Heliyon 7 (2021) e08561

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Antidiabetic activity and acute toxicity of combined extract of *Andrographis paniculata*, *Syzygium cumini*, and *Caesalpinia sappan*

Eem Masaenah [a,b], Berna Elya [a,*], Heri Setiawan [a], Zahra Fadhilah [a], Febrika Wediasari [a], Gumilar A. Nugroho [a], Elfahmi [c], Tjandrawati Mozef [d]

[a] *Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia*
[b] *Sekolah Tinggi Teknologi Industri dan Farmasi, Bogor, Indonesia*
[c] *School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia*
[d] *Indonesian Institute of Sciences, Jakarta, Indonesia*

ARTICLE INFO

Keywords:
Acute toxicity
Alternative medicine
Andrographis paniculata
Antidiabetic
Caesalpinia sappan
Syzygium cumini

ABSTRACT

Andrographis paniculata, *Syzygium cumini*, and *Caesalpinia sappan* are used as traditional medicines to treat diabetes mellitus. Therefore, this study aims to examine the antidiabetic effects and the acute toxicity of combined extract (1:1:1) of *A. paniculata*, *S. cumini*, and *C. sappan* (ASCE). The antidiabetic effect was tested using the rats model, induced by a high-fat diet and a double dose of streptozotocin injection of 35 mg/kg BW. Subsequently, diabetic rats in the experimental group were treated with 75 mg/kg BW and 150 mg/kg BW of ASCE, and those in the diabetic control group were treated with metformin 250 mg/kg BW. After seven days of treatment, fasting blood glucose (FBG), pancreatic β-cells numbers, and lipid profiles were used to analyze the antidiabetic effect. The results showed that the administration of 150 mg/kg BW ASCE significantly reduced FBG ($p < 0.01$), cholesterol levels ($p < 0.05$), LDL levels ($p < 0.05$), but not triglycerides, compared to diabetic control, this effect was comparable to metformin treatment. In addition, the pancreatic β-cells numbers were likely increased after ASCE treatment in a dose-dependent manner. The oral administration of a single dose of ASCE was safe up to 5000 mg/kg BW and did not result in any significant difference in body weight, relative organ weight, hematological and biochemical parameters compared with the control group. Therefore, it can be concluded that ASCE has a potential antidiabetic effect and can be safely developed as alternative medicine.

1. Introduction

Diabetes mellitus (DM) is a chronic disease characterized by a progressive increase in blood glucose levels (WHO, 2016). Furthermore, it is characterized by polydipsia, polyurea, ketonemia, and ketonuria (Aba and Asuzu, 2018). The prevalence of diabetes mellitus, which increases by 90–95%, is type 2 DM (T2DM), known as non-insulin-dependent diabetes. The inefficient use of insulin by the body is due to the reduced response of target tissues to regular circulating insulin levels (Salehi et al., 2019). T2DM is a complex metabolic disorder in which changes in lipid metabolism, insulin resistance, and pancreatic β-cell failure occur. Obesity is the common risk factor for the development of T2DM. It can increase triglyceride levels, blood pressure, and insulin resistance (Gheibi et al., 2017). Prolonged insulin resistance leads to chronic hyperglycemia and hyperlipidemia in diabetic patients (Schofield et al., 2016). Chronic hyperglycemia can lead to complications, increase the risk of death or disability, and reduce the quality of life (WHO, 2016; Tanty et al., 2018; Salehi et al., 2019).

The most commonly prescribed medication for T2DM is metformin (Wang et al., 2017). Metformin's mechanism increases insulin sensitivity and high-density lipoprotein (HDL) levels, reduces triglyceride and low-density lipoprotein (LDL) levels, and ultimately reduces blood glucose levels (Schofield et al., 2016). The most common side effects are gastrointestinal symptoms, which include nausea and vomiting. The most severe side effect is lactic acidosis, specifically in diabetic patients with hepatic and renal dysfunction (Wang et al., 2017). This side effect requires specific attention because it takes a long time to treat DM.

Several plants have hypoglycemic properties, therefore, they can become a source of new drugs to complement oral hypoglycemic agents in the management of DM. Their biological activity is related to their chemical composition. Plants rich in secondary metabolites such as phenol compounds, glycosides, flavonoids, alkaloids, steroids, and

* Corresponding author.
E-mail address: berna.elya@farmasi.ui.ac.id (B. Elya).

https://doi.org/10.1016/j.heliyon.2021.e08561
Received 27 April 2021; Received in revised form 24 May 2021; Accepted 3 December 2021

terpenoids, tend to lower blood glucose levels. The antidiabetic effect of phytochemicals can be prevented by various mechanisms. Plants with antidiabetic activity include *Andrographis paniculata* (Burm.f.) Nees, *Syzygium cumini* (L.) Skeel., and *Caesalpinia sappan* L. (Preethi, 2013; Rosalie and El, 2016; Salehi et al., 2019).

A. paniculata is one of the most popular medicinal herbs in Indonesia known as Sambiloto. Andrographolide, the main bioactive chemical constituent of *A. paniculata*, has been reported to have antidiabetic potential (Yu et al., 2008; Zhang et al., 2009; Sari et al., 2015). *S. cumini* is a native Indian tree that is widespread around the world and is also used as an antidiabetic (Chagas et al., 2018; França et al., 2019). In Indonesia, the tree is referred to as Jamblang. All parts of the *S. cumini* plant contain high polyphenols, one of the main ingredients in *S. cumini* leaves is quercetin and it is used as antidiabetic (Chagas et al., 2018). *C. sappan,* known in Indonesia as Secang, is an indigenous plant in southern India and many parts of Southeast Asia. The part that is often used is the heartwood. It has brazilin as the main active compound, and it is potentially antidiabetic (Setyaningsih et al., 2019; Sindu et al., 2019). The extracts of these plants were reported to be safe. Several acute toxicity studies stated that extract of *A. paniculata* (Worasuttayangkurn et al., 2019), extract of *S. cumini* (Ayyanna et al., 2015), and extract of *C. sappan* (Athinarayanana et al., 2017) are safe up to a dose of 5000 mg/kg BW. However, the acute toxicity of combined extract of *A.paniculata, S. cumini,* and *C. sappan* has not yet been elucidated.

The complex pathological condition of diabetes mellitus certainly requires treatment with many biological targets, therefore, combinations of drugs with different mechanisms become an option in its treatment (Clemmensen et al., 2019). Jamu is traditional medicine in Indonesian, which contains a mixture of natural ingredients that have been used by Indonesian society for centuries to maintain health and treat diseases such as diabetes (Elfahmi et al., 2014). Furthermore, it is believed that the combination of many ingredients in the herbal formula can improve therapeutic outcomes, reduce toxicity, and systematically manage complex conditions (Zhou et al., 2019). Several scientific studies reported that a herbal combination for the treatment of diabetes offers additional advantages over herbs with only one ingredient (Widharna et al., 2015; Nugroho et al., 2013; Deepa et al., 2018).

Most animal models induced by diabetes were administered toxic chemicals, which target the pancreatic β-cells. The chemical commonly used to induce diabetes in streptozotocin (STZ) (Gheibi et al., 2017). STZ was first isolated from a soil microorganism called *Streptomyces acromogenes* and showed a broad-spectrum antibiotic activity. It is a nitrosourea compound that penetrates the β-cells through the glucose transporter type 2 (GLUT2) (Goyal et al., 2016). A high-fat diet (HFD) with induction of STZ was administered to model and characterize the many complications of T2DM in humans (Furman, 2015). The administration of HFD leads to obesity and insulin resistance, while the administration of low doses of STZ will damage some pancreatic β-cells. The administration of both HFD and STZ induction will result in a stable state of hyperglycemia (Husna et al., 2019).

The combination of *A. paniculata* and *S. cumini* was used in several jamu products in Indonesia (Elfahmi et al., 2014). In a previous study, it was reported that the combination of extracts of *A. paniculata* and *C. sappan* had moderate antihyperglycemic effects (Wediasari et al., 2020). However, there was no prior study which examined the antidiabetic effect of the combination of *A. paniculata*, *S. cumini*, and *C. sappan* extracts. Hence, this study was carried out to determine the antidiabetic effect by combining the aerial parts extract of *A. paniculata*, the leaves extract of *S. cumini*, and the heartwoods extract of *C. sappan* with the ratio 1:1:1 and analyzing its antidiabetic activity in rats administered with HFD and STZ induction. The aim was to achieve an equivalent antidiabetic activity compared to metformin. Test parameters include fasting blood glucose (FBG) level, the number of pancreatic β-cells by immunohistochemistry, and lipid profiles. The safety of the extract combination was evaluated using an oral acute toxicity test.

2. Materials and methods

The experimental protocol was in compliance with and approved by the Ethics Committee of the Faculty of Medicine Universitas Indonesia (KET-136/UN2.F1/ETIK/PPM.00.02/2020).

2.1. Plant material and extract preparation

A. paniculata and *C. sappan* were obtained from Wonogiri, Central Java, Indonesia, while *S. cumini* was obtained from Serpong, West Java, Indonesia. The plants were authenticated by The Research Center for Plant Conservation, The Indonesian Institute of Sciences, Bogor, West Java, Indonesia. A verification specimen (B-809/IPH.3/KS/VII/2020) was deposited in Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Universitas Indonesia. The dried aerial parts of *A. paniculata*, the leaves of *S. cumini*, and the heartwoods of *C. sappan* were powdered and then stored in an airtight container for further use.

Each powdered material of *A. paniculata, S. cumini*, and *C. sappan* was macerated by soaking in 70% ethanol solvent (1:10 b/v) in a round bottom flask for 24 h at room temperature. The solvent was stirred for 6 h, then allowed to stand. After 24 h, the mixture was filtered, then the filtrate was separated, and the residue was soaked again with the same solvent and procedure. The obtained filtrates were combined and concentrated on a rotary vacuum evaporator (Buchi, Switzerland) at 50 °C. The final extract was stored in a refrigerator for further analysis.

2.2. Determination of A. paniculata, S. cumini, and C. sappan biomarker compound with high-performance liquid chromatography (HPLC)

The content of biomarker compounds, which are considered to be an antidiabetic activity, was tested for each extract. The determination of andrographolide in *A. paniculata* ethanolic extract (AE), quercetin in *S. cumini* ethanolic extract (SE), and brazilin in *C. sappan* ethanolic extract (CE) were determined using an HPLC gradient system, LC-20AT pump (Shimadzu, Japan), column RP-C18 (150 mm x 4,6 mm, 5 μm, GL Sciences, Japan), and UV detector SPD 20A. Furthermore, the HPLC method was performed with reference to Setyaningsih et al. (2019) and Sindu et al. (2019), with modification. The optimal wavelength was set at 254 nm. The binary gradient elution was performed using mobile phase A = acetic acid in water; B = acetonitrile. Gradient: 0 min 84.5% A (0.3% acetic acid in water) - 14.5% B; 8 min 74.5% A (0.3% acetic acid in water) - 24.5% B; 16 min 50% A (0.04% acetic acid in water) - 50% B; 24 min 40% A (0.04% acetic acid in water) - 60% B; and 30 min stop. The injection volume was 20 μL, and the flow rate was 1 mL/min.

Andrographolide (≥90%), quercetin (≥90%), and brazilin (≥90%) standards were purchased from The School of Pharmacy, Bandung Institute of Technology (West Java, Indonesia). Methanol, acetonitrile, and concentrated acetic acid were obtained from Merck-Germany (HPLC grade). Andrographolide, quercetin, and brazilin were combined and dissolved in methanol with shaking and sonication. The concentrations were 155, 115, 75, 55, and 27.5 μg/mL, respectively. A calibration curve was prepared by using peak areas and concentrations of standard solutions. The peak areas were recorded and the extracts combinations of ASCE solutions were analyzed. The concentration of andrographolide, quercetin, and brazilin in the ASCE solutions was determined using the calibration curve.

2.3. Animals

In the antidiabetic activity study, the white male *Sprague-Dawley* (SD) rats (6–8 weeks old, 160–210 g) were used, and in the acute oral toxicity study, the white male and female SD rats were used (6–8 weeks old, male 230–260 g, female 180–210 g). The rats were obtained from The National Agency of Drug and Food Control, Jakarta, Indonesia. During the first week, all rats were acclimatized with food and water *ad libitum* in the room under controlled conditions of temperature 22 ± 2 °C, humidity 55

± 5%, and 12:12 h light/dark cycle Laboratory Animal House, Health Sciences Cluster, Universitas Indonesia. The animals were cared for and used in accordance with the procedures established by the National Agency of Drug and Food Control Republic of Indonesia No.7 of 2014.

2.4. Induction of diabetes

After one week of acclimatization, five rats were administered with a standard pellet diet, and the rest were administered with HFD (20.07% fat, 17.78% protein, and 2.75% crude fiber). After three weeks of the HFD administration, the rats (N = 20) were fasted overnight and were induced with diabetes by intraperitoneal injection of STZ at a double dose of 35 mg/kg BW at a one-week interval. Normal control rats were treated with citrate buffer. Furthermore, STZ (Wako, Fujifilm, Japan) was freshly prepared in cold citrate buffer (pH 4.5, 0.1 M) each time of injection. Their fasting blood glucose (FBG) level was measured one week after the 2nd STZ injection. Blood was obtained from the tip of the rat's tail and smeared on a test strip. The FBG level was measured using a glucometer (Accu-Check Active, Roche Diagnostics, Mannheim Germany). Rats with FBG level ≥150 mg/dL (King, 2012; Gheibi et al., 2017; Wediasari et al., 2020) were selected as diabetic rats and used in this study. HFD was administered to the induction rats group until the end of the study.

2.5. Antidiabetic activity test

Twenty-five rats were divided into 5 groups, as follows:

1. Normal control (NC), non-diabetic rats administered with vehicle solution (0.5 % Na-CMC).
2. Diabetic control (DC), diabetic rats administered with vehicle solution (0.5% Na-CMC).
3. Positive control (MET), diabetic rats treated with metformin 250 mg/kg BW.
4. ASCE75, diabetic rats treated with the combination of ASCE 75 mg/kg BW (25 mg/kg BW each extract).
5. ASCE150, diabetic rats treated with the combination of ASCE 150 mg/kg BW (50 mg/kg BW each extract).

All treatments were administered orally once daily, for seven consecutive days. The FBG levels were measured on days 0 and 7 after treatment. At the end of the study, the rats were euthanized with ketamine and xylazine (Aamir et al., 2019). Rat blood samples were obtained via cardiac puncture and then incubated for 30 min at room temperature. The serum was collected by centrifugation at 4000 rpm for 20 min at 25 °C. The serum lipid profiles (cholesterol, triglyceride, HDL, and LDL) were measured using assays kits purchased from Biomaxima 164 (Poland). Subsequently, the rats were sacrificed with an overdose combination of ketamine and xylazine. The pancreas was collected and fixed with 4% Buffer Neutral Formalin (BNF) for 24 h. Pancreatic tissue was dissected in the incisors and analyzed by immunohistochemistry (IHC) using the primary anti-insulin antibody (clone K36AC10) (Wediasari et al., 2020). The slides were also analyzed and evaluated under a light microscope (Olympus BX51, Japan).

2.6. Acute oral toxicity test

This study was conducted in accordance with the OECD test guideline No. 420 (Acute Oral Toxicity-Fixed Dose Procedure) (Organization of Economic Co-operation and Development, 2001). Furthermore, 20 male and female rats were randomly divided into 4 groups (5 rats of each sex per group), consisting of 3 treatment groups (300, 2000, or 5000 mg/kg BW of ASCE) and 1 control group (0.5% Na-CMC). Before the dose was administered, all rats were fasted and weighed for 3–4 h (water *ad libitum*). The volume of the doses was calculated based on the bodyweight of the individual rat, administered by oral gavage in the suspension of 0.5 % Na-CMC with a total volume of 10 ml/kg BW. The first dose of 300 mg/kg BW of ASCE was orally administered to the group 1 rats. Furthermore, Group 2 rats were administered with a single dose of ASCE 2000 mg/kg BW after 24 h of group 1 treatment. Group 3 rats were administered with a single dose of ASCE 5000 mg/kg BW after 24 h of group 2 treatment. Meanwhile, the control group was administered with 0.5% Na-CMC for comparative analysis. All rats were carefully observed for any changes in skin and fur, eyes, salivation, respiration, urination, diarrhea, behavior pattern, and mortality during the first 0.5 h and 4 h after treatment and once every 24 h for two weeks. The rats were weighed after one week and two weeks of dose administration.

On the 15th day, the rats were euthanized using ketamine and xylazine (Aamir et al., 2019). Blood samples were obtained via cardiac puncture and transferred into K_3-EDTA plain vacutainers. Hematological analysis was carried out using an automated hematological analyzer (Celltac Alpha MEK-6450K, Japan). The parameters measured include white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), thrombocyte, red cell distribution width (RDW), mean platelet volume (MPV), and platelet distribution width (PDW). However, biochemical analysis was performed using rat serum. Rat blood samples in plain vacutainers were reserved for 30 min at room temperature to let blood coagulate. Then centrifuged at 4 °C, 4000 rpm for 20 min to obtain serum. The parameters measured include aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine (CRN). Subsequently, the rats were sacrificed, and the organs of the rats (heart, liver, and kidneys) were removed and weighed. Abnormalities in color, size, and morphology of the organs were documented.

2.7. Statistical analysis

All data were expressed as the mean ± standard error of the mean (SEM). Normality and homogeneity were tested with the Shapiro-Wilk and the Levene test, respectively. Normally distributed and homogeneous variance data were analyzed using one-way analysis of variance (ANOVA) followed by the least significant difference test (LSD). Non-normal data were analyzed using the Kruskal-Wallis test followed by Dunn's test. The differences were considered significant at $p < 0.05$. The concentrations of the biomarker in the ASCE were calculated using linear regression analysis.

3. Result and discussion

3.1. Determination of A. paniculata, S. cumini, and C. sappan biomarker compound

The biomarker compounds in AE, SE, and CE are determined simultaneously by HPLC. The applied gradient system succeeded in separating compounds marker contained in a combination of ASCE. Figure 1A shows the chromatogram of the combined andrographolide, quercetin, and brazilin standards. Figure 1B shows the chromatogram of the combined extract of ASCE. The chromatograms of quercetin (Rt = 5.383 min), andrographolide (Rt = 6.567 min), and brazilin (Rt = 7.333 min) marker compounds can be observed simultaneously at the same time. This simultaneous test used aqueous phases containing acetic acid, 0.04%–0.3%, and acetonitrile. The linear equation for andrographolide was $y = 1653.11x + 4961.86$ ($R^2 = 0.9945$); quercetin was $y = 1537.38x + 23440.88$ ($R^2 = 0.9990$); and brazilin was $y = 4085.97x - 13501.19$ ($R^2 = 0.9992$). The test result showed that andrographolide content in AE was 3.00%, quercetin content of SE was 3.84 %, and brazilin content of CE was 4.74%.

The ASCE chromatogram detected unknown compound peaks. Several phytochemical compounds from each extract (AE, SE, and CE) were also detected at a wavelength of 254 nm. Other main components presented in AE were 14-deoxy-11,12-didehydro andrographolide, neoandrographolide, and 14-deoxyandrographolide (Worasuttayangkurn

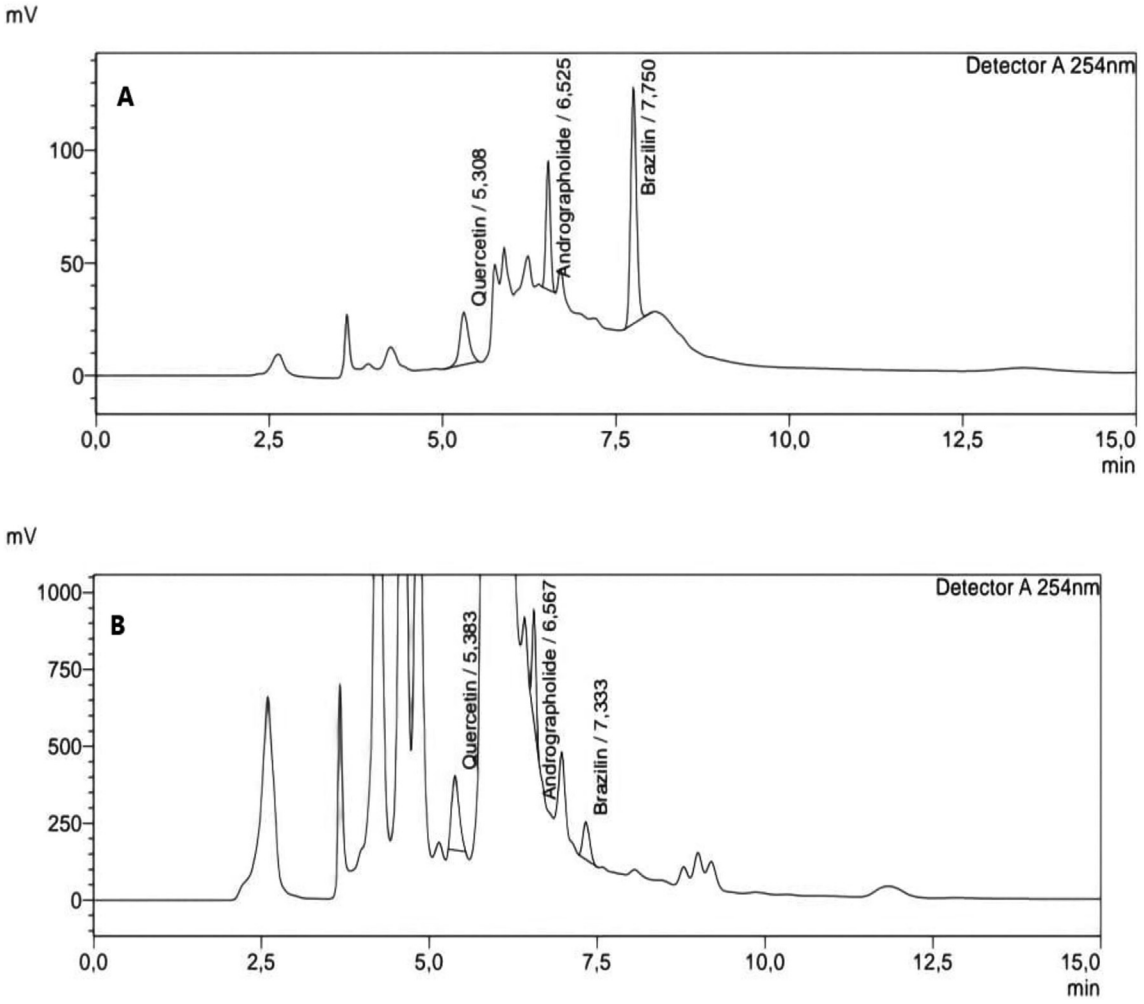

Figure 1. Representative (A) Chromatograms of combined andrographolide, quercetin, and brazilin standards (B) Chromatograms of a combined 70% ethanolic extract of *A. paniculata, S. cumini,* and *C. sappan* (ASCE).

et al., 2019). In SE are gallic acid, myristetin, and their derivatives (dos Santos et al., 2018; Chagas et al., 2018; Chavan et al., 2019). In CE are protosappanin B, protosappannin A, C-E, sappanchalcone and hematoxylin (Xia et al., 2017; Dapson and Bain, 2015). Additionally, further study is needed to identify these unknown compounds, specifically those suspected to have antidiabetic potential.

3.2. Antidiabetic activity

3.2.1. Effect of induced diabetic

The administration of HFD to animals leads to insulin resistance, and low-dose STZ induction will damage some of the pancreatic β-cells, which results in a stable state of hyperglycemia and creates T2DM (Husna et al., 2019). This model is considered to be the best for the characteristics of the many complications associated with Diabetes Mellitus in humans (Furman, 2015). The result of the statistical analysis showed that the FBG levels before (67,35 ± 3,52 mg/dL) and after (337,9 ± 27,72 mg/dL) induction had a significant difference ($p < 0.001$). The STZ selectively damages β-cells. Furthermore, it causes insulin deficiency, hyperglycemia, polydipsia, and polyuria resembling human diabetes (Goyal et al., 2016).

3.2.2. Effect of ASCE on fasting blood glucose and the number of pancreatic β-cells by immunohistochemistry

Seven days after the second STZ injection, the FBG level in the DC group increased by 135 ± 75.92 mg/dL, but the change in FBG level in the DC group was not significantly different from that of the NC group. The treatment of diabetic rats ASCE75, ASCE150, and metformin showed a significant decrease in FBG ($p < 0.05$; $p < 0.01$; $p < 0.01$; respectively) compared to the DC group. The decrease in FBG level from the MET group (110.6 ± 46.14 mg/dL), ASCE75 (76.20 ± 37.34 mg/dL) and ASCE150 (115.4 ± 71.33 mg/dL) was not significant compared to the NC group. The most significant decrease in FBG level was shown by the ASCE150 (Table 1). This study showed that ethanolic extract of ASCE (150 mg/kg BW) significantly reduced the FBG level of diabetic rats, equivalent to metformin.

The influence of the number of β-cells in the pancreas was detected using immunohistochemical methods. Observations using staining techniques showed that β-cells, which produce insulin on the Langerhans islands were considered as cells with brown-colored cytoplasm

Table 1. Fasting blood glucose level before and after treatment.

Group (n = 5)	FBG d-0 (mg/dL)	FBG d-7 (mg/dL)	Decrease FBG (mg/dL)
NC	77.40 ± 2.99	76.80 ± 2.25	0.60 ± 2.99
DC	215.20 ± 47.84	350.20 ± 59.92	-135.00 ± 75.92
MET	396.00 ± 14.63	285.40 ± 49.65	110.60 ± 46.14$^{\#\#}$
ASCE75	260.00 ± 21.60	183.80 ± 45.31	76.20 ± 37.34$^{\#}$
ASCE150	480.20 ± 20.97	364.80 ± 62.50	115.40 ± 71.33$^{\#\#}$

Significantly different from DC group ($^{\#}p < 0.05$; $^{\#\#}p < 0.01$).
Data are expressed as the mean ± SEM (n = 5).

(Figure 2A). The β-cell number of the STZ-induced rats was less intense than the NC rats. The improvement results were observed in pancreatic insulin expression after treatment with metformin and ASCE. The number of pancreatic β-cells in the groups of NC, DC, MET, ASCE75, and ASCE150 were 134.1 ± 12.4; 49.87 ± 14.34; 68.17 ± 11.88; 65.0 ± 17.44; and 90.4 ± 8.74, respectively. All treatment groups showed a better trend in β-cell number than DC rats, with the ASCE150 treatment showing the highest number. However, it was still significantly different from the NC group (Figure 2B). The combination of ASCE might have a restorative effect on pancreatic β-cells as it showed a dose-dependent tendency. Hence, further study with higher doses and more prolonged treatment is needed to observe a significant β-cell number compared to DC rats.

The phytochemical content of each extract helps to lower the blood glucose levels and the repair of pancreatic β-cells from ASCE. Several possible synergy mechanisms in combination therapy include an increased yield of bioactive compounds, chemical reactions between compounds, multi-target behavior, or an increase in bioavailability (Zhou et al., 2019).

In this study, the ethanolic extract of *A. paniculata* contains andrographolide by 3.00%. It is the main compound of *A. paniculata* responsible for its hypoglycemic activity. In the previous study, andrographolide was successful in lowering blood glucose levels by increasing glucose utilization and stimulating GLUT4 transcriptions (Yu et al., 2008; Nugroho et al., 2012), Langerhans enhancement is a condition that increases the number of β-cells, and increase pancreatic insulin levels (Zhang et al., 2009; Nugroho et al., 2014; Sari et al., 2015).

Quercetin, including flavonol, may increase antioxidant enzymes, decrease lipid peroxidation, reduce the intestinal absorption of glucose by inhibiting GLUT 2, block tyrosine kinase, and restore cell proliferation (Aba and Asuzu, 2018). Furthermore, it can lower plasma glucose because of its ability to regenerate pancreatic β-cells and increase insulin release (Vessal et al., 2003). In this study, ethanolic extract of *S. cumini* contained quercetin by 3.84%.

Brazilin, found in ethanolic extract of *C. sappan* plays a role in lowering blood glucose levels through several physiological pathways: increasing glucose transportation through GLUT4; affecting the enzymatic pathways in glucose metabolism, thereby increasing glucose oxidation, glucose-6 phosphate dehydrogenase activity, and glycolysis, reducing glyconeogenesis, and increasing insulin receptor function (Dapson and Bain, 2015). In this study, ethanolic extract of *C. sappan* contained brazilin by 4.74%.

3.2.3. *Effect of ASCE on lipid profiles*

The ASCE150 produced a significant decrease in total cholesterol level ($p < 0.05$) compared to the DC group (Table 2). The two treatments

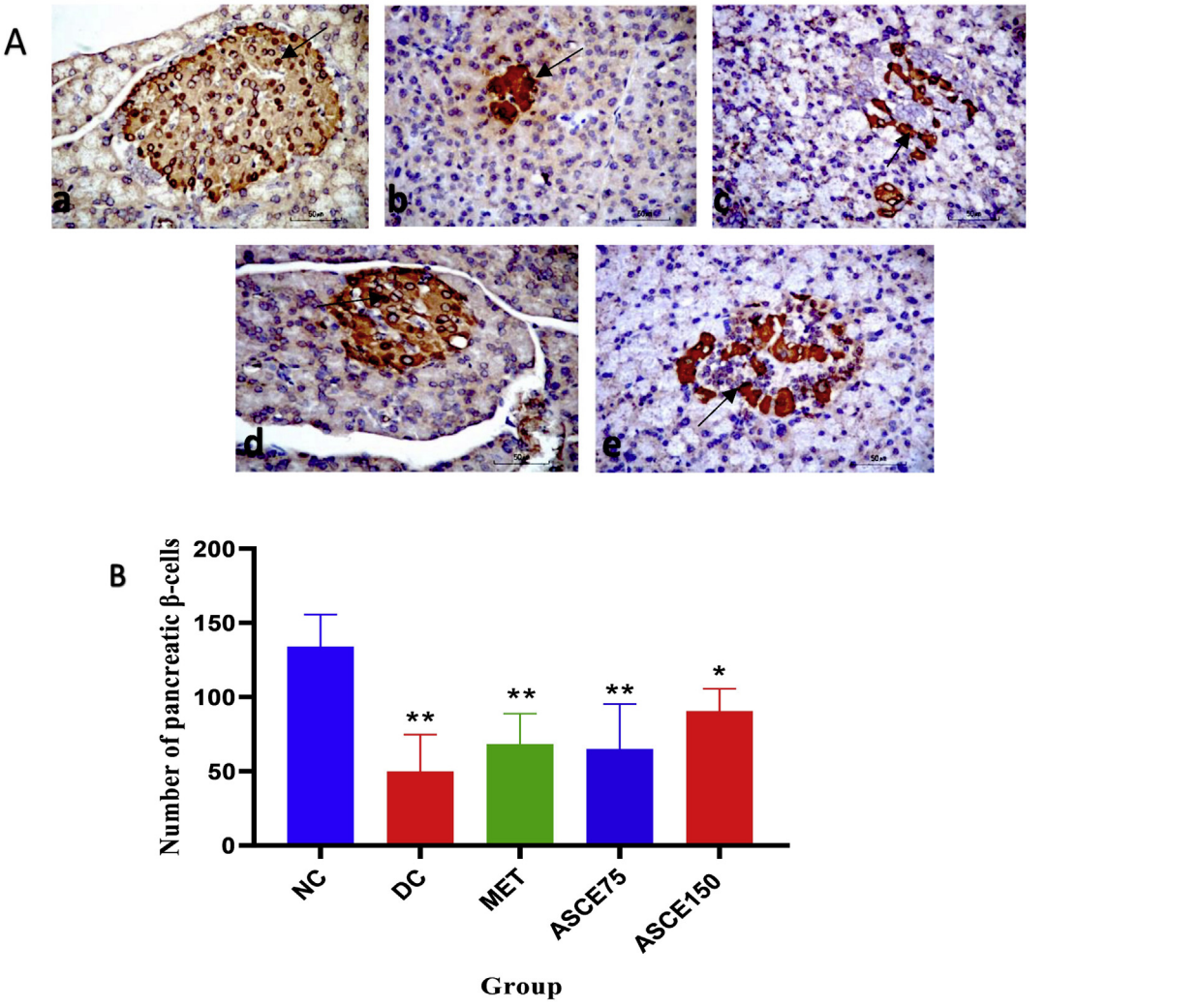

Figure 2. (A) Photomicrographs of immunohistochemical-stained sections of insulin rat pancreas. (a) Normal rat. (b) Diabetic rats. (c) Diabetic rats + metformin. (d) Diabetic rats + ASCE 75 mg/kg BW. (e) Diabetic rats + ASCE 150 mg/kg BW. → It is showed insulin immunoreactive pancreatic β-cells. —magnification 40x. The brown area indicates insulin staining (B) The number of pancreatic β-cells per 10 visual fields. Significantly different from NC group (*$p < 0.05$; **$p < 0.01$). Data are expressed as the mean ± SEM (n = 3).

Table 2. Lipid profile after treatment.

Group (n = 4)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
NC	83.43 ± 4.53	82.04 ± 1.92	76.60 ± 3.81	53.11 ± 3.66
DC	88.05 ± 4.83	95.54 ± 7.56	66.35 ± 3.64	54.36 ± 3.26
MET	73.13 ± 6.51	95.39 ± 7.43	68.14 ± 8.83	43.02 ± 4.53
ASCE75	72.30 ± 4.42	117.0 ± 6.20 **	59.15 ± 5.70 *	36.41 ± 4.01*#
ASCE150	70.56 ± 6.81#	107.4 ± 11.61 *	65.55 ± 0.87	37.50 ± 3.24*#

Significantly different from NC group (*$p < 0.05$; **$p < 0.01$) and significantly different from DC group (#$p < 0.05$). Data are expressed as the mean ± SEM (n = 4).

Table 3. Bodyweight of rats during the acute oral toxicity test.

Week	Sex	Body Weight of rats (g)			
		Control	ASCE 300	ASCE 2000	ASCE 5000
Week 0	Male	239.10 ± 3.06	240.70 ± 6.01	253.30 ± 5.47	255.00 ± 7.39
	Female	201.70 ± 5.82	190.70 ± 5.58	181.40 ± 2.46	196.20 ± 4.35
Week 1	Male	262.60 ± 3.34	261.50 ± 5.07	273.00 ± 11.16	275.60 ± 9.07
	Female	219.20 ± 7.06	198.7 ± 8.023 *	199.10 ± 1.18	211.70 ± 5.52
Week 2	Male	306.20 ± 3.73	306.3 ± 7.09	316.10 ± 8.91	323.20 ± 11.84
	Female	229.60 ± 7.01	218.10 ± 6.49	216.90 ± 1.21*	228.80 ± 2.64

Significantly different from control group (*$p < 0.05$).
Data are expressed as the mean ± SEM (n = 5). Control, 0.5% Na-CMC.

of ASCE75 and ASCE150 significantly decreased the LDL level ($p < 0.05$) compared to DC or NC groups. However, a significant increase in triglycerides level was observed in the treatment of ASCE75 and ASCE150 ($p < 0.01$, $p < 0.05$, respectively) compared to the NC group but not the DC group.

In this study, the use of the STZ induction did not affect the lipid profiles. The animal model often shows instability in diabetes due to different responses in the metabolic process (Goyal et al., 2016). Although the lipid profiles of the treatment group changed, all values were still within normal limits. Schofield et al. (2016) reported that metformin is not only a hypoglycemic agent but can also lower triglycerides and lipids. The phytochemical content of the ASCE combination plays a role in maintaining the lipid profiles. According to Nugroho et al. (2012), andrographolide plays an important role in hypoglycemic and hypolipidemic effects. Nugroho et al. (2013) stated that the combination of *A. paniculata* extract and *Centella asiatica* extract (70:30) synergizes blood cholesterol and HDL levels. The administration of *S. cumini* extract containing quercetin can affect lipid profiles, which include inhibition of LDL oxidation (dos Santos et al., 2018). Similarly, Dapson & Bain (2015) also reported that brazilin inhibits the accumulation of lipid droplets in adipocytes.

3.3. Evaluation of acute oral toxicity

3.3.1. Effect of ASCE on signs of toxicity, mortality, body weight, macroscopic of organ, and relative organ weight

The results showed there are no signs of toxicity, no symptoms of behavioral changes, and no death in any of the groups until the end of the study. This shows that the oral administration of a single dose of ASCE (1:1:1) of up to 5000 mg/kg BW was safe.

The rats with an average body weight of 239.10 ± 3.06 g and 201.70 ± 5.82 g, between the males and females were observed respectively. While, ASCE (300, 2000, and 5000 mg/kg BW) for the male groups were 240.70 ± 6.01 g; 253.30 ± 5.47 g; and 255.00 ± 7.39 g respectively, and the female rats were 190.70 ± 5.58 g; 181.40 ± 2.46 g; and 196.20 ± 4.35 g respectively. Meanwhile, during two weeks of observation, there was an increase in the bodyweight of all groups (Table 3). This shows that the content in the ASCE extracts is safe for treating disease. An increase in the bodyweight of experimental animals during the study is a sign of animal welfare, conversely, a decrease in the body weight can be a sign of toxicity (Adekola et al., 2020).

The average organ weight and as seen from the regular morphology, it showed no abnormalities in the macroscopic lesion recorded for all rats. The result shows no significant change in the relative organ weights of the heart, liver, and kidneys in all treatment groups compared to the control group ($p > 0.05$). It is suggested that oral administration of ASCE did not affect average growth and metabolism (Waty et al., 2017).

3.3.2. Effect of ASCE on hematological and biochemical profile

The treatment group showed insignificant changes in hematological parameters compared to the control group. These results suggest that ASCE may play a regular role in the production and development of blood cells. Hematological parameters can be used as toxicity markers because they are sensitive to toxic substances that enter living systems. Any damage to the hematological system of humans or animals can impair the function of the organs (Aamir et al., 2019).

The liver and kidneys play an important role in detoxification and excretion in the body (Ebohon et al., 2020). Therefore, liver and kidney function tests are necessary and reliable for toxicity studies. Plant extracts contain various phytochemical compounds that may react with enzymes in the liver and kidneys due to synergism (Aamir et al., 2019). The biochemical parameters of the treatment group (AST, ALT, CRN, and BUN) showed values that were not significantly different from the control group. These results indicate that oral administration of a single dose of ASCE (1: 1: 1) was safe up to a dose of 5000 mg/kg BW was safe and did not cause liver and kidney damages.

4. Conclusion

The combined treatment of ASCE in a 1: 1: 1 ratio for seven days in rats administered with HFD and STZ-induced potentially reduces fasting blood glucose levels and moderately improves pancreatic β-cells. In addition, the lipid profile was within the normal limits. ASCE combination of up to the dose of 5000 mg/kg BW was safe after oral administration of a single dose. Bodyweight, relative organ weight (heart, liver, and kidneys), hematological and biochemical parameters were not significantly different from the control group.

The combination of ASCE can be developed as a safe antidiabetic agent. Hence, further study is needed to examine the variations in the dose ratio of the ASCE and the antidiabetic mechanism of action of this combination. Also, further sub-chronic and chronic toxicology studies to confirm the safe use of ASCE are needed.

Declarations

Author contribution statement

Eem Masaenah: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Berna Elya, Heri Setiawan: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Zahra Fadhilah, Febrika Wediasari, Gumilar A. Nugroho: Conceived and designed the experiments; Performed the experiments.

Elfahmi, Tjandrawati Mozef: Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by KRUPT 2020 [Grant No. NKB-1/UN2.RST/HKP.05.00/2020] from Ministry of Research and Technology/National Research and Innovation Agency, Indonesia.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Aamir, K., Khan, H.U., Hossain, C.F., RejinaAfrin, M., Shaik, I., Salleh, N., Giribabu, N., Arya, A., 2019. Oral toxicity of arjunolic acid on hematological, biochemical and histopathological investigations in female *Sprague Dawley* rats. PeerJ 2019 (11), 1–24.

Aba, P.E., Asuzu, I.U., 2018. Mechanisms of actions of some bioactive anti-diabetic principles from phytochemicals of medicinal plants: a review. Indian J. Nat. Prod. Res. 9 (2), 85–96.

Adekola, M.B., Areola, J.O., Omisore, N.O., Asaolu, F.T., Ogunleye, S.G., Apalowo, O.E., Babalola, O.O., 2020. Sub-chronic toxicity study of ethanol stem-bark extract of *Blighia sapida* (Sapindaceae) in wistar rats. Heliyon 6 (2), e02801.

Athinarayanana, G., Ranjitsingh, A.J.A., Raja, A.U., Padmalatha, C., 2017. Toxicological studies of *Caesalpinia sappan* wood derived dye in Wister albino rats. Food Sci. Human Wellness 6 (1), 34–38.

Ayyanna, C., Sekar, M., Niranjan, R., Veera, P., 2015. Nephrotoxic effect of ethanolic extract of *Syzygium cumini.* Linn leaves on experimental animals. Int. J. Biol. Pharmaceut. Res. 6 (8), 678–683.

Chagas, V.T., Moraes, R., De Sousa, R., Gaspar, R.S., Abdalla, S., Mastrogiovanni, M., Trostchansky, A., 2018. Protective effects of a polyphenol-rich extract from *Syzygium cumini* (L.) Skeels leaf on oxidative stress-induced diabetic rats. Oxidative Med. Cell. Longev 2018, 1–14.

Chavan, V., Chache, T.K., Yadav, V., 2019. Simultaneous quantification of gallic acid , myricetin and quercetin in the extract of *Syzygium cumini* plant and its formulation using HPLC. Asian J. Pediatr. Pract. 5 (1), 85–88.

Clemmensen, C., Finan, B., Müller, T.D., DiMarchi, R.D., Tschöp, M.H., Hofmann, S.M., 2019. Emerging hormonal-based combination pharmacotherapies for the treatment of metabolic diseases. Nat. Rev. Endocrinol. 15 (2), 90–104.

Dapson, R.W., Bain, C.L., 2015. Brazilwood, sappanwood, brazilin and the red dye brazilein: from textile dyeing and folk medicine to biological staining and musical instruments. Biotech. Histochem. 90 (6), 401–423.

Deepa, J., Aleykutty, N., Harindran, J., 2018. Evaluation of combined effect of *Psidium guajava* and *Syzygium cumini* leaves on inflammation. Int. J. Pharm. Sci. 10 (4), 49–52.

dos Santos, M.M., Prestes, A.S., de Macedo, G.T., Ecker, A., Barcelos, R.P., Boligon, A.A., Barbosa, N.V., 2018. *Syzygium cumini* leaf extract inhibits LDL oxidation, but does not protect the liproprotein from glycation. J. Ethnopharmacol. 210, 69–79. February 2017.

Ebohon, O., Irabor, F., Omoregie, E.S., 2020. Sub-acute toxicity study of methanol extract of *Tetrorchidium didymostemon* leaves using biochemical analyses and gene expression in Wistar rats. Heliyon 6 (6), e04313.

Elfahmi, Woerdenbag, H.J., Kayser, O., 2014. Jamu : Indonesian traditional herbal medicine towards rational phytopharmacological use. J. Herb. Med. 2014, 1–23.

França, L.M., Fernando, C., Coêlho, F., Nara, L., Freitas, C., Letícia, I., Paes, D.A., 2019. *Syzygium cumini* leaf extract reverts hypertriglyceridemia via downregulation of the hepatic XBP-1s/PDI/MTP axis in monosodium L-glutamate-induced obese rats. Oxidative Med. Cell. Longev 2019, 1–14.

Furman, B.L., 2015. Streptozotocin-induced diabetic models in mice and rats. Curr. Protoc. Pharmacol. 70 (1), 5.47.1–5.47.20.

Gheibi, S., Kash, K., Ghasemi, A., 2017. A practical guide for induction of type-2 diabetes in rat : incorporating a high-fat diet and streptozotocin. Biomed. Pharmacother. 95 (24), 605–613.

Goyal, S.N., Reddy, N.M., Patil, K.R., Nakhate, K.T., Ojha, S., Patil, C.R., Agrawal, Y.O., 2016. Challenges and issues with streptozotocin-induced diabetes- A clinically relevant animal model to understand the diabetes pathogenesis and evaluate therapeutics. Chem. Biol. Interact. 244, 49–63.

Husna, F., Suyatna, F.D., Arozal, W., Purwaningsih, E.H., 2019. Model hewan coba pada penelitian diabetes. Pharmaceut. Sci. Res. 6 (3), 131–141.

King, A.J.F., 2012. The use of animal models in diabetes research. Br. J. Pharmacol. 166, 877–894.

Nugroho, A.E., Rais, I.R., Setiawan, I., Pratiwi, P.Y., Hadibrata, T., Tegar, M., Pramono, S., 2014. Pancreatic effect of andrographolide isolated from *Andrographis paniculata* (Burm.F.) Nees. Pakistan J. Biol. Sci. 17 (1), 22–31.

Nugroho, A.E., Andrie, M., Warditiani, N.K., Siswanto, E., Pramono, S., Lukitaningsih, E., 2012. Antidiabetic and antihiperlipidemic effect of *Andrographis paniculata* (Burm. f.) Nees and andrographolide in high-fructose-fat-fed rats. Indian J. Pharmacol. 44 (3), 377–381.

Nugroho, A.E., Lindawati, N.Y., Herlyanti, K., Widyastuti, L., Pramono, S., 2013. Anti-diabetic effect of a combination of andrographolide-enriched extract of *Andrographis paniculata* (Burm f.) Nees and asiaticoside-enriched extract of *Centella asiatica* L. in high fructose-fat fed rats. Indian J. Exp. Biol. 51 (12), 1101–1108.

Organization of Economic Co-operation and Development, 2001. The OECD Guideline for Testing of Chemical: 420 Acute Oral Toxicity-Fixed Dose Procudure. OECD., pp 1–14. December.

Preethi, P.J., 2013. Herbal medicine for diabetes mellitus : a review. AJP Res. 3 (2), 57–70.

Rosalie, I.O., El, E., 2016. Antidiabetic potentials of common herbal plants and plant products : a glance. Int. J. Herb. Med. 4 (4), 90–97.

Salehi, B., Ata, A., Kumar, N.V.A., Sharopov, F., Ramírez-Alarcón, K., Ruiz-Ortega, A., Sharifi-Rad, J., 2019. Antidiabetic potential of medicinal plants and their active components. Biomolecules 9 (551), 1–121.

Sari, K.R.P., Sudarsono, Nugroho, A.E., 2015. Effect of herbal combination of *Andrographis paniculata* (Burm . f) Ness and Gynura procumbens (Lour.) Merr ethanolic extracts in alloxan-induced hyperglycemic rats. Int. Food Res. J. 22 (4), 1332–1337.

Schofield, J.D., Liu, Y., Rayaz, P.R., 2016. Diabetes dyslipidemia. Diab. Ther. 7 (2), 203–219.

Setyaningsih, E.P., Saputri, F.C., Mun'im, A., 2019. The antidiabetic effectivity of Indonesian plants extracts via DPP-IV inhibitory mechanism. J. Young Pharm. 11 (2), 161–164.

Sindu, A., Saputri, F.C., Mun'im, A., 2019. Optimization of choline chloride-glycerol based natural deep eutectic solvent for extraction bioactive substances from *Cinnamomum burmannii* barks and *Caesalpinia sappan* heartwoods. Heliyon 5 (October), e02915.

Tanty, H., Permai, S.D., Pudjihastuti, H., 2018. In vivo anti-diabetic activity test of ethanol extract of the leaves of *Cassia Siamea* Lamk. Procedia Comp. Sci. 135, 632–642.

Vessal, M., Hemmati, M., Vasei, M., 2003. Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. Comp. Biochem. Physiol. C Comp. Pharmacol. Toxicol. 135 (3), 357–364.

Wang, Y.W., He, S., Feng, X., Cheng, J., Luo, Y., Tian, L., Huang, Q., 2017. Metformin : a review of its potential indications. Drug Des. Dev. Ther. 2017 (II), 2421–2429.

Waty, D.R., Saputri, F.C., Mun'im, A., 2017. Secondary metabolites screening and acute toxicity test of *Peperomia pellucida* (L.) Kunth methanolic extracts. Int. J. PharmTech Res. 10 (1), 31–38.

Wediasari, F., Nugroho, G.A., Fadhilah, Z., Elya, B., Setiawan, H., Mozef, T., 2020. Hypoglycemic effect of a combined *Andrographis paniculata* and *Caesalpinia sappan* extract in streptozocin-induced diabetic rats. Adv. Pharmacol. Pharmaceut. Sci. 2020, 1–9.

World Health Organization, 2016. Global Report on Diabetes. World Health Organization, France.

Widharna, R., Ferawati, Tamayanti, W., Hendriati, L., Hamid, I., Widjajakusuma, E., 2015. Antidiabetic effect of the aqueous extract mixture of *Andrographis paniculata* and *Syzygium polyanthum* leaf. Eur. J. Med. Plants 6 (2), 82–91.

Worasuttayangkurn, L., Nakareangrit, W., Kwangjai, J., Sritangos, P., Pholphana, N., Watcharasit, P., Satayavivad, J., 2019. Acute oral toxicity evaluation of *Andrographis paniculata*-standardized first true leaf ethanolic extract. Toxicol. Rep. 6 (August 2018), 426–430.

Xia, Z., Li, D., Li, Q., Zhang, Y., Kang, W., 2017. Simultaneous determination of brazilin and protosappanin B in *Caesalpinia sappan* by ionic - liquid dispersive liquid - phase microextraction method combined with HPLC. Chem. Cent. J. 1–11.

Yu, B.C., Chang, C.K., Su, C.F., 2008. Mediation of β -endorphin in andrographolide-induced plasma glucose-lowering action in type I diabetes-like animals. Naunyn-Schmiedeberg's Arch. Pharmacol. 377 (2008), 529–540.

Zhang, Z., Jiang, J., Yu, P., Zeng, X., Larrick, J.W., Wang, Y., 2009. Hypoglycemic and beta cell protective effects of andrographolide analogue for diabetes treatment. J. Transl. Med. 7 (62), 1–13.

Zhou, X., Razmovski-Naumovski, V., Kam, A., Chang, D., Li, C.G., Chan, K., Bensoussan, A., 2019. Synergistic study of a Danshen (Salvia Miltiorrhizae Radix et Rhizoma) and Sanqi (Notoginseng Radix et Rhizoma) combination on cell survival in EA.hy926 cells. BMC Compl. Alternative Med. 19 (1), 1–13.