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Original Article

Hypoglycemic and antioxidant activities of *Clerodendrum inerme* leaf extract on streptozotocin-induced diabetic models in mice

Hai Trieu Ly[a,b], Thi Thu Huong Nguyen[a], Thi Thanh Loan Tran[c], Bich Thao Lam[a], Thi Thu Huong Phung[d], Van Minh Le[a,*]

[a] *Research Center of Ginseng and Medicinal Materials (CGMM), National Institute of Medicinal Materials, Ho Chi Minh City 700000, Viet Nam*
[b] *University of Science, Vietnam National University, Ho Chi Minh City 700000, Viet Nam*
[c] *University of Medicine and Pharmacy, Ho Chi Minh City 700000, Viet Nam*
[d] *NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City 700000, Viet Nam*

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ABSTRACT

Objective: Recently, much attention has been paid to natural product-derived compounds for antidiabetic drug discovery. More recent studies are being focused on clarifying the bioactivity of plants and derived products. The aim of the present study was to investigate the anti-oxidant and antidiabetic activities of *Clerodendrum inerme* leaf extract (CILE) in streptozotocin-induced diabetic mice.

Methods: *C. inerme* leaves were analyzed for preliminary phytochemical properties and the content of total phenolic and flavonoid were determined. *In vitro* anti-oxidant activity was measured using DPPH assay. Streptozotocin-induced diabetic model in mice was applied for *in vivo* study by the effect of CILE at two dose levels (343 and 686 mg/kg b.w.).

Results: The results showed that *C. inerme* leaves contained the major constituents of flavonoids, alkaloids, tannins, triterpenes, and saponins. CILE exhibited the total polyphenol and flavonoid content with 120.458 mg gallic acid equivalent/g dry weight and 4.494 mg hispidulin equivalent/g dry weight, respectively. The anti-oxidant activity of CILE was expressed with $IC_{50} = 25.28$ μg/mL. CILE at the doses of 343 mg/kg and 686 mg/kg after 7 d administration exerted a decrease in plasma glucose, protected the liver, kidneys against oxidation stress via increasing glutathione content in the liver, and reduced malondialdehyde content in the liver and kidneys. Pancreatic histological analysis in diabetic mice treated with CILE also showed the pancreatic β-cells regeneration via increasing the size and number of pancreatic islets.

Conclusion: These findings suggested that *C. inerme* leaves had potent antidiabetic and anti-oxidant activities. The results provide reliable scientific base, which is the premise for further research and development of CILE as supplements.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder, characterized by hyperglycemia and secondary metabolic disorders. It is considered as one of the leading causes of death in the world. Thus, the control and treatment of diabetes mellitus is a global problem. Previous studies suggested that diabetes is an oxidative stress disorder and hyperglycemia is known in mediating oxidative damage and impairing the endogenous anti-oxidant defense systems in many different ways. The drugs in the treatment of diabetes are often reported to associate with side effects (Chaudhury et al., 2017). Therefore, finding other antidiabetes agents, especially those made from natural sources is interested. Natural substances with therapeutic properties have been used in ancient times. Previous studies also demonstrated that the plant extract and natural compounds showed antidiabetic and anti-oxidant activities (Soares et al., 2017; Testa et al., 2016; Yashin, Yashin, Xia & Nemzer, 2017; Vinayagam et al., 2015).

Clerodendrum inerme L. Gaertn (Verbenaceae) has been used as a traditional herb on the treatment of various ailments (National Institute of Medicinal Materials, 2016). Pharmacological studies have shown that the compounds and extracts from *C. inerme* have extensive activities, such as antibacterial, antifungal, anti-oxidant, antitumor, anti-inflammatory, antidiabetic, hepatoprotective, neuroprotective, and other activities. *C. inerme* leaves have been reported to contain flavonoids, alkaloids, tannins,

* Corresponding author.
E-mail address: lvminh_hcm@nimm.org.vn (V.M. Le).

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triterpenes, and saponins (Chethana, Hari Venkatesh & Gopinath, 2013; Esmail, 2016). Among phytochemicals, polyphenols compounds such as flavonoids and tannins, are considered as some of bioactive compounds which have been demonstrated to possess various therapeutic properties. Previous studies have shown that *C. inerme* leaf extracts presented inhibitory activities, hypoglycemic/antidiabetic and antioxidant effect on carbohydrate-hydrolyzing enzymes (Thirumal & Muthusamy, 2016; Bhushan, Sardana & Gulshan, 2015; Biman, Kumar & Kumar, 2015; Li et al., 2015). However, most of the previous reports focused on plasma glucose levels, and other parameters related to diabetes have not been evaluated. Therefore, the objective of this study was to investigate the anti-oxidant and antidiabetic activities of *C. inerme* leaf extract.

2. Materials and methods

2.1. Plant material and extraction

C. inerme leaves were collected on May 2017 in Phu Quoc Island, Kien Giang Province and identified by the Southern Institute of Ecology, Vietnam Academy of Science and Technology. The dried samples (Loss on drying was 7.92%) were ground to a fine powder and stored individually in airtight PVE bag at the Research Center Ginseng and Medicinal Materials in Ho Chi Minh City.

Dried powdered material was extracted with 45% (volume percent) ethanol by percolation method (ratio of materials/solvent as 1: 15). Briefly, dried powdered material was soaked with 45% ethanol at room temperature for 24 h in percolator apparatus. The extract was collected at a rate of 2 mL/min and the solvent was continuously added until reaching the above extract rate. The extract was concentrated using a rotary evaporator at 60 °C under reduced pressure, and crude 45% ethanol extract was obtained. The extract yield and the loss on drying were 27.43% and 15.25%, respectively. The extract was dissolved in distilled water for oral injection in mice. The test doses were expressed as mg extract per kg mouse body weight (mg/kg) and be equivalent to 1.25–2.50 g raw materials/kg.

2.2. Chemicals, reagents, apparatus and equipment

Hispidulin (HPLC ≥ 98%, China), Folin-Ciocalteu's phenol reagent, gallic acid, DPPH reagent (1,1-diphenyl-2-picrylhydrazyl), ascorbic acid, streptozotocin, glibenclamide, malondialdehyde, glutathione, thiobarbituric acid, and Ellman's reagent [5,5'-dithiobis(2-nitrobenzoic acid)] were purchased from Sigma-Aldrich® Co., Ltd (USA).

2.3. Determination of content of total phenolic and flavonoid

The total phenolic content was estimated by Folin Ciocalteu's method as previously described using gallic acid as a standard (Chumark, Panya, Yupin, Srichan & Noppawan, 2008). The total polyphenol content was expressed as mg of gallic acid equivalent/g of dry weight.

The flavonoids content was determined based on previously described method using hispidulin as reference compound (Nguyen et al., 2015). Briefly, 1 mL test extract was mixed with 9 mL methanol. Then, the absorbance was measured at 353 nm and all tests were performed in triplicate. The blank was performed using methanol. Hispidulin was used as standard. The total flavonoids content was expressed as mg of hispidulin equivalent/g of dry weight.

2.4. In vitro *anti-oxidant activity assay*

The anti-oxidant activity was determined by DPPH free radical scavenging assay based on a previously described method (Alhakmani, Kumar & Khan, 2013). Briefly, 0.5 mL of various concentrations of extract or ascorbic acid (1.0, 0.5, 0.25, 0.125, 0.0625 mmol/L) was added into a tube containing 0.5 mL of 0.6 mmol/L DPPH solution dissolved in methanol and the volume was made uniformly to 4 mL using methanol. The solution was mixed and kept in the dark at room temperature for 30 min. Then, the absorbance was measured at 515 nm and all tests were performed in triplicate. Ascorbic acid was used as a positive control. The results were expressed as IC_{50} values for each sample. Percentage of the radical scavenging activity was estimated using the equation:

$$(\%\,\text{scavenging effect}) = \frac{A_c - A_t}{A_c} \times 100$$

Where A_c is the absorbance of the blank (without test extract) and A_t is the absorbance of the sample (with test extract).

2.5. Test animals

Healthy Swiss albino male mice, 5 – 6 weeks old and with an average weight of (25 ± 2) g, were obtained from the Institute of Vaccines and Medical Biologicals in Nha Trang City. Mice were adapted at least one week prior to experiment under the same laboratory conditions of temperature (22 ± 2) °C, relative humidity (70 ± 4)%, a 12 h light/dark cycle, and received a nutritionally standard diet and tap water. The sample volume being administered (intraperitoneally or orally) was of 10 mL/kg mouse body weight (b.w.). The total number of animals used in this study was one hundred. Animal studies were adhered to principles stated in the Guide for Care and Use of Laboratory Animals.

2.6. Oral glucose tolerance test (OGTT)

The OGTT was performed on overnight fasting normal mice ($n = 6$). The mice were administered single doses or repeated dose for seven consecutive days of distilled water, plant extracts (343 mg/kg and 868 mg/kg) and glibenclamide (5 mg/kg) to four groups of mice, respectively. One hour after the last administration, tail vein blood samples were taken to determine initial plasma glucose levels. Glucose at dose of 2 g/kg b.w. was then orally administered and plasma glucose levels were measured at 30 and 120 min after glucose overload using glucose meter kit of Human Co., Ltd. (Germany) according to GOD-PAP (Glucose oxidase-Phenol and 4 aminophenazone) method.

Glucose (2 mg/kg) and glibenclamide (5 mg/kg) were selected as a standard drug based on reports of previous studies (Tesfaye, Makonnen & Gedamu, 2016; Yashwant Kumar, Nandakumar, Handral, Talwar & Dhayabaran, 2010; Zhang, Feng, Chen, Li & Shen, 2016). The two doses of the plant extract were determined based on the result of the acute oral toxicity study.

2.7. Determination of plasma glucose levels

Blood samples (0.5 mL) were collected from tail veins of 12 h fasted mice, mixed with EDTA, and then centrifuged at 3000 r/min for 10 min. The level of glucose was determined by using GOD-PAP method (Glucose Liquicolor kit, Human, Germany). In brief, the glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxidase reacts under catalysis of peroxidase with phenol and 4-aminophenazone to a red-violet quinoneimine dye as indicator.

2.8. Experimental design

Mice were subjected by a single intraperitoneal injection of streptozotocin (170 mg/kg) after overnight fasting. Streptozotocin was freshly prepared with 0.1 mol/L sodium citrate (pH = 4.5) just prior to injection (Yamamoto et al., 2008). Seven days after streptozotocin injection, mice with plasma glucose levels higher than 126 mg/dL (Anjani et al., 2018; Sharma et al., 2010) were chosen and randomly divided into five different groups ($n = 6 - 12$) as follows: Group I: Physiological control (distilled water, p.o.); Group II: Pathological control (streptozotocin 170 mg/kg, i.p. + distilled water, p.o); Group III: Pathological 1st test (streptozotocin 170 mg/kg, i.p. + 343 mg/kg CILE, p.o.); Group IV: Pathological 1st test (streptozotocin 170 mg/kg, i.p. + 686 mg/kg CILE, p.o.); Group V: Positive control (streptozotocin 170 mg/kg, i.p. + glibenclamide STADA 5 mg/kg, p.o.).

All groups were administered orally from 8:00 a.m. to 9:00 a.m. for seven consecutive days. At the end of the experiment, mice were fasted overnight and plasma glucose levels were determined 1 h after the last administration. Then, the OGTT was performed as above. Finally, animals were sacrificed and liver, kidneys and pancreas were dissected and rinsed in saline buffer (0.9% NaCl).

2.8.1. Determination of malondialdehyde (MDA) and glutathione (GSH)

Lipid peroxidation was estimated by measuring MDA in the liver and kidney tissue homogenate and the activity of reduced GSH in the liver tissue homogenate. The liver or kidney tissue from each mouse was homogenized with 1.15% KCl buffer solution. The reaction mixture containing 1 – 2 mL of tissue homogenate and Tris–HCl (pH 7.4), just enough 3 mL, were incubated at 37 °C for 60 min. Then, the reaction was stopped by 1 mL of 10% trichloroacetic acid and centrifuged at 1500 r/min (10 min, 4 °C).

For determination of MDA, after centrifugation, 2 mL of the clear fluid (supernatant) was mixed with 1 mL of 0.8% thiobarbituric acid. The reaction mixture was incubated at 100 °C for 15 min and measure at $\lambda = 532$ nm.

For determination of GSH, after centrifugation, taking 1 mL of the clear fluid to react with 0.2 mL of Ellman's reagent and adding EDTA phosphate buffer to make into 3 mL. The reaction mixture was kept at room temperature for 3 min and measured at $\lambda = 412$ nm.

The content of MDA and GSH (nmol/L/g protein) was calculated by the linear regression equation of an MDA and GSH standard, respectively (Cheseman et al., 1986; Hissin & Hilf, 1976).

2.8.2. Histopathological examination

Pancreatic tissues were collected and fixed in 10% Neutral Buffered Formalin, processed routinely, and embedded in paraffin. The 3 μm thick sections were prepared and stained with hematoxylin and eosin (H & E) dye for microscopic investigation. The stained sections were examined and photographed under a light microscope (Olympus BX53F) (Matsuda et al., 2011).

2.9. Statistical analysis

All the measurements were done in triplicate and results are expressed in terms of mean ± standard error of the mean and IC_{50} values were calculated using MS Excel 2016 software. Data were analyzed by Systat statistical software (version 3.5, Inc. SigmaStat Executable) using *t*-test and One-way ANOVA following by Student-Newman-Keuls test. Differences were considered significant at $P < 0.05$.

Table 1
Content of total phenol and flavonoid in extract of *C. inerme* leaves.

Samples	Polyphenols (mg GAE/g d.w.)	Flavonoids (mg HIE/g d.w.)
Dry powder	23.995 ± 0.519	2.225 ± 0.093
Extract	120.458 ± 1.100[a]	4.494 ± 0.057[a]

[a] $P < 0.05$ represmts significantly different; mg GAE/g d.w.: mg of gallic acid equivalent/1 g of dry weight; mg HIE/g d.w.: mg of hispidulin equivalent/1 g of dry weight.

3. Results

3.1. Phytochemical analysis

The extract was subjected to qualitative chemical tests for the identification of various secondary metabolites present in the leaves of *C. inerme*. Our previous studies showed that the *C. inerme* leaves contained flavonoids, alkaloids, tannins, triterpenes and saponins, coumarins, anthraquinones, volatile oils, and reducing agents (Trie et al., 2018b).

3.2. Total phenolics and flavonoids content

The results of this study showed that total phenolic and flavonoid content of CILE were much higher than those reported in previous studies (Gurudeeban, Satyavani, Ramanathan, Umamaheswari & Shanmugapriya, 2010; Khan et al., 2013; Li et al., 2015) (Table 1).

3.3. In vitro *anti-oxidant activity*

The scavenging abilities of CILE were concentration-dependent at concentrations of 12.5, 25, 37.5, 50, 62.5 µg/mL presented percentage of DPPH scavenging by (39.66 ± 0.15)%, (48.58 ± 0.11)%, (60.92 ± 0.08)%, (71.07 ± 0.18)%, (89.52 ± 0.11)%, respectively. The IC_{50} values for DPPH radicals with CILE (25.28 µg/mL) was lower than previously reported (1.34 mg/mL) (Li et al., 2015) and higher than ascorbic acid (4.37 µ g/mL).

3.4. Effect of extract on normoglycemic mice in oral glucose tolerance test (OGTT)

As shown in Fig. 1A, CILE at doses of 343 or 686 mg/kg and glibenclamide at dose of 5 mg/kg after single-dose administration exerted a significant decrease on plasma glucose which reached 17.55%, 23.68% and 40.39% of hypoglycemia as compared to control at 30 min after glucose overload, respectively, indicating the modulating effect on OGTT. As shown in Fig. 1B, CILE at dose of 343 mg/kg after 7-day administration showed a decrease on plasma glucose (12.22%) which was significantly different as compared to control at 30 min after glucose overload. CILE at dose of 686 mg/kg and glibenclamide at dose of 5 mg/kg exerted a significant decrease on plasma glucose as compared to control at 30 min and 120 min after glucose overload, indicating the modulating effect on OGTT. There was also a significant reduction in glucose levels after 120 min compared to glucose levels after 30 min. The hypoglycemic effect of glibenclamide group was observed to be significantly higher compared with that of the extracts.

3.5. Effect of extract on streptozotocin-induced diabetic mice

The effects of different dosages of CILE and glibenclamide on fasted plasma glucose in diabetic mice were illustrated in Fig. 2. The result showed that plasma glucose values of streptozotocin-injected groups reached 59% of hyperglycemia which significantly increased as compared to physiological control (group I), indicating

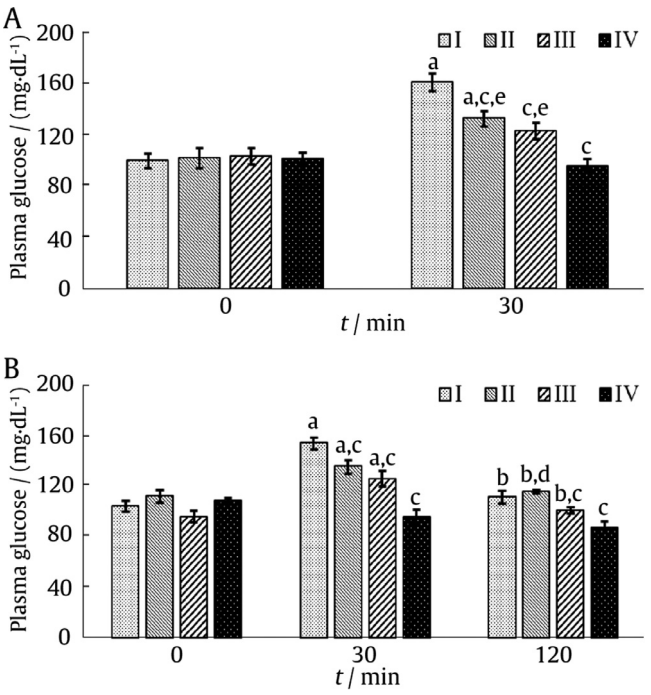

Fig. 1. Plasma glucose levels in normal mice after treatment with single dose (A) and repeated dose (B) of CILE for seven consecutive days. Group I: distilled water, p.o.; Group II: 343 mg/kg extract, p.o.; Group III: 686 mg/kg extract, p.o.; Group IV: 5 mg/kg glibenclamide, p.o. Values are expressed as mean ± SEM ($n = 6$ mice in each group). [a]$P < 0.05$ as compared with prior to tolerance. [b]$P < 0.05$ as compared with at 30 min after tolerance. [c]$P < 0.05$ as compared with group I. [d]$P < 0.05$ as compared with group III. [e]$P < 0.05$ as compared with group IV.

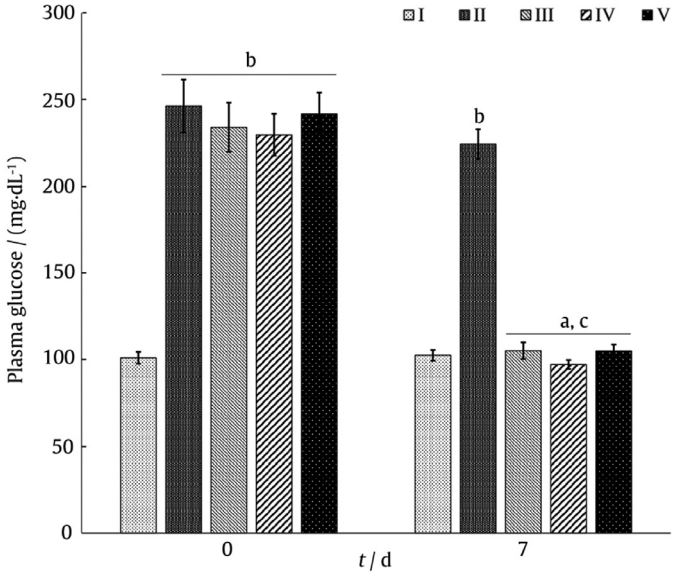

Fig. 2. Plasma glucose levels in normal and experimental mice after 7 d of treatment with CILE. Group I: distilled water, p.o.; Group II: STZ 170 mg/kg, i.p. + distilled water, p.o.; Group III: STZ 170 mg/kg, i.p. + 343 mg/kg extract, p.o.; Group IV: STZ 170 mg/kg, i.p. + 686 mg/kg extract, p.o.; Group V: STZ 170 mg/kg, i.p. + 5 mg/kg glibenclamide, p.o. Values were expressed as mean ± SEM ($n = 10–12$ mice in each group). [a]$P < 0.05$ as compared with prior to treatment. [b]$P < 0.05$ as compared with group I. [c]$P < 0.05$ as compared with group II.

diabetic condition in mice. CILE at doses of 343 and 686 mg/kg as well as glibenclamide at dose of 5 mg/kg after 7 d administration exerted a significant decrease on plasma glucose which reached 53.1%, 56.7%, and 53.2% of hypoglycemia as compared to diabetic control (group II), respectively. The CILE treatment group was not significantly different from the physiological control group. The plasma glucose levels of CILE treated group was returned to the normal range after 7 d treatment.

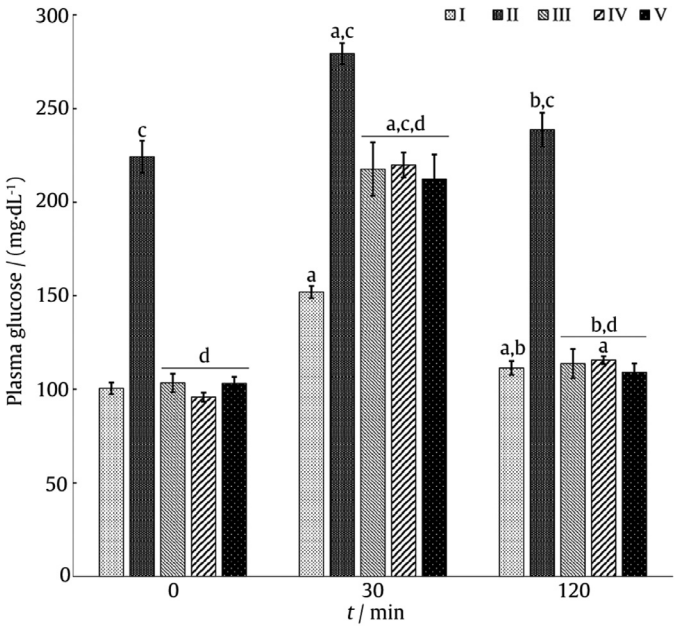

Fig. 3. Plasma glucose levels in normal and experimental mice after 7 d of treatment with CILE in the OGTT. Group I: distilled water, p.o.; Group II: STZ 170 mg/kg, i.p. + distilled water, p.o.; Group III: STZ 170 mg/kg, i.p. + 343 mg/kg extract, p.o.; Group IV: STZ 170 mg/kg, i.p. + 686 mg/kg extract, p.o.; Group V: STZ 170 mg/kg, i.p. + 5 mg/kg glibenclamide, p.o. Values were expressed as mean ± SEM ($n = 10$ mice in each group). [a]$P < 0.05$ as compared with prior to tolerance. [b]$P < 0.05$ as compared with at 30 min after tolerance. [c]$P < 0.05$ as compared with group I. [d]$P < 0.05$ as compared with group II.

The effects of CILE and glibenclamide in OGTT of diabetic mice were shown in Fig. 3. The plasma glucose values of streptozotocin-injected groups reached 55.2% (before OGTT), 45.6% (after 30 min), and 53.5% (after 120 min) of hyperglycemia which was significantly different as compared to physiological control (group I), indicating impaired glucose tolerance in diabetic mice. CILE at doses of 343 and 686 mg/kg taken in diabetic mice exerted a decrease on plasma glucose which was significantly different as compared to diabetic control (group II). The plasma glucose values in 343 and 683 mg/kg CILE group (group III and IV) reached 22.1% and 21.3% (after 30 min) to 52.39% and 51.6% (after 120 min) of hypoglycemia. There was no significant difference between groups III and IV. There was also a significant reduction in glucose levels after 120 min compared to glucose levels after 30 min. Glibenclamide at dose of 5 mg/kg taken in diabetic mice exerted a significant decrease on plasma glucose which reached 24% (after 30 min) to 51.6% (after 120 min) of hypoglycemia as compared to diabetic control.

As shown in Fig. 4, the diabetic control group (group II) showed an increase of MDA content (marker of lipid peroxidation damages of cell membrane) in the homogenates of liver or kidneys, reaching statistical significance compared with respective physiological controls (group I) (45.1% for liver and 23.8% for kidneys). The streptozotocin-induced diabetic mice treated with 5 mg/kg body weight of glibenclamide as well as 343 or 686 mg/kg b.w. of CILE showed a significant reduction in MDA content in liver or kidney homogenates compared to the diabetic controls.

In parallel, the diabetic control group also demonstrated a reduction of GSH content (endogenous anti-oxidant) in the homogenates of liver, reaching statistical significance compared with physiological controls (43.3%). The diabetic mice administered with CILE at dose 343 or 686 mg/kg b.w. as well as glibenclamide

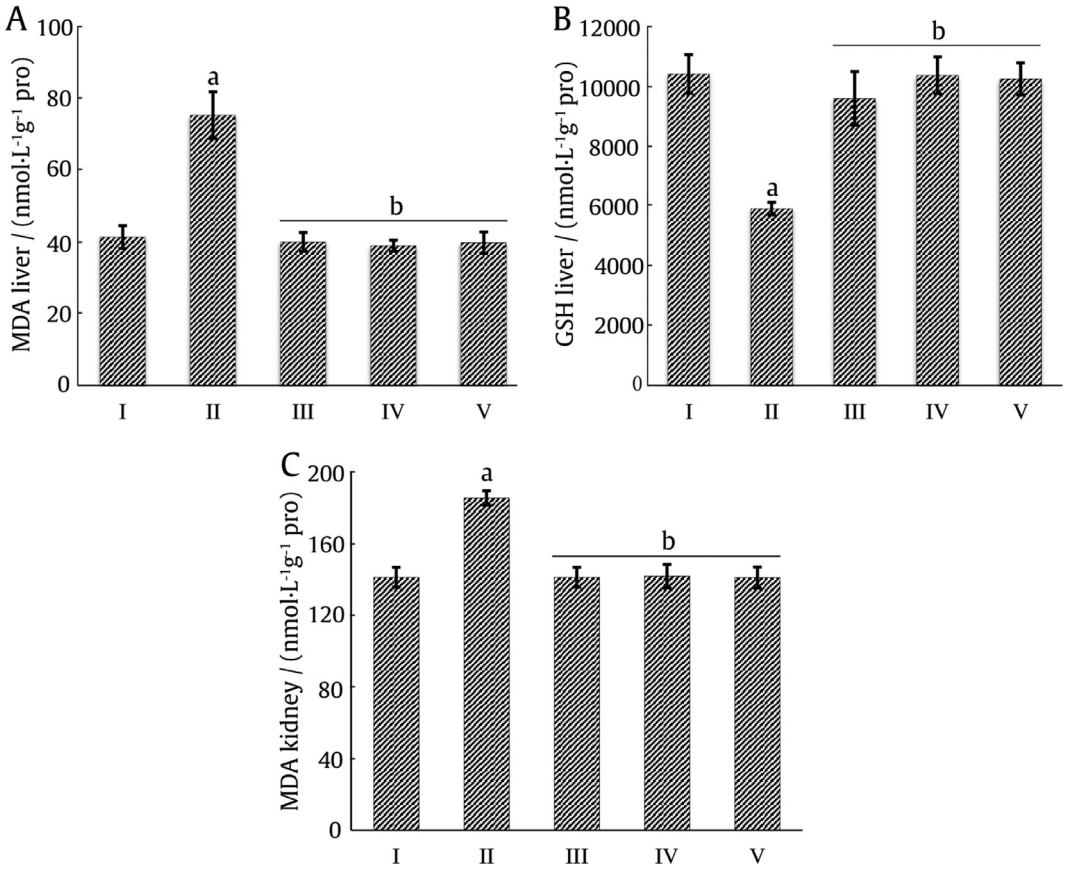

Fig. 4. Levels of MDA in liver (A), GSH in liver (B), and MDA in kidney (C) in normal and experimental mice after 7 d of treatment with CILE. Values were expressed as mean ± SEM ($n = 9 - 10$ mice in each group). [a]$P < 0.05$ *vs* group I. [b]$P < 0.05$ *vs* group II.

Table 2
Size and number of pancreatic islets in normal and experimental mice following CILE treatment for seven consecutive days.

Groups	Islet size /μm^2	Number of islets
I	31742.31 ± 10249.94	14.70 ± 1.89
II	2758.73 ± 499.95[a]	6.17 ± 1.11[a]
III	6753.67 ± 1691.50[b]	13.11 ± 1.60[b]
IV	7269.16 ± 2468.89[b]	10.38 ± 1.44[b]
V	5096.77 ± 1122.91[b]	10.14 ± 1.50[b]

Group I: distilled water, p.o.; Group II: STZ 170 mg/kg, i.p. + distilled water, p.o.; Group III: STZ 170 mg/kg, i.p. + 343 mg/kg extract, p.o.; Group IV: STZ 170 mg/kg, i.p. + 686 mg/kg extract, p.o.; Group V: STZ 170 mg/kg, i.p. + 5 mg/kg glibenclamide, p.o. Values are expressed as mean ± SEM ($n = 6 - 10$ mice in each group). [a]$P < 0.05$ *vs* group I. [b]$P < 0.05$ *vs* group II.

showed an increase of GSH levels in liver homogenates that were statistically significant difference compared with diabetic controls.

The content of MDA and GSH in CILE groups was not different as compared to physiological control indicating CILE restored MDA and GSH of liver or kidney levels to normal values.

As shown in Table 2 and Fig. 5, the diabetic control group (group II) showed a decrease of the size and number of pancreatic islets, reaching statistical significance compared with respective physiological controls (group I). After 7 d treatment, the pancreatic islets of diabetic mice were recovered. The size and number of pancreatic islets in the treatment groups with 343 or 686 mg/kg b.w. of CILE were increased, reaching statistical significance compared with diabetic control group and there were no statistically significant differences between the two groups. Glibenclamide at dose of 5 mg/kg taken in diabetic mice exerted a significant increase on size and number of pancreatic islets as compared to diabetic control and there was no significant difference compared with test groups (group III and IV).

4. Discussion

Streptozotocin (STZ), a toxic agent produced by Streptomyces achromogenes, is well known for its selective pancreatic islet β cell cytotoxicity and has been typically used to induce hyperglycemia in experimental mice model. Previous studies have demonstrated that STZ can induce apoptosis of cells and inhibit insulin biosynthesis via enhancing the over-production of ROS and causing oxidative damage. STZ causes tissues degeneration such as liver, kidneys, and pancreas (Szkudelski, 2001; Ren et al., 2015).

A large amount of studies indicated that natural products and plant extracts have played an important role in diabetes mellitus treatment (Sobeh et al., 2017a, 2017b; Youssef et al., 2017). Our study firstly demonstrated the potential hepatoprotective, renoprotective and pancreatic protective activities of CILE in STZ-induced diabetic mice. A significant increased level of MDA and decreased GSH level in STZ-induced diabetic mice kidneys or liver were found. These results illustrated the enhanced oxidative stress in these organs. Our studies demonstrated that CILE at doses of 343 and 686 mg/kg can protect the liver and kidneys from toxic agent through increasing the content of GSH in the liver, reducing the MDA content in the liver and kidneys. At the same time, the results of histopathological observations of CILE-treated pancreas in streptozotocin-induced diabetic mice also illustrated that treatment with CILE has the potential to recover pancreas through in-

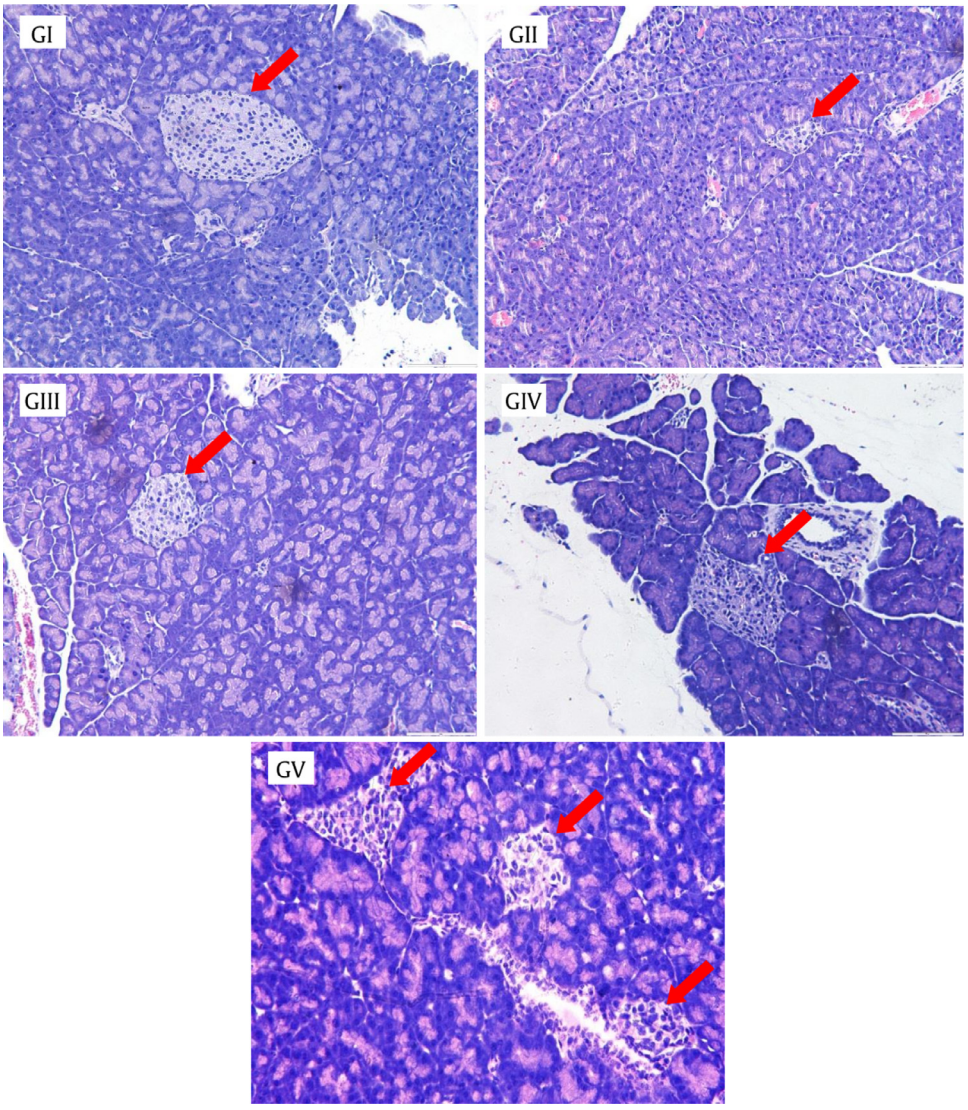

Fig. 5. Histopathological observations of CILE and glibenclamide treated pancreas in streptozotocin-induced diabetic mice (H&E staining, 200 × magnification). Arrows point to pancreatic islet.

creasing the size and number of pancreatic islets. The results of the *in vitro* anti-oxidant assay also showed that CILE had DPPH free radical scavenging activity with the IC_{50} value of 25.28 μg/mL, which was stronger than previous study (Li et al., 2015). According to these results, we demonstrated that the hypoglycemic effects of CILE may be due to their effect on palliation of oxidative stress.

The results showed that streptozotocin-injected mice reached 59% of hyperglycemia which significantly increased as compared to physiological control. It is suggested that CILE at the doses of 343 and 686 mg/kg have a marked hypoglycemic activity by improvement of the glucose tolerance test in normoglycemic mice and by lowering the blood glucose levels in STZ-induced diabetic mice after 7 d of treatment. In our study, we used dose of CILE which was equivalent to 1.25 g and 2.5 g dried medicinal materials powder with the doses of 343 and 686 mg/kg, respectively, but we found higher antidiabetic activity than previously reported study (at doses of 400 and 600 mg/kg). The present study detected the effect after 7 d of treatment, while previously it was 14 and 21 d (Bhushan et al., 2015). This study showed that CILE produced a marked decrease in blood glucose, protected liver, kidneys and recovered pancreas at the doses of 343 and 686 mg/kg in STZ-induced diabetic mice after 7 d of treatment. At the same time, our previous results of the *in vitro* α-amylase and α-glucosidase inhibition assay displayed that CILE had an inhibitory effect with lower IC_{50} value in comparison with standard drug acarbose (Trie et al., 2018a). The present study suggests that one of the mechanisms by which *C. inerme* leaves exhibited its hypoglycemic potential is through the inhibition of α-amylase and α-glucosidase.

The biological effects of CILE might be due to the presence of several phytochemicals in it. Our previous study results indicated that preliminary phytochemical screening of the extract of *C. inerme* leaves contains flavonoids, alkaloids, tannins, triterpenes and saponins, coumarins, anthraquinones, volatile oils, and reducing agents (Trie et al., 2018a). Previous reports suggested that potential antidiabetic and anti-oxidant compounds isolated from medicinal plants belong to flavonoid class (Soares et al., 2017; Testa et al., 2016; Vinayagam et al., 2015; Panche, Diwan & Chandra, 2016). Moreover, polyphenolics, alkaloids, saponins and tannins that were reported to have antidiabetic and anti-oxidant activities (Barky, Hussein, AlmEldeen, Hafez & Mohamed, 2017; Uzor & Osadebe, 2016 ; Umeno, Horie, Murotomi, Nakajima & Yoshida, 2016). Thus, the significant antidiabetic and anti-oxidant effects of CILE could be due to the possible presence of the phytochemical constituents. Some of the bioactive constituents could act syner-

gistically or independently to enhance the antidiabetic and anti-oxidant activities.

On the whole, the crude 45% ethanol extract of *C. inerme* leaves was found to display antidiabetic and anti-oxidant activities in STZ-induced diabetic mice, which having the scavenging effect of free radicals, inhibiting α-amylase and α-glucosidase, increasing glucose uptake, reducing plasma glucose levels, improving antioxidant systems, and regenerating pancreas. This indicated that *C. inerme* leaves have potential for use in the treatment of diabetes mellitus. However, further studies are necessary to illuminate the molecular mechanism by which *C. inerme* leaf extract exhibits its effects.

5. Conclusion

Ethanol extract of *C. inerme* leaves has antidiabetic and protective effects against oxidative stress-induced damage in liver and kidneys of diabetic mice.

Declaration of Competing Interest

All the authors of this manuscript declare that there is no conflict of interest.

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