestingly, despite the sustained hyperglycemic condition, those treated with *V. gracilis* decoction exhibited cerebellar tissue features comparable to those of non-diabetic (healthy) individuals. This finding indicates that *V. gracilis* decoction could effectively protect the cerebellum against hyperglycemia-induced degeneration. It is speculated that the bioactive compounds in the decoction might play a role in inhibiting oxidative stress, thereby preventing subsequent damage to brain tissue. In addition, the decoction could also function to enhance the activity of endogenous antioxidants

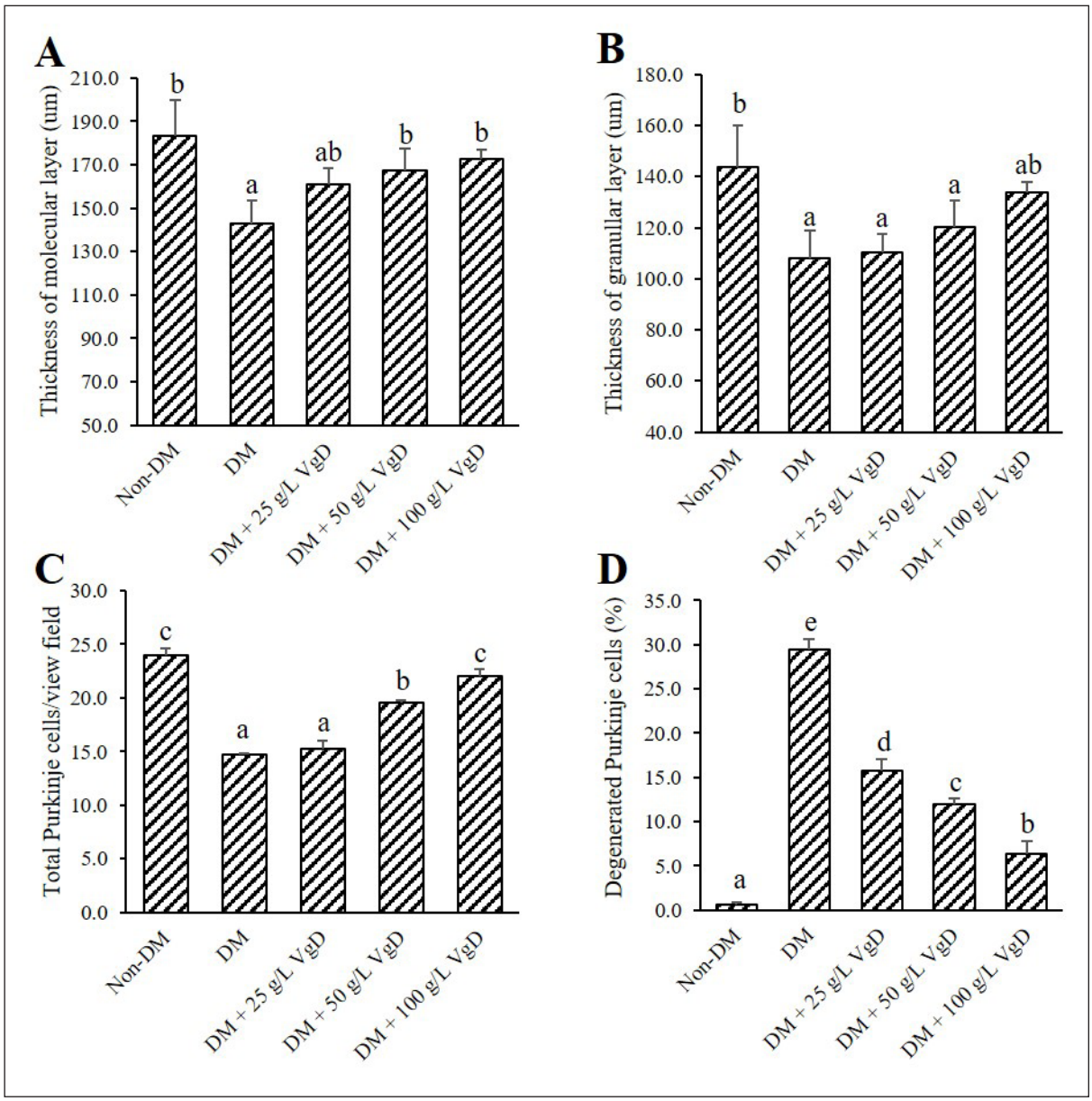

Figure 7. Effect of *V. gracilis* leaf decoction on histopathological alterations of the cerebellum in alloxan-induced diabetic mice. (A) Thickness of molecular layer, (B) thickness of granular layer, (C) total Purkinje cells, and (D) percentage of degenerated Purkinje cells. Different lower case letters above the bars represent statistical significance ($p < 0.05$).

in the brain, including catalase, superoxide dismutase, and glutathione. However, our current study did not measure the levels of endogenous antioxidants. Therefore, further research is needed to clarify the speculations.

While our present study did not investigate the inflammatory responses, their involvement in the development of central and peripheral neurological disturbances due to diabetes is highly likely [30]. Inhibiting proinflammatory cytokines is suggested as a key strategy for managing neurodegeneration caused by hyperglycemia [31]. In a prior study using a micro-colloidal preparation, we found that administering *V. gracilis* leaf extract significantly decreased the proinflammatory cytokine Tumor Necrosis Factor-alpha (TNF-α) while enhancing the anti-inflammatory cytokine Interleukin-10 in the circulatory system of rats [17]. This anti-inflammatory effect of *V. gracilis* may be attributed to certain compounds that could regulate systemic inflammatory responses. However, whether the decoction of *V. gracilis* leaf can also regulate inflammation in brain tissue to mitigate the pathological consequences of hyperglycemia remains to be elucidated.

The health benefits of *V. gracilis* decoction in managing the detrimental outcomes of diabetes on the brain, as indicated in this study, may be attributed to the various bioactive compounds found in the leaves of the plant. General phytochemical screening conducted by previous studies revealed that *V. gracilis* leaves contained flavonoids,

alkaloids, glycosides, tannins, saponins, and terpenoids [14,15]. Furthermore, our previous study, employing a high-performance liquid chromatography-mass spectrometry technique [20], identified 26 phytochemical compounds in the leaf decoction of *V. gracilis*. Among these compounds, some potential substances include carthamoleusteron, methylbutanoate, valporic acid, curcubiten glucoside, lauric acid, dipropyleneglycol methyl ether acetate, arbutin, tetrahydroxypentanoic acid, phenyl salicylate, ribonic acids, ethylparaben, phenyl 2-hydroxybenzoate, arbutin, and norethindrone acetate. The common bioactivity of these compounds is as antioxidants, specifically acting as lipid peroxidase inhibitors, free radical scavengers, and NADPH peroxidase inhibitors. Alternatively, some compounds, such as phenyl 2-hydroxybenzoate, arbutin, lauric acid, and norethindrone acetate, may act as anti-inflammatory agents. Therefore, it is reasonable to suggest that the leaf decoction of *V. gracilis* exerts antioxidant and anti-inflammatory effects to protect the brain from damage caused by diabetes mellitus. Further studies emphasizing this mechanistic aspect are required to clarify these speculations.

Hyperglycemia is a pathological cause of various deleterious outcomes related to diabetes mellitus [4,5]. In this study, daily oral administrations of *V. gracilis* leaf decoction failed to manage hyperglycemia as indicated by the markedly higher blood glucose levels (> 400 mg/dl) until the end of treatment. Moreover, the exogenous administration of insulin was not effective in bringing blood glucose down to the normoglycemic level, indicating an insulin intolerance. Although we did not examine the histopathological alterations in the pancreatic tissue, we suggest that *V. gracilis* decoction might not be effective in alleviating the degeneration of pancreatic β cells caused by alloxan induction. As a consequence, the blood glucose homeostasis was not properly managed due to insulin insufficiency. Moreover, the fact that insulin intolerance remained observed could indicate the impairment of insulin receptors in the insulin-targeted cells (including myocytes, adipocytes, hepatocytes, and other cells). To our knowledge, there was no previous study investigating the effect of *V. gracilis* leaves on blood glucose profile under diabetes conditions. However, a study of another species in the same genus namely *Vitis vinifera* (red grape) demonstrated its ability to suppress blood glucose levels in individuals with diabetes mellitus [32]. Moreover, *V. vinifera* was also reported to exert an ameliorative effect on insulin sensitivity in human subjects with metabolic syndrome [33]. Therefore, besides species difference (*V. gracilis vs. V. vinfera*), it is assumed that this discrepancy may be also due to several plausible reasons. First, in our present study, the sample was prepared as a decoction, adopting a common traditional practice of the Karo tribe (in North Sumatra) in consuming *V. gracilis* leaves as a medicinal herb. In contrast, a previous study in *V. vinifera* employed extraction procedures. This might be implicated in the phytochemical constituents of the sample thereby affecting their effectivity in counteracting hyperglycemia and insulin intolerance. Second, the experimental treatment in our present study was conducted in a relatively short period (30 days). It is speculated that decoction may require a longer period of treatment (more than 30 days) to effectively exert alleviative effects against hyperglycemia and insulin intolerance under diabetic conditions. Future investigations with a longer period of administration and deploying different extraction techniques followed by comprehensive phytochemical screenings are needed to justify our assumptions.

Despite having comparable food intakes to those of non-treated groups, our current study also found that diabetic mice treated with *V. gracilis* decoction did not show a substantial body weight gain during the course of treatment. Under normal physiological circumstances, the energy from the food will be manifested in the body weight gain [34]. However, the hyperglycemia state in diabetes impairs the energy metabolic homeostasis [35]. Accordingly, instead of depositing as tissue biomass, including muscle and adipose tissue mass, energy from the food in diabetic individuals will be largely compensated to overcome physiological dysregulations promoted by hyperglycemia-associated disturbances [36]. Hence, a lesser body weight gain observed in diabetic mice treated with *V. gracilis* decoction could be a result of the inability of decoction treatment to manage hyperglycemia. In addition, the water intake was also remained higher in those treated with *V. gracilis* leaf decoction, suggesting the consequence of unmanageable hyperglycemia. As commonly observed in diabetic individuals, excessive water intake is associated with the physiological mechanism to compensate for elevated urine output due to glycosuria [36]. Therefore, as long as blood glucose levels remain in the state of hyperglycemia, the water intake also would be sustained at higher levels.

Although our current findings suggest the promising potential of *V. gracilis* leaf decoction in preventing neurological issues related to diabetes, it is important to acknowledge a notable limitation, namely the absence of a standard drug as a control group. The inclusion of such a standard drug group would have offered a benchmark for comparison, enabling a more comprehensive assessment of the efficacy of *V. gracilis* decoction in managing diabetes-related complications, including neurological problems. Future studies that incorporate a standard drug would contribute to a more robust evaluation of the potential therapeutic effects of *V. gracilis* decoction in comparison to established medical interventions for diabetes and associated outcomes.

CONCLUSION

In summary, this study investigated the effects of *V. gracilis* leaf decoction on various parameters in alloxan-induced diabetic mice. The findings indicated that the decoction demonstrated its efficacy in ameliorating sensory and motoric disorders as well as reducing oxidative stress in the brain tissue. In addition, an examination of the cerebellum histopathology revealed that *V. gracilis* decoction safeguarded against hyperglycemia-induced degeneration, preserving the integrity of cerebellar tissue. However, the decoction did not significantly affect blood glucose levels, insulin tolerance, or body weight, suggesting a lack of substantial anti-hyperglycemic effects. Therefore, despite its limited impact on hyperglycemia, *V. gracilis* leaf decoction demonstrated potential neuroprotective properties, indicating a potential role in alleviating neurological complications associated with

diabetes. Subsequent research could delve into the underlying mechanisms, including potential anti-inflammatory effects, and investigate the long-term effects of decoction administration on hyperglycemia and its associated complications.

AUTHOR CONTRIBUTION

PS and RM designed the study and prepared the manuscript; PS, RM, and AY performed animal experiments; AT and AY conducted histopathological analysis; PS and DFB analyzed the data.

FINANCIAL SUPPORT

This study was funded by Research for Reputable Publication Grant of Andalas University 2023 (RPT, Contract No. T/71/UN16.19/PT.01.03/KO-RPT/2023).

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Andalas University, Padang, Indonesia with approval number 5210/2022.

DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Koye DN, Magliano DJ, Nelson RG, Pavkov ME. The global epidemiology of diabetes and kidney disease. Adv Chronic Kidney Dis. 2018;25(2):121–32. doi: https://doi.org/10.1053/j.ackd.2017.10.011
2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, *et al.* IDF diabetes atlas committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843. doi: https://doi.org/10.1016/j.diabres.2019.107843
3. Bonner R, Albajrami O, Hudspeth J, Upadhyay A. Diabetic kidney disease. Prim Care. 2020;47(4):645–59. doi: https://doi.org/10.1016/j.pop.2020.08.004
4. Hamed AE, Elwan N, Naguib M, Elwakil R, Esmat G, El Kassas M, *et al.* Diabetes association with liver diseases: an overview for clinicians. Endocr Metab Immune Disord Drug Targets. 2019;19(3):274–80. doi: https://doi.org/10.2174/1871530318666181116111945
5. Damaskos C, Garmpis N, Kollia P, Mitsiopoulos G, Barlampa D, Drosos A, *et al.* Assessing cardiovascular risk in patients with diabetes: an update. Curr Cardiol Rev. 2020;16(4):266–74. doi: https://doi.org/10.2174/1573403x15666191111123622
6. Asrar MM, Kumari S, Sekhar BC, Bhansali A, Bansal D. Relative efficacy and safety of pharmacotherapeutic interventions for diabetic peripheral neuropathy: a systematic review and Bayesian network meta-analysis. Pain Physician. 2021;24(1):E1–E14
7. Srinivasan A, Dutta P, Bansal D, Chakrabarti A, Bhansali AK, Hota D. Efficacy and safety of low-dose naltrexone in painful diabetic neuropathy: A randomized, double-blind, active-control, crossover clinical trial. J Diabetes. 2021;13(10):770–8. doi: https://doi.org/10.1111/1753-0407.13202
8. Khasbage S, Shukla R, Sharma P, Singh S. A randomized control trial of duloxetine and gabapentin in painful diabetic neuropathy. J Diabetes. 2021;13(7):532–41. doi: https://doi.org/10.1111/1753-0407.13148
9. Derry S, Bell RF, Straube S, Wiffen PJ, Aldington D, Moore RA. Pregabalin for neuropathic pain in adults. Cochrane Database Syst Rev. 2019;1(1):CD007076. doi: https://doi.org/10.1002/14651858.cd007076.pub3
10. Pathak R, Sachan N, Chandra P. Mechanistic approach towards diabetic neuropathy screening techniques and future challenges: a review. Biomed Pharmacother. 2022;150:113025. doi: https://doi.org/10.1016/j.biopha.2022.113025
11. Pan Y, Davis PB, Kaebler DC, Blankfield RP, Xu R. Cardiovascular risk of gabapentin and pregabalin in patients with diabetic neuropathy. Cardiovasc Diabetol. 2022;21(1):170–8. doi: https://doi.org/10.1186/s12933-022-01610-9
12. Sicras-Mainar A, Rejas-Gutiérrez J, Perez-Paramo M, Navarro-Artieda R. Cost of treating peripheral neuropathic pain with pregabalin or gabapentin at therapeutic doses in routine practice. J Comp Eff Res. 2018;7(7):615–25. doi: https://doi.org/10.2217/cer-2018-0008
13. Wasnis NZ, Ilyas S, Hutahaean R, Silaban R, Situmorang PC. Effect of *Vitis gracilis* Wall (gagatan harimau) in the recovery of gastrocnemius muscle cells and cytochrome c expression of Mus musculus. J Pharm Pharmacogn Res. 2022;10(2):303–9. doi: https://doi.org/10.56499/jppres21.1208_10.2.303
14. Aththorick TA, Berutu L. Ethnobotanical study and phytochemical screening of medicinal plants on Karonese people from North Sumatra, Indonesia. J Phys Conf Ser. 2018;116(5):55–62. doi: https://doi.org/10.1088/1742-6596/1116/5/052008
15. Wasnis NZ, Ilyas S, Hutahaean S, Silaban R, Situmorang PC. Analysis of apoptotic cells and lung inflammation after given by *Vitis gracilis*. Pak J Biol Sci. 2022;25(11):1033–9. doi: https://doi.org/10.3923/pjbs.2022.1033.1039
16. Midoen YH, Ilyas S, Santoso P, Situmorang PC. Effect of maximal physical exercise on apoptosis via cytochrome c in hippocampus cells after administration of *Vitis gracilis* Wall. J Pharm Pharmacogn Res. 2023;11(2):297–307. doi: https://doi.org/10.56499/jppres22.1563_11.2.297
17. Santoso P, Ilyas S, Midoen YH, Situmorang PC. Effect of *Vitis gracilis* Wall administration on maximal swimming exercise apoptosis via cytochrome c in rat lung cells. J Pharm Pharmacogn Res. 2023;11(3):381–90. doi: https://doi.org/10.56499/jppres23.1603_11.3.381
18. Mary AC, Ireen C, Vijayalakshmi P, Indhu S, Divyabharathi S, Felix KS, *et al.* Phytochemical screening and anti-obesity, anti- diabetic and anti-oxidant properties of *Scoparia dulcis* leaf decoction (crude). Bioinformation. 2023;19(3):238–42. doi: https://doi.org/10.6026/97320630019238
19. Yin P, Wang Y, Yang L, Sui J, Liu Y. Hypoglycemic effects in alloxan-induced diabetic rats of the phenolic extract from mongolian oak cups enriched in ellagic acid, kaempferol and their derivatives. Molecules. 2018;23(5):1046. doi: https://doi.org/10.3390/molecules23051046

20. Santoso P, Ilyas S, Midoen Y, Yuniarti A Protective Effect of *Vitis gracilis* Wall (Vitaceae) leaf decoction on sexual vitality and testis of alloxan-induced diabetic mice. Trad Integr Med. 2023;8(3):256–68. doi: https://doi.org/10.18502/tim.v8i3.13711.
21. Ostovar M, Akbari A, Anbardar MH, Iraji A, Salmanpour M, Hafez GS, *et al.* Effects of *Citrullus colocynthis* L. in a rat model of diabetic neuropathy. J Integr Med. 2020;18(1):59–67. doi: https://doi.org/10.1016/j.joim.2019.12.002
22. Orenduff MC, Rezeli ET, Hursting SD, Pieper CF. Psychometrics of the balance beam functional test in C57BL/6 mice. Comp Med. 2021;71(4):302–8. doi: https://doi.org/10.30802/AALAS-CM-21-000033
23. González P, Lozano P, Ros G, Solano F. Hyperglycemia and oxidative stress: an integral, updated and critical overview of their metabolic interconnections. Int J Mol Sci. 2023;24(11):9352. doi: https://doi.org/10.3390/ijms24119352
24. Darenskaya MA, Kolesnikova LI, Kolesnikov SI. Oxidative stress: pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. Bull Exp Biol Med. 2021;171(2):179–89. doi: https://doi.org/10.1007/s10517-021-05191-7
25. D'Angelo E. Physiology of the cerebellum. Handb Clin Neurol. 2018;154:85–108. doi: https://doi.org/10.1016/b978-0-444-63956-1.00006-0
26. Sedaghat-Nejad E, Pi JS, Hage P, Fakharian MA, Shadmehr R. Synchronous spiking of cerebellar Purkinje cells during control of movements. Proc Natl Acad Sci USA. 2022;119(14):e2118954119. doi: https://doi.org/10.1073/pnas.2118954119
27. Kilteni K, Ehrsson HH. Functional connectivity between the cerebellum and somatosensory areas implements the attenuation of self-generated touch. J Neurosci. 2020;40(4):894–906. doi: https://doi.org/10.1523/jneurosci.1732-19.2019
28. Deng CK, Mu ZH, Miao YH, Liu YD, Zhou L, Huang YJ, *et al.* Gastrodin ameliorates motor learning deficits through preserving cerebellar long-term depression pathways in diabetic rats. Front Neurosci. 2019;13(2):1239. doi: https://doi.org/10.3389/fnins.2019.01239
29. Medras ZJH, Mostafa YM, Ahmed AAM, El-Sayed NM. Arctigenin improves neuropathy via ameliorating apoptosis and modulating autophagy in streptozotocin-induced diabetic mice. CNS Neurosci Ther. 2023;29(10):3068–80. doi: //doi.org/10.1111/cns.14249
30. Baum P, Toyka KV, Blüher M, Kosacka J, Nowicki M. Inflammatory mechanisms in the pathophysiology of diabetic peripheral neuropathy (DN)-new aspects. Int J Mol Sci. 2021;22(19):10835. doi: https://doi.org/10.3390/ijms221910835
31. Zhou G, Yan M, Guo G, Tong N. Ameliorative effect of berberine on neonatally induced type 2 diabetic neuropathy via modulation of BDNF, IGF-1, PPAR-γ, and AMPK Expressions. Dose Response. 2019;17(3):1559325819862449. doi: https://doi.org/10.1177/1559325819862449
32. Huamán-Castilla NL, Campos D, García-Ríos D, Parada J, Martínez-Cifuentes M, Mariotti-Celis MS, *et al.* Chemical properties of *Vitis Vinifera Carménère* pomace extracts obtained by hot pressurized liquid extraction, and their inhibitory effect on type 2 diabetes mellitus related enzymes. Antioxidants (Basel). 2021;10(3):472. doi: https://doi.org/10.3390/antiox10030472
33. Martínez-Maqueda D, Zapatera B, Gallego-Narbón A, Vaquero MP, Saura-Calixto F, Pérez-Jiménez J. A 6-week supplementation with grape pomace to subjects at cardiometabolic risk ameliorates insulin sensitivity, without affecting other metabolic syndrome markers. Food Funct. 2018;9(11):6010–9. doi: https://doi.org/10.1039/c8fo01323c
34. Argyrakopoulou G, Simati S, Dimitriadis G, Kokkinos A. How important is eating rate in the physiological response to food intake, control of body weight, and glycemia? Nutrients. 2020;12(6):1734. doi: https://doi.org/10.3390/nu12061734
35. Roden M, Shulman GI. The integrative biology of type 2 diabetes. Nature. 2019 Dec;576(7785):51–60. doi: https://doi.org/10.1038/s41586-019-1797-8
36. Stella AB, Yardley J, Francescato MP, Morrison SA. Fluid intake habits in type 1 diabetes individuals during typical training bouts. Ann Nutr Metab. 2018;73(1):10–8. doi: https://doi.org/10.1159/000489823

How to cite this article:
Santoso P, Yuniarti A, Maliza R, Tofrizal A, Belahusna DF. Ameliorative effect of *Vitis gracilis* leaf decoction against sensory and motoric disorders, oxidative stress, and cerebellar degeneration in diabetic mice. J Appl Pharm Sci. 2024;14(10):141–151.