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Antidiabetic effect of combined extract of *Coccinia grandis* and *Blumea balsamifera* on streptozotocin-nicotinamide induced diabetic rats

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ABSTRACT

Background: *Coccinia grandis* and *Blumea balsamifera* are two medicinal plants that have been known to have good antidiabetic properties. Combining these two plant extracts may generate a greater effect that can increase efficacy and decrease the dose.
Objective: This research investigated the antidiabetic activity of the combination of *C. grandis* and *B. balsamifera* leaves extracts on experimental diabetic rats.
Materials and methods: The dried leaves of *C. grandis* and *B. balsamifera* were powdered and macerated with ethanol 70% (v/v). A diabetic condition in male Wistar albino rats was generated by intraperitoneal injection of a single dose of streptozotocin (65 mg/kg) followed by nicotinamide (110 mg/kg). Diabetes-confirmed rats were then given glibenclamide (4.5 mg/kg), *C. grandis* extract (300 mg/kg), *B. balsamifera* extract (150 mg/kg), and the combined extracts with a dose ratio of 1:1, 1:3, and 3:1. The treatment was performed for 28 days and fasting blood glucose was tested once a week. The pancreas and liver organs were taken on day 29 for antioxidant, histological, and immunohistochemical assessment.
Results: Among all the extracts, the combined extract with a ratio of 1:3 showed the greatest glucose lowering effect. This combination also lowered malondialdehyde levels while increasing superoxide dismutase and catalase levels in the pancreas and liver organs. Histological examination showed this combination regenerated the islet of Langerhans. It also increased pancreatic insulin expression in immunohistochemical evaluation.
Conclusion: This study revealed that the combined extracts of *C. grandis* and *B. balsamifera* exhibited enhanced antidiabetic activity via ameliorating oxidative stress, regenerating β-cells, and increasing insulin expression.

1. Introduction

Diabetes mellitus (DM) is a devastating metabolic condition involving numerous consequences. Chronic elevation in blood glucose level becomes the primary cause of DM [1]. Type 2 diabetes mellitus (T2DM) represents the most frequent type of DM, accounting for more than 90% of all DM cases globally [2]. Even though DM is not infectious, this disease is one of the significant reasons for mortality, as well as creating additional medical issues such as high blood pressure, heart disease, stroke, and chronic renal disease [3]. In animal models, various diabetogenic agents are employed to generate T2DM, including streptozotocin (STZ), alloxan, a high-fat diet, a fructose diet, and combinations of these ingredients [4,5]. Among these diabetogenic agents, STZ is the most frequently used since it selectively damages pancreatic β-cells and causes insulin deficiency. In rats, the early phases of STZ-induced DM showed the elevation of oxidative stress as well as mitochondrial dysfunction, which is evidence that the development of DM was initiated by oxidative stress. Since STZ damages pancreatic β-cells in rats and nicotinamide (NA) only partially protects pancreatic β-cells from STZ, it has been postulated that administering STZ in combination with NA in rats will promote T2DM [6,7]. Given the close link between oxidative stress and cellular events related to T2DM onset, progression, and complications, reducing oxidative stress in animal models could be a strategy to treat T2DM [8].

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In general, the treatment of T2DM is done by administrating some conventional drugs, including sulfonylureas, α-glucosidase inhibitors, biguanides, thiazolidinediones, incretin mimetics, meglitinides, and DPP-4 inhibitors [9]. These medications need to be used with caution because of their limitations, such as decreased efficacy and undesirable effects over a longer term of medication [10]. Many herbs can be utilized as antidiabetic therapy, and medicinal plants are thought to be a great alternative. These herbs are known for their beneficial effects and lower side effects [11]. Due to their effectiveness and lack of toxicities compared to conventional medications, WHO advised evaluating traditional plant treatments used to treat DM [12]. According to estimates, 80% of people still primarily obtain their medical care from herbal remedies [13].

Coccinia grandis (L.) Voigt is a species of plant found in India, Indonesia, Malaysia, the Philippines, and Thailand that has historically been known to cure DM. The bioactive compounds such as β-sitosterol, stigmast-7-en-3-one, tritriacontane, cephalandrol cephalandrine a, cephalandrine b, lupeol, and taraxerol are all present in this plant [14]. According to Mohammed et al. [15], *C. grandis* leaves extract ameliorated DM condition in STZ-induced diabetic rats by restoring antioxidant enzymes and increasing the population of pancreatic islets. Giving *C. grandis* aqueous leaves extract at a dose of 750 mg/kg to diabetic rats induced by alloxan for 30 days lowered the blood glucose and repaired the pancreatic β-cells [16]. *Blumea balsamifera* is a plant that has excellent antidiabetic and antioxidant activity. In Southeast Asian nations, including Vietnam, Thailand, Malaysia, and the Philippines, this plant has been employed in traditional medicine for a long time. *B. balsamifera* contains polyphenolic compounds including xanthoxylin, eugenol and dimethoxydurene, and flavonoids, and dihydroflavones [17]. In streptozotocin-induced diabetic rats, the leaf extract of *B. balsamifera* reduced blood glucose levels, improved lipid profile, and enhanced antioxidant enzymes [18].

The combination of plant has been widely used to improve efficacy, reduce toxicity, lower dosage concentration, and decrease adverse effects [19]. Combining two extracts (or more) frequently results in a synergistic effect in their therapeutic potential. Synergism is the effect of a combination of drugs that seems to be higher than the total of the activities of a single drug. The implication is that the phytochemicals in the combination of plant extracts work on various target receptors in the disease pathogenesis, increasing overall treatment efficacy [20]. *C. grandis* and *B. balsamifera* are two plants that contain various phytochemicals so they have the potential to be antidiabetic agents. The phytochemical contents of these two plants are expected to offer a stronger effect than a single extract with complimentary mechanism of action. The preclinical study in order to investigate the synergism of the combined extracts of *C. grandis* and *B. balsamifera* in controlling T2DM was very limited. Indeed, there were no reports found related to this. In this paper, we report the findings about the synergistic effect of the combined extracts of *C. grandis* and *B. balsamifera* in ameliorating T2DM conditions. The possible mechanisms of action of combined extract related to T2DM were also investigated.

2. Materials and method

2.1. Plant materials and extracts preparation

C. grandis and *B. balsamifera* plants were obtained in two separate areas in Indonesia (Bali and East Java, respectively) and these two plant materials were determined by an expert Botanist in Bali Botanic Garden, Indonesian Institute of Science (Identification number: B-192/IPH.7/AP/VII/2020). The extraction processes of the plant leaves have previously been published [21]. The extracts were then stored at 4°C for further investigations.

2.2. Phytochemical screening of the extracts using TLC

The bioactive components contained in *C. grandis* extract (CGE) and *B. balsamifera* extract (BBE) were analyzed qualitatively using thin layer chromatography (TLC). The group of compounds tested in this study included phenolics, flavonoids, steroids, saponins, terpenoids, alkaloids, and tannins. The mobile phases and spraying reagents used in this experiment are described in Table 1. As the stationary phase, Supelco TLC silica gel 60 F254 (Merck KGaA, Darmstadt, Germany) was utilized.

2.3. Ethical approval

All animals experimental were carried out according to the protocol and guidelines of the Institutional Animal Ethics Committee and approved by The Commission of Ethical Clearance for Preclinical Research, LPPT Universitas Gadjah Mada, Certificate No. 00005/04/LPPT/III/2022.

2.4. Animals

40 male Wistar albino rats, weighing 200–250 g and aged 10–12 weeks were used for screening of antidiabetic activity. These rats were obtained from Animal Research House, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada. The animals were randomly split into eight groups (five rats per group) and acclimatized for one week. The animals were housed in cages (dimensions of 50 cm × 45 cm x 15 cm) and each cage was labeled with the group name. The animals were maintained in the animal housing facility at constant temperature (22 °C ± 2 °C) with relative humidity of 50–70%, and a 12 h light–dark cycle. Each cage accommodated four rats, and they were free access the standard pellets and water *ad libitum*.

2.5. Induction of diabetes

A single intraperitoneal injection of streptozotocin (Cayman Chemical, Michigan, USA) at a dose of 65 mg/kg followed by nicotinamide (Sigma, St. Louis, MO, USA) injection at a dose of 110 mg/kg was used to

Tabel 1
Rf values of the extracts and their spot/band colour

Groups	Mobile phase	Spraying reagent	Spot/band colour	Rf	
				CGE	BBE
Phenolics[a]	Methanol: ammonia (100:1.5)	$FeCl_3$	Grey[h]	0.59	0.59
Flavonoids[b]	Butanol: acetic acid: water (3:1:1)	$AlCl_3$	Yellow[i]	0.94	0.94
Terpenoids[c]	Toluene: ethyl acetate (93:7)	Vanillin-sulfuric acid	Pink[i]	0.45, 0.86	0.4, 0.44, 0.88
Steroids[d]	Hexane: ethyl acetate (70:30)	Lieberman Bucard	Yellow grey[i]	0.51, 0.67	0.53, 0.67
Saponins[e]	Chloroform: methanol (95:5)	Anisaldehid-sulfuric acid	Violet blue[i]	0.62	0.62
Alkaloids[f]	Chloroform: methanol: ammonia (80:20:1)	Dragendorff	ND[i]	ND	ND
Tannins[g]	Ethyl acetate: formic acid: acetic acid: water (100:5:5:13)	$FeCl_3$	ND[i]	ND	ND

Standards used: [a]gallic acid (Rf = 0.85, grey), [b]quercetin (Rf = 0.94, yellow), [c]terpineol (Rf = 0.41, pink), [d]β-sitosterol (Rf = 0.49, yellow grey), [e]β-sitosterol (Rf = 0.41, violet blue), [f]quinine (Rf = 0.72, orange), [g]tannic acid (Rf = 0.47, blue grey), observed at [h]UV 254 and [i]visible light; CGE: *Coccinia grandis* extract; BBE: *Blumea balsamifera* extract; ND: Not detected.

induce T2DM in rats that had fasted for 12 h [22]. On day 7, fasting blood glucose (FBG) was measured using the Glucose GOD FS Kit (DiaSys Diagnostic, Germany). Rats with FBG levels more than 250 mg/dl were deemed to have DM and eligible for use in future research.

2.6. Experimental design

A total of 35 diabetic rats were split into one diabetes control group and six treatment groups. Also, five non-diabetic rats were set as a normal control group. The diabetes induction procedure using STZ and NA in this study has strictly followed previous research. However, during the research, many rats died due to the toxic effects of STZ. At the end of the experiment, the number of rats remaining was only four per group. For the NC group, one rat appeared weak and did not show any increase in body weight, therefore we sacrificed it. Fig. 1 illustrates the experimental design and the treatment schedule of various groups. As seen in Fig. 1, the groups are: NC (normal control) group (regular food and drinking water *ad libitum*), DC (diabetic control) group (2 ml of Na-CMC 5%, per oral), GLI group (4.5 mg/kg of glibenclamide, per oral), CGE 300 group (300 mg/kg of CGE, per oral), BBE 150 group (150 mg/kg of BBE, per oral), and three groups treated with extract combination with dosage ratio of 1:1, 1:3, and 3:1. All treatments were given via oral gavage once a day for 28 days. All rats were given commercial food pellets (Comfeed BR-1) and drinking water *ad libitum* during treatment. The FBG and body weight of rats were assessed every week prior to the administration of the extracts. All rats were sacrificed on day 29, and their pancreas and liver organs were taken for antioxidant, histological, and immunohistochemical investigations.

2.7. Determination of pancreatic and liver antioxidants

The frozen pancreas and liver tissues (previously stored at −80°C), were immersed in cold phosphate-buffered saline (PBS) solution to remove excess blood and weighed before homogenizing. The tissue was chopped into small pieces and homogenized in lysis buffer (250 µL lysis buffer for 100 µg tissue). The suspension formed was then sonicated with an ultrasonic cell disrupter until the solution was clear. The resulting homogenate was then centrifuged at 10.000×*g* for 5 min until separated into supernatant and residue. For the antioxidant assessment, the supernatant was stored at −20°C. The levels of malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) were determined using the ELISA kit, and the procedures were based on the protocols described in the kit manual. The assay kits for the MDA and CAT were obtained from ABclonal Technology (Cummings Park Ste. 6500, Woburn, MA 01801, USA), and the SOD was purchased from Abbkine (Optics Valley International Biomedicine Park, Wuhan, China).

2.8. Histological analysis of the pancreas

On day 29, the pancreas organs were removed from rats and washed using PBS. The organs were then fixed overnight in a neutral 10% buffered formalin solution (Merck KGaA, Darmstadt, Germany), dehydrated via a graduated succession of alcohol, cleared in xylol, and infiltrated with liquid paraffin. Furthermore, the organs were embedded in a paraffin block and sliced into thin sections (5 µm). Staining was done with hematoxylin and eosin (H&E). Histopathological alterations were investigated using a CX43 Biological Microscope (Olympus Corporation, Tokyo, Japan), with images acquired at a magnification of 400×.

2.9. Immunohistochemistry (IHC) analysis

The paraffin blocks of pancreas tissue were subjected to incubation, deparaffinization, and rehydration processes. The antigen retrieval process is carried out using a decloaking chamber device. The antibody used to detect the insulin was the mouse anti-insulin clone HB125 (BioGenex, Fremont, CA 94538, USA). Following primary and secondary antibody incubation, the slides were treated with diaminobenzidine (DAB) till brown and counterstained with hematoxylin (Mayer's). The tissues were observed under the same microscope that was used for the histological examination at a magnification of 400× and 1080p RGB resolution. Insulin expression in pancreatic islets was determined using a modified histologic score (H-score) from Cass et al. [23]. In this particular instance, the H-scores of insulin expression were calculated by an expert pathologist by multiplying the percentage of positive β-cells with the staining intensity (0–3).

2.10. Statistical analysis

The findings of this study were presented as mean ± standard error of the mean (SEM). All the data were statistically evaluated using one-way ANOVA and paired *t*-test. Statistical significance between treatment groups was calculated by Tukey post hoc test at a significant level of $p < 0.05$. The GraphPad Prism version-8 was used for all statistical analyses (GraphPad Software, Inc., San Diego, California, USA).

3. Results

3.1. Phytochemical profile of the extracts

The results of the TLC analysis of CGE and BBE showed the appearance of phenolics, flavonoids, terpenoids, steroids, and saponins (Table 1, Supplementary Fig. 1). Meanwhile, the presence of alkaloids

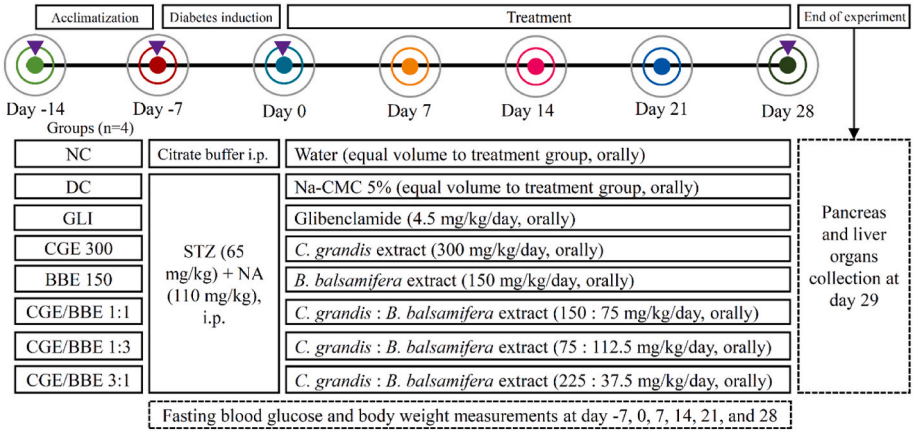

Fig. 1. Group treatment and experimental design. NC: normal control, DC: diabetic control, GLI: glibenclamide, CGE: *Coccinia grandis* extract, BBE: *Blumea balsamifera* extract, STZ: streptozotocin, NA: nicotinamide, Na-CMC: sodium-carboxymethyl cellulose, i. p.: intraperitoneal

and tannins was not visible on the TLC plate even after being sprayed with a spraying reagent (Dragendorff and $FeCl_3$, respectively). Quantitatively, total phenolics and total flavonoids of CGE and BBE have been determined and have been published previously [21]. From the phytochemical analysis we found that the total phenolics and total flavonoid content of BBE were much greater than that of CGE.

3.2. Effect of treatment on FBG

Determination of FBG levels was carried out based on the glucose oxidase-peroxidase aminoantipyrine (GOD-PAP) method. Table 2 outlines the effect of treatment on FBG of STZ-NA-induced diabetic rats for 28 days. After STZ induction (day 0), the FBG level of the DC group significantly raised in comparison to the NC group ($p < 0.05$), and this value raised significantly ($p < 0.05$) at the end of the experiment period. Diabetic rats treated with 4.5 mg/kg of glibenclamide (GLI group), 300 mg/kg of CGE (CGE 300 group), and 150 mg/kg of BBE (BBE 150 group) depicted a significant reduction in FBG levels in comparison to the DC group ($p < 0.05$). FBG levels in CGE/BBE 1:1, CGE/BBE 1:3, CGE/BBE 1:1 group were also decreased significantly when compared to the DC group ($p < 0.05$). However, the effect produced by the extract combinations was not significantly different in comparison to the single extract ($p > 0.05$). All treatments caused a significant decrease in FBG levels at day 28 in comparison to day 0 ($p < 0.05$). Fig. 2 depicts the percentage decrease in FBG of treated diabetic rats at the end of the experiment period. It was discovered that all treatments demonstrated a significant difference when compared to the DC group ($p < 0.05$). The CGE/BBE 1:3 group showed the strongest hypoglycemic effect with a percentage decrease of 72.79 ± 5.66%, followed by the GLI, CGE, BBE, CGE/BBE 3:1, and CGE/BBE 1:1 with a percentage decrease of 70.78 ± 2.71%, 58.83 ± 5.42%, 57.37 ± 5.97%, 55.52 ± 7.12%, and 44.60 ± 3.25%, respectively. However, the percentage decrease in FBG level of CGE/BBE 1:3 group was not significantly different compared to the other treatment groups ($p > 0.05$).

3.3. Effect of treatment on body weight

Table 2 shows the body weight values of rats before and after STZ-NA induction. The induction of rats using STZ and NA led to drastic weight loss. There were no differences in body weight between groups that appeared prior to treatment (day 0), as reported in Table 2. On day 28, the body weight of the DC group was seen to decrease significantly when compared to day 0 ($p < 0.05$). Furthermore, the body weight of the rats in the GLI, CGE, BBE, CGE/BBE 1:1, CGE/BBE 1:3, and CGE/BBE 3:1 increased after 28 days of treatment. However, only the GLI group differed significantly from the DC group ($p < 0.05$).

Table 2
Effect of treatment on fasting blood glucose and body weight of STZ-NA-induced diabetic rats for 28 days

Group (n = 4)	Fasting blood glucose (mg/dl)		Body weight (g)	
	Day 0 (initial)	Day 28 (final)	Day 0 (initial)	Day 28 (final)
NC	92.3 ± 3.1^{a}	89.9 ± 8.2^{a}	188.13 ± 0.70	223.58 ± 6.02$^{bc, \#}$
DC	379.4 ± 14.8^{b}	480.3 ± 27.9$^{d, \#}$	179.49 ± 3.08	159.21 ± 1.97^{a}
GLI	410.4 ± 37.6^{b}	118.7 ± 13.1$^{a, \#}$	174.90 ± 4.79	226.70 ± 8.61$^{c, \#}$
CGE 300	496.7 ± 27.7^{b}	208.8 ± 37.6$^{ab, \#}$	163.00 ± 12.85	188.78 ± 23.93^{abc}
BBE 150	450.1 ± 38.6^{b}	189.9 ± 25.4$^{ab, \#}$	168.88 ± 9.24	182.75 ± 9.34^{abc}
CGE/BBE 1:1	487.5 ± 30.1^{b}	268.8 ± 17.8$^{c, \#}$	156.54 ± 2.12	175.28 ± 5.68^{ab}
CGE/BBE 1:3	474.9 ± 40.0^{b}	129.9 ± 29.8$^{a, \#}$	157.32 ± 11.46	193.69 ± 6.07^{abc}
CGE/BBE 3:1	450.2 ± 71.0^{b}	194.0 ± 36.9$^{ab, \#}$	184.93 ± 12.88	200.80 ± 8.47^{abc}

All data were presented as mean ± standard error of the mean (SEM), n = 4. Different letters (a, ab, abc, bc, and c) indicate significant differences based on the ANOVA followed by the Tukey post hoc test ($p < 0.05$). $^{\#}p < 0.05$ vs initial based on paired *t*-test. NC: normal control; DC: diabetic control; GLI: glibenclamide; CGE: *C. grandis* extract; and BBE: *B. balsamifera* extract.

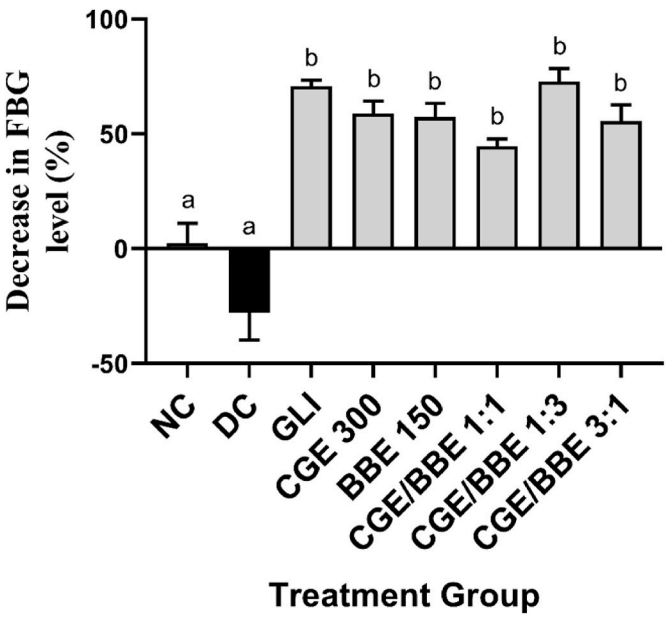

Fig. 2. Percentage decrease in FBG levels of STZ-NA-induced diabetic rats after treatment for 28 days. All data were presented as mean ± standard error of the mean (SEM), n = 4. Different letters (a and b) indicate significant differences based on the ANOVA followed by the Tukey post hoc test ($p < 0.05$). FBG: fasting blood glucose; NC: normal control; DC: diabetic control; GLI: glibenclamide; CGE: *C. grandis* extract; and BBE: *B. balsamifera* extract.

3.4. Effect of treatment on pancreatic and liver antioxidants

Determination of MDA, SOD, and CAT levels in the pancreas and liver of experimental rats was performed using an ELISA kit. Based on the data in Figs. 3A and 4A, the DC groups depicted a significant increase in MDA levels in comparison to the NC groups ($p < 0.05$). Treatment of diabetic rats with glibenclamide, CGE 300, BBE 150, CGE/BBE 1:1, CGE/BBE 1:3, and CGE/BBE 3:1 lowered MDA levels in diabetic rats ($p < 0.05$). Among the three combination groups, the CGE/BBE 1:3 group seemed to have the lowest MDA level. The levels of SOD and CAT of DC group decreased significantly in the pancreas (Fig. 3B and C, respectively) and liver (Fig. 4B and C, respectively) when compared to the NC ($p < 0.05$), which proved the development of severe oxidative stress status. Treatment of glibenclamide (GLI group) for 28 days raised the levels of antioxidant enzymes in both organs in comparison to the DC group ($p < 0.05$). The level of pancreatic SOD was significantly increased as a result of CGE 300 ($p < 0.05$) and BBE 150 ($p \leq 0.01$) administration to diabetic rats for 28 days. The same result was also observed in liver SOD which indicated the potent antioxidant activity of CGE and BBE. A significant increase in CAT levels in both organs was demonstrated by the CGE 300 ($p < 0.05$) and BBE 150 ($p < 0.05$) groups in comparison to the DC group. Among the combination groups, the CGE/BBE 1:3 group showed the most potent antioxidant activity. This was demonstrated by a considerable reduction in MDA level and a significant elevation in antioxidant enzymes level ($p < 0.05$) in both pancreas and liver of CGE/BBE 1:3 group in comparison to the DC group. In the pancreas, the MDA level of the CGE/BBE 1:3 group was lower than that of the CGE 300 group ($p < 0.05$). Still in the pancreas, the CGE/BBE 1:3 group had greater SOD levels compared to the CGE 300 group ($p <$

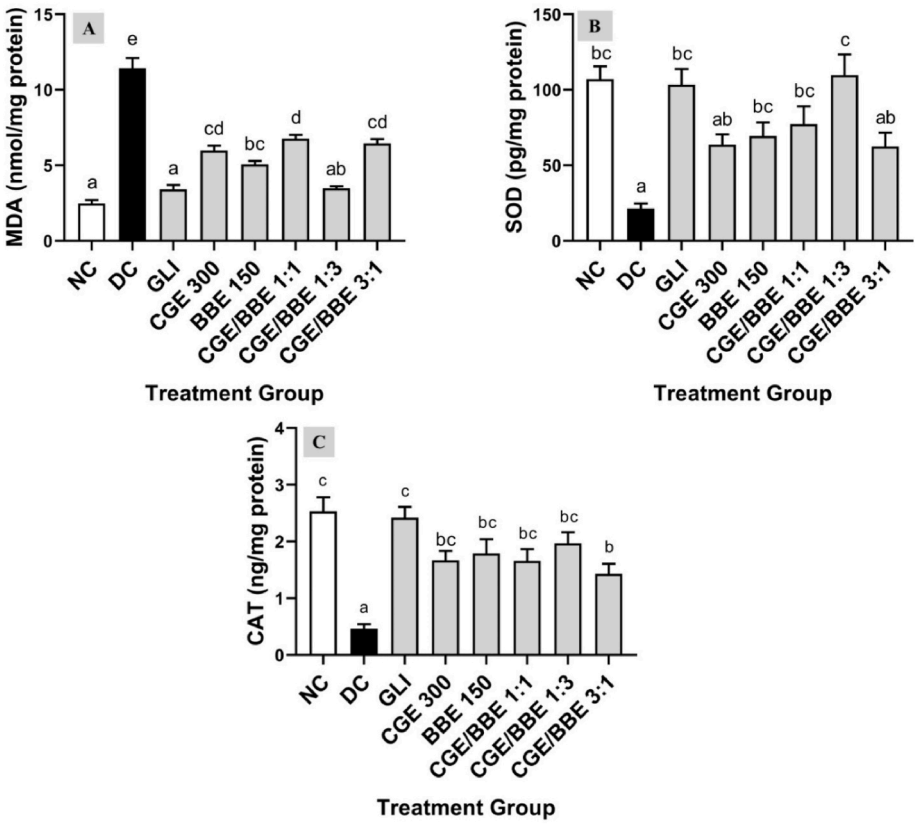

Fig. 3. Effect of the treatment on the levels of pancreatic MDA (A), SOD (B), and CAT (C) of STZ-NA-induced diabetic rats. All data were presented as mean ± standard error of the mean (SEM), n = 4. Different letters (a-e, ab, bc, and cd) indicate significant differences based on the ANOVA followed by the Tukey post hoc test ($p < 0.05$). MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; NC: normal control; DC: diabetic control; GLI: glibenclamide; CGE: *C. grandis* extract; and BBE: *B. balsamifera* extract.

0.05). In the liver, the CAT level of the CGE/BBE 1:3 group was found to be lower than that of the CGE 300 group ($p < 0.05$).

3.5. Effect of treatment on a histological view of the pancreas

Fig. 5 shows the pancreatic histology of the experimental rats. In the NC group (Fig. 5A), the islet displayed a regular structure with a huge core structure composed of β-cells with granulated cytoplasm. Induction of STZ caused the severe injury of the pancreas of DC group, which can be observed by the huge reduction of islet dimension and β-cells population (Fig. 5B). Cytoplasmic degeneration of β-cells has also occurred. The administration of CGE 300 and BBE 150 (Fig. 5D and E, respectively) resulted in moderate improvement of islets dimension and β-cells number. The combination of CGE and BBE also expanded the dimension of the islet and restored the number of islet cells. Among the three combinations (Fig. 5F–H), CGE/BBE 1:3 showed the best effect in regenerating β-cells of the diabetic pancreas (Fig. 5G). The morphological alteration caused by CGE/BBE 1:3. As a positive control, glibenclamide (GLI group) also showed an effect similar to the effect shown by the extract combination, indicating the β-cell regeneration effect of glibenclamide (Fig. 5C).

3.6. Effect of treatment on pancreatic insulin expression

Insulin is produced by β-cells in the islet of Langerhans and is found in the cytoplasm. It was found to have a positive reactivity to immunohistochemical staining, as evidenced by the brown colour. Fig. 6 shows the insulin immunochemical stained islets of experimental rats. A semi-quantitative calculation on immunohistochemical staining (H-scores) was performed in order to reveal the pancreatic insulin expression (Table 3). In the NC group, insulin was normally distributed in all β-cells of the islet (Fig. 6A) with H-score of 270. A markedly decrease in insulin expression (H-score of 10) was observed in the DC group, indicating severe injury to β-cells caused by STZ administration (Fig. 6B). Glibenclamide strongly ameliorated the insulin expression of diabetic rats (Fig. 6C) with H-score of 210. The decrease in insulin expression was also slightly ameliorated by CGE 300 and BBE 150 (Fig. 6D and E, respectively). Surprisingly, more prominent increases in insulin expression were shown by the combinations of CGE and BBE in various ratios (Fig. 6F–H). Among the three combinations, the CGE/BBE 1:3 showed the best improvement in insulin expression (H-score of 210), indicating the synergistic effect (Fig. 6G). Based on the H-score value, the effect shown by CGE/BBE 1:3 is similar to the effect shown by GLI.

4. Discussion

In the phytochemical assessment of plant extracts, the characterization of the active ingredients plays a crucial role and is essential in determining how they might affect the body. This can be performed using thin-layer chromatography (TLC). This approach is quick and efficient, combining sensitivity and simplicity at a minimal cost. In this research, the existence of phenolics, flavonoids, terpenoids, steroids, and saponins in CGE and BBE is indicated by the appearance of colored spots/bands on certain Rf values (Table 1). However, alkaloids and tannins were not detected on the TLC plate, which may be due to its very low concentration in CGE and BBE.

In this study, diabetes induction in Wistar rats was conducted by injection of STZ and NA at a dose of 65 mg/kg and 110 mg/kg, respectively, successfully performed (Table 2). This was evidenced by the FBG levels of all treatment groups being higher than 250 mg/dl at

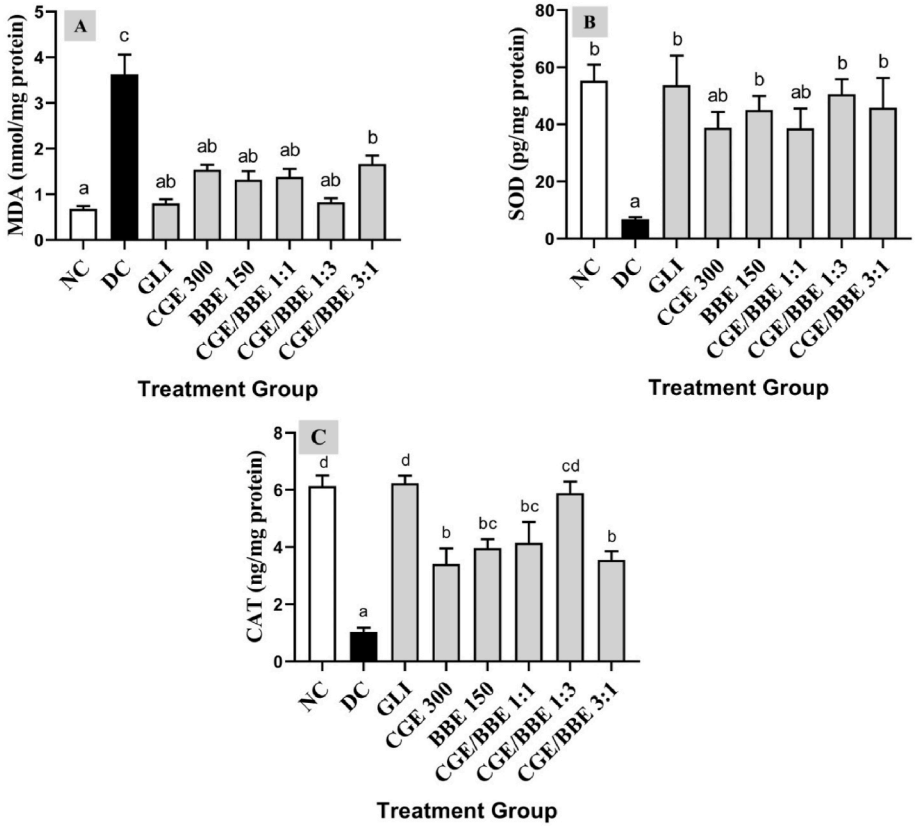

Fig. 4. Effect of the treatment on the levels of liver MDA (A), SOD (B), and CAT (C) of STZ-NA-induced diabetic rats. All data were presented as mean ± standard error of the mean (SEM), n = 4. Different letters (a-d, ab, bc, and cd) indicate significant differences based on the ANOVA followed by the Tukey post hoc test ($p < 0.05$). MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; NC: normal control; DC: diabetic control; GLI: glibenclamide; CGE: *C. grandis* extract; and BBE: *B. balsamifera* extract.

the initial treatment period (7th day after induction). In the course of the treatment period (28 days), the FBG level of the DC group remained high and increased significantly ($p < 0.05$). This indicates that STZ exerts a deleterious effect on pancreatic β-cells, and this effect persisted until the end of the experiment. STZ can cause hyperglycemia by selectively destroying pancreatic β-cells. Increasing the STZ dose results in increased cytotoxicity and greater pancreatic β-cell damage [24]. Conversely, NA is an antioxidant that can protect pancreatic β-cells from the cytotoxic effects of STZ. Since STZ destroys pancreatic β-cells and NA in rats only partially protects pancreatic β-cells from STZ, it has been established that administration of STZ in combination with nicotinamide in rats leads to the development of T2DM [6,7]. The administration of glibenclamide (4.5 mg/kg) orally per day for 28 days significantly reduced the FBG levels of the GLI group in comparison to the DC group ($p < 0.05$). As a second-generation antidiabetic drug of sulfonylurea, glibenclamide lowers blood sugar levels in T2DM patients [25]. The blood sugar-lowering effect of glibenclamide is produced by restricting the ATP-sensitive K^+ channels of pancreatic β-cells. As a result, intracellular calcium level rises and further stimulates insulin release from β-cells and increases glucose uptake by adipocytes and skeletal muscle cells [26]. This finding is in agreement with the results reported by Krishnasamy et al. [27] and Sayeli and Shenoy [28]. In their study, glibenclamide was reported to significantly reduce FBG levels of STZ-NA-induced diabetic rats in comparison to negative controls ($p < 0.05$).

The diabetic rats treated with CGE and BBE for 28 days reduced FBG significantly compared to DC ($p < 0.05$). The FBG-lowering effect of CGE and BBE in this study is related to its bioactive content. Phytochemical profiles of CGE and BBE depicted the existence of phenolics, flavonoids, steroids, saponins, and terpenoids. Among these groups of compounds, phenolics and flavonoids are the most frequently studied because of their potent hypoglycemic effect [29]. The result of FBG lowering effect of CGE in this study is supported by Mohammed et al. [15]. In their study, the 21 days treatment of CGE (500 mg/kg) significantly reduced FBG levels of STZ-NA-induced diabetic rats when compared to the negative control ($p < 0.05$). These findings were probably caused by a combination of factors such as: (1) reduced intestinal absorption, (2) increased glucose uptake by peripheral tissues, (3) increased glycolytic regulation, (4) increased glycogenic processes, and (5) increased insulin secretion from residual or recovered pancreatic β-cells. Among the extract combination treatment groups, it was seen that the CGE/BBE 1:3 group showed the strongest hypoglycemic effect with a decrease in FBG of 72.79 ± 5.66%, which indicated that the combination of CGE and BBE reduced FBG levels of diabetic rats synergistically.

The FBG lowering effect of CGE/BBE 1:3 is similar to glibenclamide which indicates CGE/BBE 1:3 works by improving insulin secretion. The combination of CGE and BBE improved insulin secretion after the damage of pancreatic β-cells due to the toxic effect of STZ. This could be possible considering that the two extracts showed a synergistic antioxidant effect ($p < 0.05$) when they were combined with a low concentration ratio [21]. Antioxidants are essential in the etiology of DM as they can reduce oxidative stress as a consequence of hyperglycemic conditions. Interactions between herbal extracts can be in the form of pharmacokinetic or pharmacodynamic interactions. Pharmacodynamic interactions occur when herbal extract produces synergistic or antagonistic effects when combined with other drugs or herbs [30]. The synergistic effect is produced by various phytochemicals in the extracts working on the same target or different targets. Some phytochemicals also regulate the activity of transporters and enzymes related to metabolism so that the bioavailability of herbal medicines increases. In

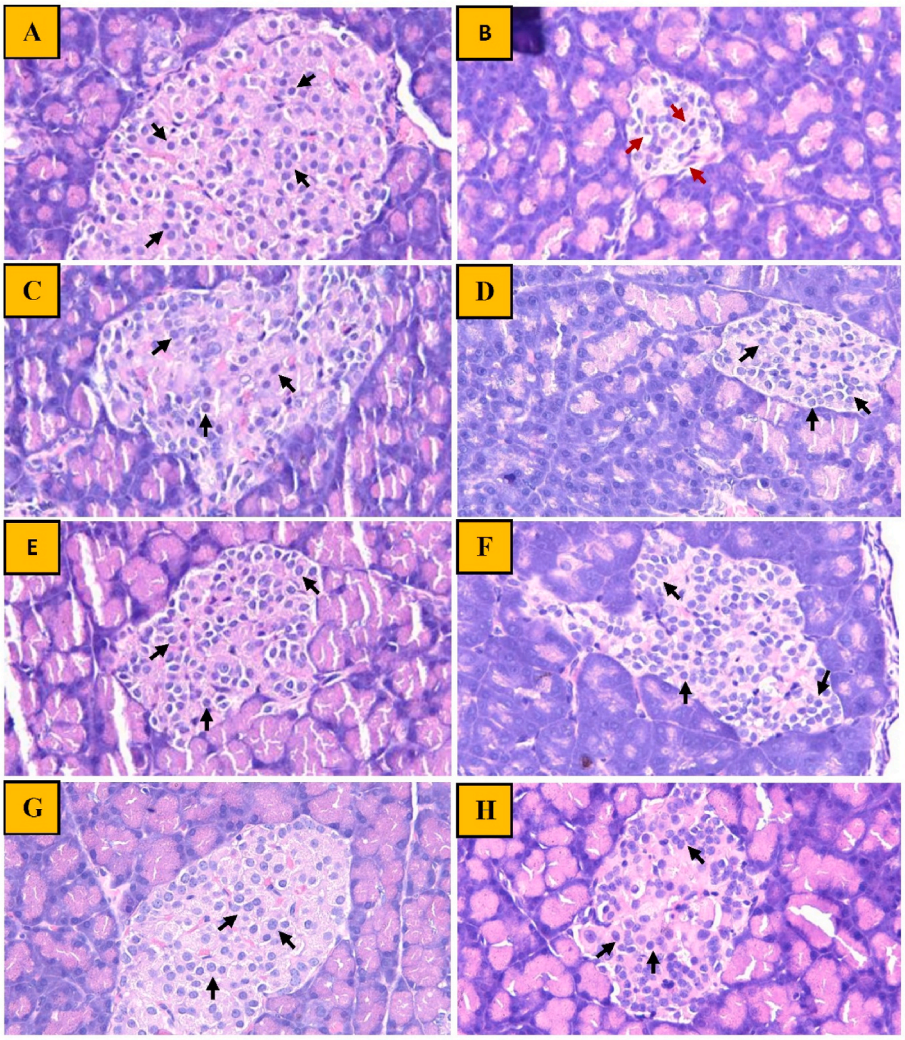

Fig. 5. Experimental rat pancreatic histology of NC (A), DC (B), GLI (C), CG 300 (D), BB 150 (E), CG/BB 1:1 (F), CG/BB 1:3 (G), and CG/BB 3:1 (H) groups. Red arrows represent cytoplasmic degeneration, while black arrows represent cytoplasmic restoration. All sections were stained with hematoxylin and eosin. The images were recorded under a magnification of 400×.

addition, the phytochemicals of one extract extend the pharmacological activity of the other extracts in the combination [31]. These findings are supported by Perumal et al. [32], who showed that the combination of *Taraxacum officinale* and *Momordica charantia* extracts at a dose of 250 mg/kg had a glucose-lowering impact in STZ-NA-induced diabetic rats. Meanwhile, the combination of extracts at doses of 62.5 and 1000 mg/kg had no glucose-lowering effect and tended to have antagonistic interactions.

According to studies, diabetic animals that experience hyperglycemia also tends to lose weight. STZ-induced diabetic rats appear sick and lose weight due to the effect of STZ, which causes DNA alkylation and produces hyperglycemia and necrotic lesions [33]. Some researchers have shown that intraperitoneal injection of STZ and NA in rats caused drastic weight loss [28,34]. In this research, diabetic induction with STZ and NA led to drastic weight loss in rats. This weight loss may be caused by impaired glucose use due to damage β-cells damage [35]. Weight loss is one of the main signs of DM, but the mechanism is not fully understood. This may be related to loss of appetite, decreased muscle mass, and loss of tissue protein [36]. All extract treatments, including CGE 300, BBE 150, CGE/BBE 1:1, CGE/BBE 1:3, and CGE/BBE 3:1 raised the diabetic-rats body weight but not remarkably against the DC group ($p > 0.05$). This result is probably caused by the β-cell damage caused by STZ, which has not been fully recovered by the extract treatment, so glucose metabolism is still disturbed. In a previous study, it was shown that the ethanol extract of *C. grandis* leaves with various doses (50, 250, and 500 mg/kg) raised the body weight of STZ-induced diabetic rats in a dose-dependent manner [15]. Among the treatment groups, only the GLI group differed significantly in comparison to the DC group ($p < 0.05$). This indicates that glibenclamide is able to improve impaired glucose use in diabetic rats, resulting in a substantial rise in body weight. Sayeli and Shenoy [28] reported a 23% increase in body weight of STZ-NA-induced diabetic rats due to administration of glibenclamide. This is related to the activity of glibenclamide to stimulate the release of insulin and the accompanying anabolic effects. Unlike glibenclamide, the increase in body weight caused by the extracts was not significantly different when compared to the DC group ($p > 0.05$). This indicated that single extracts and its combinations might be used in DM therapy to reduce blood glucose without significantly increasing body weight.

The early phase of DM in STZ-induced rats exhibits increased oxidative stress as well as mitochondrial dysfunction, which is evidence that the development of DM is significantly influenced by oxidative stress [6]. Hyperglycemia, in fact, causes oxidative stress by increasing the generation of ROS, which alters glucose metabolism [37,38]. In general, the body has a self-defense system that protects itself from ROS. This system is known as an anti-oxidative system, which can be enzymatic (SOD, CAT, and GPx) and non-enzymatic (GSH). However, under

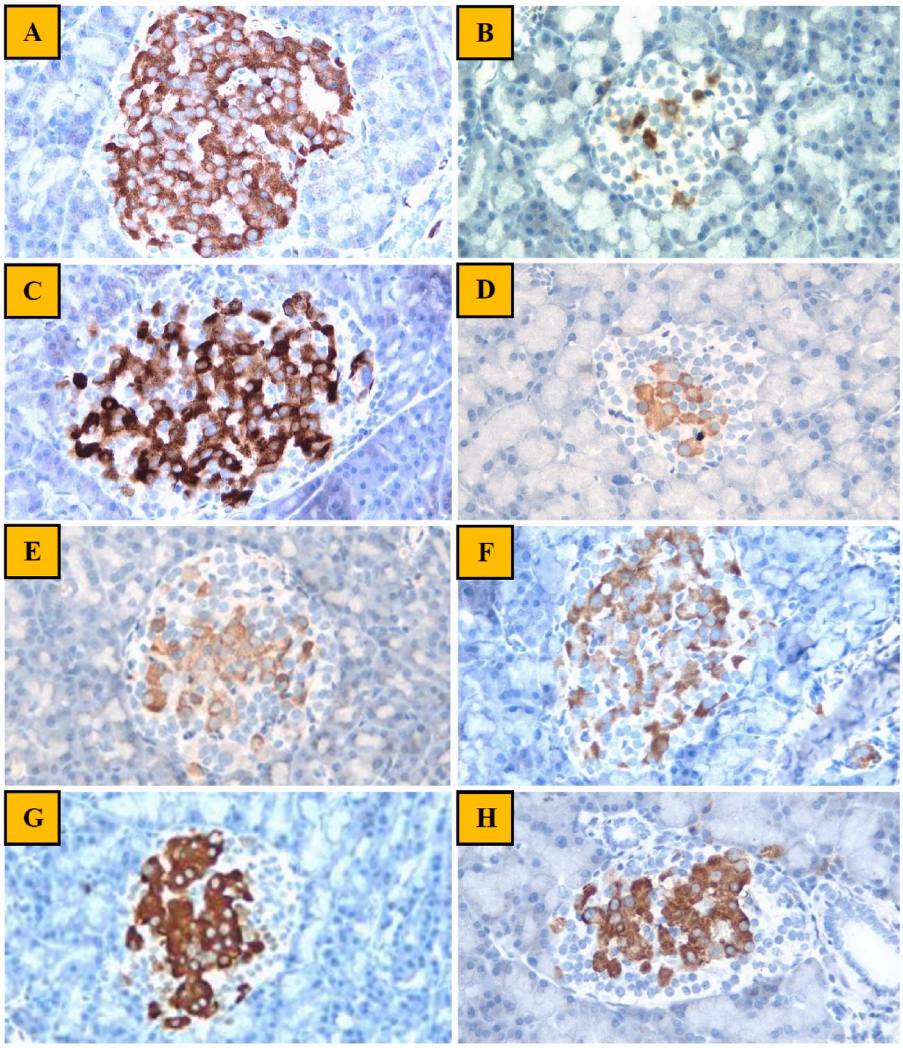

Fig. 6. Immunohistochemical staining of insulin in pancreatic islet of NC (A), DC (B), GLI (C), CG 300 (D), BB 150 (E), CG/BB 1:1 (F), CG/BB 1:3 (G), and CG/BB 3:1 (H) groups. The images were recorded under a magnification of 400×.

Table 3
H-scores of immunohistochemical staining of insulin in pancreatic islet of experimental groups

Groups	Intensity of staining	Percentage of positive cells (%)	H-Score
NC	3	90	270
DC	2	5	10
GLI	3	70	210
CG	2	25	50
BB	3	30	90
CG/BB 1:1	3	45	135
CG/BB 1:3	3	70	210
CG/BB 3:1	3	45	135

Intensity of staining (0–3): 0 = non-staining; 1 = weak; 2 = median; and 3 = strong; H-score: histology score; The H-score shows the result of multiplying the intensity of staining with the percentage of positive cells.

hyperglycemic conditions, the amount of ROS produced can exceed the amount that can be controlled by the body's antioxidants [39,40]. The ROS:antioxidant ratio imbalance causes changes in the redox signal of cells which in turn leads to impaired cell metabolism [41]. As a result, anti-oxidative stress treatment strategies can effectively stop or delay the progression of DM [42]. ROS can cause extensive cell damage by interacting with the majority of biological macromolecules, including proteins, lipids, and DNA. The overproduction of ROS increases the production of MDA through lipid peroxidation. MDA is a by-product of the peroxidation of polyunsaturated fatty acids in cells [43]. MDA levels are frequently used in DM patients as an indicator of oxidative stress and antioxidant status. Measurement of MDA level is one of the key techniques for determining the effects of ROS on living organisms. In this study, CGE/BBE 1:3 showed the strongest antioxidant activity in terms of reducing MDA levels and increasing SOD and CAT levels of STZ-NA-induced diabetic rats (Figs. 3 and 4). These findings indicate that the combination of CGE and BBE with a ratio of 1:3 provides a synergistic effect in reducing the oxidative stress and restoring the amount of antioxidant in the pancreas and liver of diabetic rats. The synergistic effect shown by CGE/BBE 1:3 occurred at a dose of CGE (75 mg/kg), which was smaller than the dose of BBE extract (112.5 mg/kg) in combination. This may be due to the regeneration effect produced by CGE with respect to BBE, as the antioxidant activity of the extract combination increases. The regeneration refers to a synergy that arises when distinct extracts work together to regenerate, with a weaker one regenerating a stronger one. The regeneration mechanism can be explained as follows. Compounds in the extract with stronger antioxidant activity scavenge free radicals by donating hydrogen so that they are in an oxidized form and lose their antioxidant ability. Furthermore, the compounds in the extract with weaker antioxidant activity reduce the oxidized compounds back to their initial form, allowing them to scavenge more free radicals [44]. The abovementioned mechanism is

relevant to the results of this study considering that in the previous research, it was found that BBE is a very strong antioxidant, while CGE is a very weak antioxidant [21]. In addition, the phytochemicals in an extract can also protect the other phytochemicals that are responsible for the degradation of metabolic enzymes [20]. At a higher dose ratio of CGE (lower dose of BBE), the regeneration effect decreases, so it leads to competition between the phytochemicals contained in the two extracts. This can be observed in the CGE/BBE 1:1 and CGE/BBE 3:1, which had higher MDA levels than the single extract. This indicates that these two groups have an antagonistic effect.

STZ penetrates the pancreatic cells via glucose transporter-2 (GLUT-2) and destroys the islets in multiple ways, leading to a noticeable drop in intracellular insulin [45]. Histology examination on the pancreas of diabetic rats (DC group) showed that STZ partially damaged the islet of Langerhans, causing islet shrinkage, β-cells reduction, and cytoplasmic degeneration (Fig. 5B). Induction with STZ also causes a drastic reduction in insulin expression by pancreatic β-cells which can be observed by immunohistochemical staining. This decrease can be observed through the reduced intensity of the brown color in the islet of Langerhans of the DC group (Fig. 6B). In this study, CGE and BBE were able to regenerate the islet of Langerhans causing enlargement of islet dimensions and improvement of pancreatic β-cells number. Insulin expression was also observed to elevate slightly. It was confirmed that CGE and BBE contain phenolics and flavonoids that are responsible to antidiabetic activity. Gallic acid belongs to the phenolic group (phenolic acid subclass), which has been shown to regenerate β-cells and increase insulin expression [46,47]. Quercetin (a part of the flavonoid group) also has similar activities. Quercetin is able to prevent pancreatic β-cell apoptosis during diabetic conditions [48]. Moreover, quercetin also raised the pancreatic β-cells mass [49] and stimulated insulin expression [50]. Indeed, the effect produced by an extract combination is higher than the effect generated by an individual extract.

Although this study followed the procedures of previous researches, the death of rats could not be avoided. This caused the sample size to be small, making it difficult to find significant differences between groups. The decrease in the number of rats during treatment was likely caused by the administration of STZ before NA, which caused a lack of NA protection against STZ attacks. To overcome this limitation in future research, induction with STZ should be carried out after NA administration. The CGE/BBE 1:3 revealed the most potent effect in ameliorating diabetic conditions in the diabetic pancreas. This effect is similar to the effect shown by glibenclamide, indicating the synergistic interaction occurred. In this case, the CGE and BBE synergistically worked together to regenerate β-cells and restore the islet dimension. The expression of insulin also increased markedly. These findings are in agreement with the findings stated by Atangwho et al. [51], who reported that the combination of *Vernonia amygdalina* and *Azadirachta indica* extracts regenerated islet cells and improved the insulin expression of diabetic rats induced by STZ. Masaenah et al. [52] also reported the synergistic effect of a combined extract of *Andrographis paniculata, Syzygium cumini*, and *Caesalpinia sappan* on diabetic rats induced by high-fat diet (HFD) and STZ. The administration of extracts in combination with a ratio of 1:1:1 for seven days increased β-cells number and insulin expression in the pancreas.

5. Conclusion

In this study, the extract combinations showed antidiabetic activity by reducing oxidative stress, regenerating the islet of Langerhans, and increasing pancreatic insulin expression. A notable result was shown by the combination of CGE and BBE with a ratio of 1:3 which confirms the regeneration effect between the active compounds. This combined extract showed higher antidiabetic activity than the single extracts, indicating CGE and BBE worked together to produce better antidiabetic action than either CGE or BBE alone. The effects produced by this combined extract can be said to be comparable to those of the standard drug, glibenclamide. Considering the effects produced by this combination of extracts, it could be improved to become a possible treatment for T2DM with minimal or completely no side effects. An in-depth investigation related to phytochemicals with antidiabetic activity in CGE and BBE needs to be developed. It is also necessary to explore the antidiabetic effect of this combination preclinically with different mechanisms of action.

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Declaration of generative AI in scientific writing

The authors state that any help from generative AI or AI-assisted technology has not been obtained in writing this manuscript.

Author contributions

I Made Wisnu Adhi Putra: Conceptualization, Methodology, Software, Investigation, Data curation, Writing – original draft, Visualization, Project administration, Funding acquisition. Nanang Fakhrudin: Validation, Formal analysis, Writing - review & editing, Supervision. Arief Nurrochmad: Conceptualization, Methodology, Software, Resources, Writing - review & editing, Supervision. Subagus Wahyuono: Writing – review & editing, Visualization, Supervision. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that there were no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Editor instruction to typesetter: Delete these supplementary files

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References

[1] Harreiter J, Roden M. Diabetes mellitus – Definition, Klassifikation, Diagnose, Screening und Prävention (Update 2019). Wien Klin Wochenschr 2019;131:6–15. https://doi.org/10.1007/s00508-019-1450-4.
[2] Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of type 2 diabetes mellitus. Int J Mol Sci 2020;21:6275. https://doi.org/10.3390/ijms21176275.
[3] Chen R, Ovbiagele B, Feng W. Diabetes and stroke: Epidemiology, pathophysiology, pharmaceuticals and outcomes. Am J Med Sci 2016;351:380–6. https://doi.org/10.1016/j.amjms.2016.01.011.
[4] Kottaisamy CPD, Raj DS, Prasanth Kumar V, Sankaran U. Experimental animal models for diabetes and its related complications-a review. Lab. Anim. Res. 2021; 37:23. https://doi.org/10.1186/s42826-021-00101-4.
[5] Noshahr ZS, Salmani H, Khajavi Rad A, Sahebkar A. Animal models of diabetes-associated renal injury. J Diabetes Res 2020;2020:1–16. https://doi.org/10.1155/2020/9416419.
[6] Cruz PL, Moraes-Silva IC, Ribeiro AA, Machi JF, de Melo MDT, dos Santos F, et al. Nicotinamide attenuates streptozotocin-induced diabetes complications and

increases survival rate in rats: role of autonomic nervous system. BMC Endocr Disord 2021;21:133. https://doi.org/10.1186/s12902-021-00795-6.
[7] Szkudelski T. Streptozotocin–nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Exp Biol Med 2012;237:481–90. https://doi.org/10.1258/ebm.2012.011372.
[8] Salazar-García M, Corona JC. The use of natural compounds as a strategy to counteract oxidative stress in animal models of diabetes mellitus. Int J Mol Sci 2021;22:7009. https://doi.org/10.3390/ijms22137009.
[9] Chaudhury A, Duvoor C, Reddy Dendi VS, Kraleti S, Chada A, Ravilla R, et al. Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. Front Endocrinol 2017;8. https://doi.org/10.3389/fendo.2017.00006.
[10] Alhadramy MS. Diabetes and oral therapies: a review of oral therapies for diabetes mellitus. J Taibah Univ Med Sci 2016;11:317–29. https://doi.org/10.1016/j.jtumed.2016.02.001.
[11] Salehi B, Ata A, Anil Kumar N V, Sharopov F, Ramírez-Alarcón K, Ruiz-Ortega A, et al. Antidiabetic potential of medicinal plants and their active components. Biomolecules 2019;9:551. https://doi.org/10.3390/biom9100551.
[12] Junejo JA, Rudrapal M, Nainwal LM, Zaman K. Antidiabetic activity of hydro-alcoholic stem bark extract of Callicarpa arborea Roxb. with antioxidant potential in diabetic rats. Biomed Pharmacother 2017;95:84–94. https://doi.org/10.1016/j.biopha.2017.08.032.
[13] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 2014;4:1–10. https://doi.org/10.3389/fphar.2013.00177.
[14] Tupe RS, Kulkarni A, Adeshara K, Shaikh S, Shah N, Jadhav A. Syzygium jambolanum and Cephalandra indica homeopathic preparations inhibit albumin glycation and protect erythrocytes: an in vitro study. Homeopathy 2015;104:197–204. https://doi.org/10.1016/j.homp.2015.02.009.
[15] Mohammed SI, Chopda MZ, Patil RH, Vishwakarma KS, Maheshwari VL. In vivo antidiabetic and antioxidant activities of Coccinia grandis leaf extract against streptozotocin induced diabetes in experimental rats. Asian Pac J Trop Dis 2016;6:298–304. https://doi.org/10.1016/S2222-1808(15)61034-9.
[16] Attanayake AP, Jayatilaka KAPW, Mudduwa LKB, Pathirana C. β-cell regenerative potential of selected herbal extracts in alloxan induced diabetic rats. Curr Drug Discov Technol 2019;16:278–84. https://doi.org/10.2174/1570163815666180418153024.
[17] Pang Y, Wang D, Fan Z, Chen X, Yu F, Hu X, et al. Blumea balsamifera—a phytochemical and pharmacological review. Molecules 2014;19:9453–77. https://doi.org/10.3390/molecules19079453.
[18] Roy K, Saha S, Biswas S, Ahmed W, Mariappan G. In vivo assessment of antidiabetic and antioxidant activities of Blumea balsamifera in streptozotocin-diabetic rats. Res J Med Plant 2013;7:48–57. https://doi.org/10.3923/rjmp.2013.48.57.
[19] Ramulondi M, De Wet H, Van Vuuren S. Toxicology of medicinal plants and combinations used in rural northern KwaZulu-Natal (South Africa) for the treatment of hypertension. J Herb Med 2019;16:100251. https://doi.org/10.1016/j.hermed.2018.12.001.
[20] Rasoanaivo P, Wright CW, Willcox ML, Gilbert B. Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. Malar J 2011;10:1–12. https://doi.org/10.1186/1475-2875-10-S1-S4.
[21] Putra IMWA, Fakhrudin N, Kusumawati IGAW, Nurrochmad A, Wahyuono S. Antioxidant properties of extract combination of Coccinia grandis and Blumea balsamifera: an in vitro synergistic effect. J Herbmed Pharmacol 2022;11:55–62. https://doi.org/10.34172/jhp.2022.06.
[22] Parmar GR, Pundarikakshudu K, Balaraman R. Antidiabetic and antihyperlipidemic activity of Euphorbia thymifolia L. extracts on streptozotocin-nicotinamide induced type 2 diabetic rats. J Appl Pharmaceut Sci 2017;7:78–84. https://doi.org/10.7324/JAPS.2017.70811.
[23] Cass JD, Varma S, Day AG, Sangrar W, Rajput AB, Raptis LH, et al. Automated quantitative analysis of p53, cyclin D1, Ki67 and pERK expression in breast carcinoma does not differ from expert pathologist scoring and correlates with clinico-pathological characteristics. Cancers 2012;4:725–42. https://doi.org/10.3390/cancers4030725.
[24] Chaudhry ZZ, Morris DL, Moss DR, Sims EK, Chiong Y, Kono T, et al. Streptozotocin is equally diabetogenic whether administered to fed or fasted mice. Lab Anim 2013;47:257–65. https://doi.org/10.1177/0023677213489548.
[25] Rambiritch V, Maharaj B, Naidoo P. Glibenclamide in patients with poorly controlled type 2 diabetes: a 12-week, prospective, single-center, open-label, dose-escalation study. Clin Pharmacol Adv Appl 2014;6:63–9. https://doi.org/10.2147/CPAA.S54809.
[26] Kewcharoenwong C, Rinchai D, Utispan K, Suwannasaen D, Bancroft GJ, Ato M, et al. Glibenclamide reduces pro-inflammatory cytokine production by neutrophils of diabetes patients in response to bacterial infection. Sci Rep 2013;3:3363. https://doi.org/10.1038/srep03363.
[27] Krishnasamy G, Muthusamy K, Chellappan DR, Subbiah N. Antidiabetic, antihyperlipidaemic, and antioxidant activity of Syzygium densiflorum fruits in streptozotocin and nicotinamide-induced diabetic rats. Pharm Biol 2016;54:1716–26. https://doi.org/10.3109/13880209.2015.1125932.
[28] Sayeli VK, Shenoy AK. Antidiabetic effect of bio-enhanced preparation of turmeric in streptozotocin-nicotinamide induced type 2 diabetic Wistar rats. J Ayurveda Integr Med 2021;12:474–9. https://doi.org/10.1016/j.jaim.2021.04.010.
[29] Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Via Medici 2018;5:1–16. https://doi.org/10.3390/medicines5030093.
[30] Gupta RC, Chang D, Nammi S, Bensoussan A, Bilinski K, Roufogalis BD. Interactions between antidiabetic drugs and herbs: an overview of mechanisms of action and clinical implications. Diabetol Metab Syndrome 2017;9:59. https://doi.org/10.1186/s13098-017-0254-9.
[31] Yang Y, Zhang Z, Li S, Ye X, Li X, He K. Synergy effects of herb extracts: Pharmacokinetics and pharmacodynamic basis. Fitoterapia 2014;92:133–47. https://doi.org/10.1016/j.fitote.2013.10.010.
[32] Perumal N, Nallappan M, Shohaimi S, Kassim NK, Tee TT, Cheah YH. Synergistic antidiabetic activity of Taraxacum officinale (L.) Weber ex F.H.Wigg and Momordica charantia L. polyherbal combination. Biomed Pharmacother Biomedecine Pharmacother 2021;145:112401. https://doi.org/10.1016/j.biopha.2021.112401.
[33] Zafar M, Naqvi SN-H. Effects of STZ-induced diabetes on the relative weights of kidney, liver and pancreas in albino rats: a comparative study. Int J Morphol 2010;28. https://doi.org/10.4067/S0717-95022010000100019.
[34] Pottathil S, Nain P, Morsy MA, Kaur J, Al-Dhubiab BE, Jaiswal S, et al. Mechanisms of antidiabetic activity of methanolic extract of punica granatum leaves in nicotinamide/streptozotocin-induced type 2 diabetes in rats. Plants 2020;9:1609. https://doi.org/10.3390/plants9111609.
[35] Guo X, Wang Y, Wang K, Ji B, Zhou F. Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection. J Zhejiang Univ - Sci B 2018;19:559–69. https://doi.org/10.1631/jzus.B1700254.
[36] Moukette BM, Ama Moor VJ, Biapa Nya CP, Nanfack P, Nzufo FT, Kenfack MA, et al. Antioxidant and synergistic antidiabetic activities of a three-plant preparation used in Cameroon folk medicine. Int Sch Res Notices 2017;2017:1–7. https://doi.org/10.1155/2017/9501675.
[37] Matough FA, Budin SB, Hamid ZA, Alwahaibi N. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ Med J 2012;12:5–18. https://doi.org/10.12816/0003082.
[38] Ullah A, Khan A, Khan I. Diabetes mellitus and oxidative stress—a concise review. Saudi Pharmaceut J 2016;24:547–53. https://doi.org/10.1016/j.jsps.2015.03.013.
[39] McClelland AD, Herman-Edelstein M, Komers R, Jha JC, Winbanks CE, Hagiwara S, et al. miR-21 promotes renal fibrosis in diabetic nephropathy by targeting PTEN and SMAD7. Clin Sci 2015;129:1237–49. https://doi.org/10.1042/CS20150427.
[40] Sharma M, Gupta S, Singh K, Mehndiratta M, Gautam A, Kalra OP, et al. Association of glutathione-S-transferase with patients of type 2 diabetes mellitus with and without nephropathy. Diabetes Metab Syndr Clin Res Rev 2016;10:194–7. https://doi.org/10.1016/j.dsx.2016.06.006.
[41] Mahmoodnia L, Aghadavod E, Beigrezaei S, Rafieian-Kopaei M. An update on diabetic kidney disease, oxidative stress and antioxidant agents. J Ren Inj Prev 2017;6:153–7. https://doi.org/10.15171/jrip.2017.30.
[42] Chen H-W, Yang M-Y, Hung T-W, Chang Y-C, Wang C-J. Nelumbo nucifera leaves extract attenuate the pathological progression of diabetic nephropathy in high-fat diet-fed and streptozotocin-induced diabetic rats. J Food Drug Anal 2019;27:736–48. https://doi.org/10.1016/j.jfda.2018.12.009.
[43] Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014;2014:360438. https://doi.org/10.1155/2014/360438.
[44] Bayram I, Decker EA. Underlying mechanisms of synergistic antioxidant interactions during lipid oxidation. Trends Food Sci Technol 2023;133:219–30. https://doi.org/10.1016/j.tifs.2023.02.003.
[45] Nahdi AMTA, John A, Raza H. Elucidation of molecular mechanisms of streptozotocin-induced oxidative stress, apoptosis, and mitochondrial dysfunction in Rin-5F pancreatic β-cells. Oxid Med Cell Longev 2017;2017:e7054272. https://doi.org/10.1155/2017/7054272.
[46] Abdel-Moneim A, Abd El-Twab SM, Yousef AI, Ashour MB, Reheim ESA, Hamed MAA. New insights into the in vitro, in situ and in vivo antihyperglycemic mechanisms of gallic acid and p-coumaric acid. Arch Physiol Biochem 2022;128:1188–94. https://doi.org/10.1080/13813455.2020.1762659.
[47] Rahimifard M, Baeeri M, Bahadar H, Moini-Nodeh S, Khalid M, Haghi-Aminjan H, et al. Therapeutic effects of gallic acid in regulating senescence and diabetes; an in vitro study. Molecules 2020;25:5875. https://doi.org/10.3390/molecules25245875.
[48] Zhuang M, Qiu H, Li P, Hu L, Wang Y, Rao L. Islet protection and amelioration of type 2 diabetes mellitus by treatment with quercetin from the flowers of Edgeworthia gardneri. Drug Des Dev Ther 2018;12:955–66. https://doi.org/10.2147/DDDT.S153898.
[49] Adewole SO, Caxton-Martins EA, Ojewole JA. Protective effect of quercetin on the morphology of pancreatic β-cells of streptozotocin-treated diabetic rats. Afr J Tradit, Complementary Altern Med 2007;4:64–74. https://doi.org/10.4314/ajtcam.v4i1.31196.
[50] Kittl M, Beyreis M, Tumurkhuu M, Fürst J, Helm K, Pitschmann A, et al. Quercetin stimulates insulin secretion and reduces the viability of rat INS-1 beta-cells. Cell Physiol Biochem 2016;39:278–93. https://doi.org/10.1159/000445623.
[51] Atangwho IJ, Ebong PE, Eyong EU, Asmawi MZ, Ahmad M. Synergistic antidiabetic activity of Vernonia amygdalina and Azadirachta indica: biochemical effects and possible mechanism. J Ethnopharmacol 2012;141:878–87. https://doi.org/10.1016/j.jep.2012.03.041.
[52] Masaenah E, Elya B, Setiawan H, Fadhilah Z, Wediasari F, Nugroho GA, et al. Antidiabetic activity and acute toxicity of combined extract of Andrographis paniculata, Syzygium cumini, and Caesalpinia sappan. Heliyon 2021;7:e08561. https://doi.org/10.1016/j.heliyon.2021.e08561.