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Research Article

Potency of Antidiabetic Effects of the Combination of Syzygium cumini and Andrographis paniculata in Rats with High-Fat Diet- and Streptozotocin-Induced Diabetes

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ABSTRACT

Andrographis paniculata (AP) and *Syzygium cumini* (SC) are known for their antihyperglycemic effects. However, the combined effects of these plants have not yet been assessed. This study evaluated the oral acute toxicity and *in vivo* antihyperglycemic effects of the extract combining AP and SC (SCAP) in rats with high-fat diet- and streptozotocin (STZ)-induced diabetes. Thirteen female DDY mice for toxicity test were divided into three groups and orally administered one dose SCAP (0, 300, or 2000 mg/kg). On day 15, animals were euthanized, their internal organs were observed, and blood samples were collected for clinical biochemistry analyses. *In vivo* antihyperglycemic activity was examined in male Sprague-Dawley rats-induced diabetes. Diabetic rats were assigned to once-daily oral treatment with metformin, AP, SC or SCAP for 1 week. Concerning toxicity, SCAP had no effects on liver and kidney and histology of these organs displayed no abnormalities. Blood glucose levels had a tendency to reduce in treatment groups compared with the findings in the diabetic control group. SCAP treatment protected rats against pancreatic damage. These results illustrated that the combined SCAP treatment had beneficial effects on blood glucose levels and pancreatic β-cell function, in rats-induced diabetes.

Key words: *Syzygium cumini*, *Andrographis paniculata*, Diabetes, Combination.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder associated with inadequate insulin production by the pancreas or the inability of the body to effectively use insulin to regulate blood glucose levels. Abnormal insulin levels in patients with DM result in metabolic disturbances, including high blood glucose levels and lipid metabolism changes, thereby increasing the risk of vascular disease.[1] A global study reported that the prevalence of DM is steadily increasing worldwide especially in Indonesia that projected diabetes patient from 10.3 million in 2017 to 16.7 million by year 2045.[1,2]

Insulin resistance (IR) and pancreatic β-cell failure contribute to the development of diabetes. Epidemiological risk factors such as a sedentary lifestyle, high-fat diet (HFD) consumption and genetic variation promote differences in the prevalence of diabetes among different countries. Some reports established IR as a predisposing element for dyslipidemia, which is described by increased total cholesterol (TC), triglyceride (TG) and low-density lipoprotein (LDL) levels accompanied by decreased high-density lipoprotein (HDL) levels.[3]

Syzygium cumini (SC, family: *Myrtaceae*), known as jamblang in Indonesia, is a tropical plant found across Southeast Asia, including Indonesia. The plant is rich in anthocyanin, glucoside, isoquercetin, kaempferol and myricetin.[4] Traditionally, SC has been used to treat conditions such as cough, dysentery and inflammation, in addition to DM.[4,5] Myricetin, as one of the bioactive chemical constituents in *Syzygium* spp., has been reported to have various pharmacological activities,[6,7] including anti-inflammatory,[8] anti-cancer effects[9,10] and antidiabetes.[11]

Andrographis paniculata (AP), which is known as sambiloto, is one of the most popularly used medicinal herbs in Indonesia. This plant also grows in many other Asian countries such as China, India, Thailand and Sri Lanka. It is mainly known for its enormously bitter properties and it is used traditionally as a therapy against fever, common cold and inflammation. Andrographolide, the main bioactive chemical constituent of AP, has been reported to have various pharmacological activities, including antidiabetic.[12]

The AP and SC had an activity for anti-diabetes through different bioactive constituents. The combination was documented in "Cabe Puyang" manuskrip,[13] this manuskrip documented many Indonesian herbs for medical purposes, but there was no scientific report found. The present study evaluated the potential antidiabetic activity of combined AP and SC (SCAP) extract in rats with HFD- and streptozotocin (STZ)-induced diabetes. The effects of SCAP were compared with those of the standard diabetes therapy metformin.

MATERIALS AND METHODS

Materials and chemicals

STZ (Wako Fujifilm, Japan), sodium citrate monohydrate, ethanol (Brataco, Indonesia), phosphate-buffered saline (PBS), citric acid (Merck, Indonesia), metformin (Kimia Farma, Indonesia),

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formaldehyde (Brataco, Indonesia), carboxymethylcellulose (Brataco, Indonesia) and sodium hydroxide (Merck, Indonesia) were obtained from commercial suppliers. Meanwhile, an Accu-Chek Active glucometer was purchased from Roche (Germany).

Collection of plant materials

AP was collected from Wonogiri, Jawa Tengah, Indonesia. SC leaves were collected from Jakarta, Indonesia. All plant materials were determined by the Indonesian Institute of Science Research Centre for Plant Conservation and Botanic Gardens (Bogor, Indonesia) and given a voucher specimen number (B-2404/IPH.3/KS/VII/2019).

Extraction

The dried herb of AP (500g) and dried leaves of SC (500g) were grounded into fine powder. Each herbs powder was extracted using 3 L of 70% ethanol *via* maceration process in room temperature (25° ± 2° C) for 24 hours with constant stirring as stated in Indonesian Herbal Pharmacopoeia.[14] Both of the yielded extracts were concentrated via evaporation using rotary evaporator and stored in an airtight container at 4°C for further study. All extracts were suspended in 0.5% CMC on the day of the experiment and administered *via* oral gavage.[15]

Experimental animals

All experiments using animals were performed according to an ethical agreement (KET-832/UN2.F1/ETIK/PPM.00.02/2019) that was issued by the Ethics Committee of the Faculty of Medicine, University of Indonesia. In total, 13 female DDY mice[15] and 25 male Sprague-Dawley rats were obtained from the National Agency of Drug and Food Control (NADFC), Indonesia. Total rats used is based on degree of freedom in ANOVA test. Animals were kept at Animal House, Rumpun Ilmu Kesehatan, University of Indonesia and allowed to acclimatize for one week before study under a 12-h/12-h light/dark cycle, the temperature of 23 ± 3°C and relative humidity of 50-70% with free access to standard chow food and water.

Acute oral toxicity of SCAP

Acute oral toxicity testing was performed following the NADFC guideline.[14] DDY mice (8–10 weeks old) were randomly assigned to control group (n = 5); 300 mg/kg (n = 3); and 2000 mg/kg SCAP (n = 5). One dose was administered orally after an overnight fast. Toxicity signs were observed for 14 days. Bodyweight was recorded before dosing and after 7 and 14 days of dosing. On day 15, all mice were fasted overnight, anesthetized (intraperitoneal injection of ketamine [100 mg/kg] and xylazine [10 mg/kg]), to obtain blood samples via cardiac puncture and sacrificed with overdose combination of ketamine and xylazine. The blood serum was used to analyze liver and kidney function.

Induction of diabetes

Male Sprague-Dawley rats (8-9 weeks old) were used in this experiment because this strain has been commonly used to assess antihyperglycemic effects of herbal medicines. As a control group, five rats were fed a standard pellet diet. The remaining 30 rats were fed an HFD (Biofarmaka, Bogor) containing 20.07% fat, 17.78% protein and 2.75% fiber. After 3 weeks of HFD feeding, rats were fasted overnight and intraperitoneally injected with STZ (35 mg/kg dissolved in 0.1 M citrate buffer [pH 4.5]) and this treatment was repeated after 7 days. At 72 h after the second STZ dose, blood glucose levels were measured in blood obtained *via* the tail vein after an overnight fast using glucometer. Rats were monitored for 10 days, and those with fasting blood glucose levels exceeding 200 mg/dl were considered diabetic as described in previous research.[16] Rats consumed the HFD until the end of the study.

Experimental design

Rats were randomly assigned into five groups (n = 5) that were orally treated once daily for 7 consecutive days as follows:

Group 1, normal control rats (C) treated with 0.5% CMC;

Group 2, diabetic rats (DM) treated with 0.5% CMC (diabetic control);

Group 3, diabetic rats treated with metformin (Met) (250 mg/kg);[17]

Group 4, diabetic rats treated with 100 mg/kg SCAP (SCAP100);

Group 5, diabetic rats treated with 200 mg/kg SCAP (SCAP200).

All doses of individual extract and combination were submaximal doses (blood glucose levels were monitored using a glucometer). On day 7, all treated rats were anesthetized (intraperitoneal injection of ketamine [80 mg/kg] and xylazine [10 mg/kg]) to collect blood samples *via* cardiac puncture. Subsequently, the rats were sacrificed with overdose combination of ketamine and xylazine to obtain the pancreas and white fat for examination. Blood serum were analyzed for total cholesterol, triglyceride, HDL and LDL using assays kits purchased from Biomaxima (Poland). The methods of diabetes induction experimental design were also referring to.[18]

Immunohistochemical and histopathological examinations

Immunohistochemical staining for counting β-cells of pancreatic tissue and histological examination of pancreatic tissue were performed by an experienced pathologist at Primata Animal Study Center, IPB, Bogor, Indonesia. Pancreatic and adipose tissues were collected immediately after sacrificing animals, washed with phosphate buffer saline and directly fixed with 10% formaldehyde. The pancreas and adipose slices were stained using hematoxylin and eosin (H&E).

Statistical analysis

Analysis of variance and Fisher's least significant difference post hoc test were used to identify significant differences among the groups. Results are presented as the mean ± SEM and $p < 0.05$ denoted statistical significance.

RESULT

SCAP was safely administered up to a dose of 2000 mg/kg, as no mortality or toxic effects were observed in the treated animals. Table 1 presents the body weight and organ weight for each treatment group. Increased body weight was observed in all treatment groups, but a significant difference versus the control was only observed in the 2000 mg/kg SCAP group ($P < 0.05$). The weights of all harvested organs were normal and ROW between the control and treatment groups exhibited non-significant variations (Table 1). Biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine concentration were not different between the control and treatment groups (Table 1).

Table 2 presents fasting glucose levels among the groups. Rats induced diabetes shows diabetic criteria with fasting blood glucose (FBG) levels above 200 mg/dL, although the differences of FBG between group was high, thus the observation of FBG also referred to before and after therapy. FBG were statistically not significantly different in treatment groups than in the diabetic control group (DM), although there was a tendency to decrease FBG levels in Met and SCAP100 groups. Table 3 presents the lipid profiles of the groups. Total cholesterol levels tended to be lower in the Met and SCAP100 groups compared to control group. There are no significant different among groups for triglyceride, HDL and LDL.

The number of pancreatic β-cells was higher in the normal control group than in the diabetic control group. Meanwhile, although some

Table 1: Body weight, organ weight and biochemical parameters of acute oral toxicity "test".

Parameters	Groups		
	Control	SCAP 300	SCAP 2000
BW-0	19.74 ± 0.75	19.97 ± 0.15	19.34 ± 0.47
BW-14	20.40 ± 0.68	24.73 ± 1.82	27.82 ± 1.03*
ALW	1.060 ± 0.03	1.39 ± 0.02	1.512 ± 0.05
AHW	0.100 ± 0.00	0.11 ± 0.01	0.112 ± 0.01
AKW	0.126 ± 0.01	0.15 ± 0.00	0.150 ± 0.01
RLW	0.052 ± 0.00	0.054 ± 0.00	0.055 ± 0.00
RHW	0.005 ± 0.00	0.004 ± 0.00	0.004 ± 0.00
RKW	0.006 ± 0.00	0.006 ± 0.00	0.005 ± 0.00
AST (U/L)	140.56 ± 10.14	128.35 ± 24.00	140.99 ± 3.22
ALT (U/L)	150.36 ± 16.91	95.68 ± 22.46	78.83 ± 6.51
Creatinine (mg/dL)	0.90 ± 0.04	1.00 ± 0.40	1.14 ± 0.28
Blood ureum (mg/dL)	66.32 ± 2.24	61.52 ± 8.40	72.65 ± 4.20

Weight in grams; BW-0, body weight before treatment day-0; BW-7, bodyweight after treatment day 14; ALW, absolute liver weight; AHW, absolute heart weight; AKW, absolute kidney weight; RLW, relative liver weight; RHW, relative heart weight; RKW, relative kidney weight; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Data presented in mean±SEM, significantly different from Control "(*p<0.05)".

Table 2: Fasting blood glucose level at day-0 and day-7 after "treatment".

Groups	FBG (mg/dL)		
	D-0	D-7	ΔFBG
C	79.5±2.8	77.5±2.8	2.0±3.4
DM	284.0±24.5	374.8±11.	-97.8±55.9
Met	396.0±14.6	332.0±22.1	110.6±46.1
SCAP100	325.6±26.2	306.8±52.1	18.8±41.26
SCAP200	221.8±28.8	331.0±3.9	-109.6±23.04

The result presenting in mean±SEM. D-0, day-0 before treatment. D-7, days-7 after treatment.

Table 3: Lipid profile of rats after "treatment".

Groups	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
C	90.12 ± 3.45	86.74 ± 1.73	79.00 ± 3.80	55.05 ± 3.43
DM	89.61 ± 4.06	92.98 ± 6.39	72.82 ± 3.69	57.58 ± 0.17
Met	87.80 ± 11.48	95.95 ± 5.78	70.50 ± 7.24	49.21 ± 4.82
SCAP 100	88.06 ± 5.71	119.0 ± 16.09	75.58 ± 4.29	48.87 ± 5.05
SCAP 200	89.29 ± 5.14	136.50 ± 15.13	74.66 ± 4.66	45.77 ± 3.22

The result presenting in mean±SEM. Significantly different from C (# p<0.05; ## p<0.01), significantly different from DM "(*p<0.05; **p<0.01)".

treatments increased pancreatic β-cell counts versus the diabetic control group, the values remained significantly lower than those in the normal control group. Among the various treatment groups, pancreatic β-cell counts were highest in the SCAP200 group (Figure 1b). In the normal control group, the pancreatic section featured normal islets, a normal pancreatic structure and normal cell sizes (Figure 1a). Meanwhile, less pancreatic cell impairment was observed in the metformin and SCAP groups than in the diabetic control group. In addition, 200 mg/kg SCAP provided better pancreatic protection than 100 mg/kg SCAP. Figure 2, shows the histological H&E staining of pancreas islet section. Groups that received treatment with SCAP showed amelioration of pancreatic islet.

DISCUSSION

The administration of AP and SC extracts alone is known to provide many benefits in diabetes therapy. In previous studies, AP extracts were known to have several therapeutic effects, such as protecting pancreatic β-cells, lowering blood glucose levels and stimulating translocation of glucose transporter subtype 4 (GLUT4) to increase glucose utilization.[18] SC extracts are also known to have anti-dyslipidemia effects, save pancreatic β cell activity and increase insulin sensitivity.[3] This study is a series of studies related to the combination of *Andrographis paniculata, Syzygium cumini*, and *Caesalpinia sappan* as an alternative therapy for diabetes treatment.

As a combination, it is crucial to examine toxicity of SCAP prior to their safety use in laboratory animals. Therefore, we examine oral acute toxicity of SCAP in female DDY mice for getting safe dosage for preclinical study on DM model. NADFC guide states that using only one species and usually the female. Result in the profile of AST, ALT, urea and creatinine level in mice showed that SCAP combination not toxic in organ level that acts as a particular test and assures serum biochemistry test as important parameters for toxicity in the study of phytochemistry. According to NADFC guide in acute toxicity study in which at a given dose 2000 mg/kg no death occurred in one or more in one group, the LD_{50} of combination SCAP can be considered greater than 2000 mg/kg.[15]

The DM group showed worsening with increasing FBG during the treatment period. This indicates that the animal model induction of diabetes shows a stable increase in blood sugar levels without treatment. The metformin standard drug administration in the MET group showed a significant decrease in blood glucose levels compared to the DM group so that standard treatment had an effect on lowering blood glucose levels. A decrease in FBG was seen in the group receiving the standard drug and low dose combined extract (SCAP100), although the decrease was not statistically significant. Treatment with SCAP for 7 days has not been able to reduce blood glucose levels to near normal (100 mg/dL), as well as therapy with the standard drug metformin, so there is a need for further studies by adding SCAP treatment time.

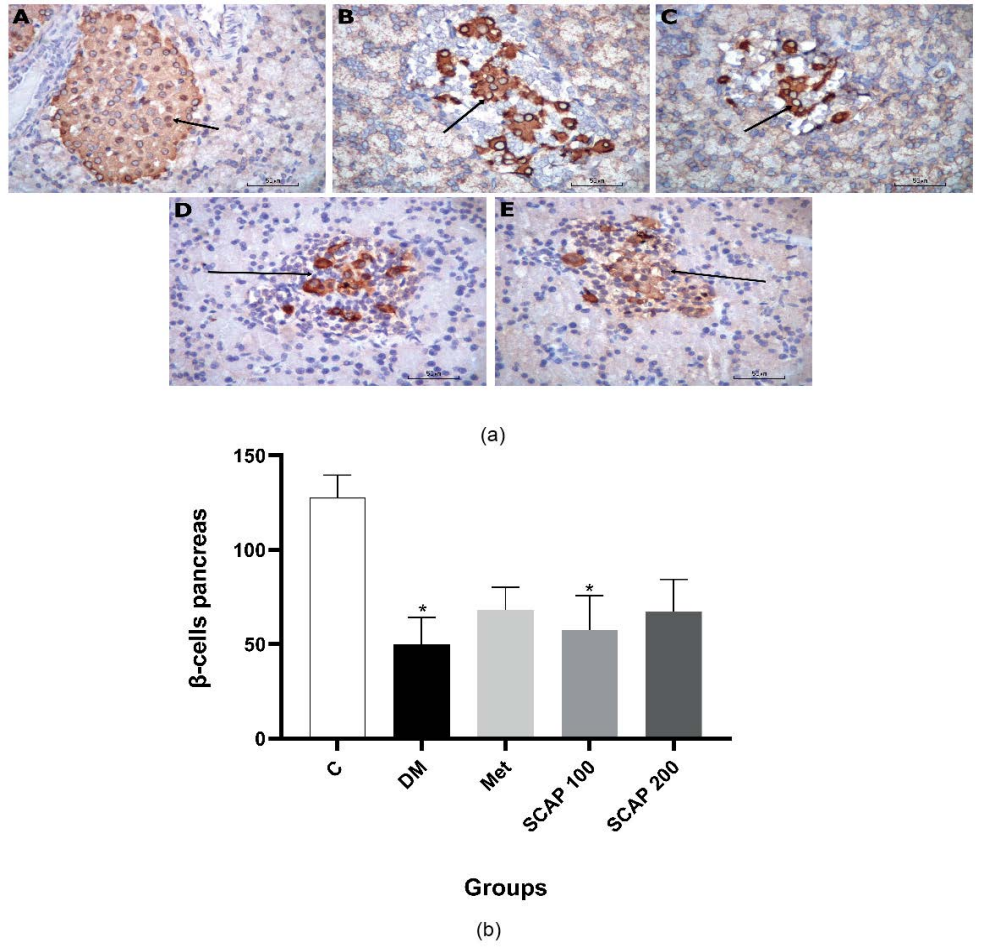

Figure 1: (a) Imunohistochemistry staining of section of pancreatic β-cells, 40x. (A) Section of pancreatic β-cells from control group; (B) Section from pancreas of group DM showing less density of pancreatic β-cells; (C) Section of pancreatic β-cells from group MET; (D) Section of pancreatic β-cells from group SCAP100; (E) Section of pancreatic β-cells from group SCAP200. (b) Number of pancreatic β-cells in all groups after treatment. Result was present in mean±SEM. Significantly different from C "(* $p<0.05$)".

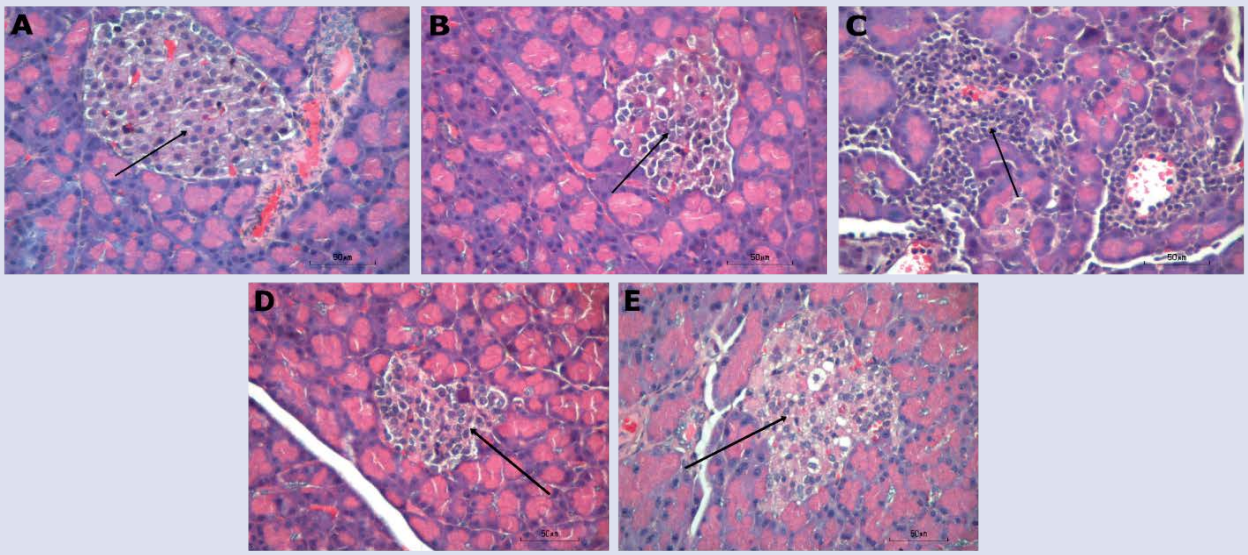

Figure 2: Histological study of pancreatic islet, H&E 40x. (A) Section of pancreas from control group; (B) Section of pancreas from group DM; (C) Section of pancreas from group MET; (D) Section of pancreas from group SCAP100; (E) Section of pancreas from group SCAP200.

STZ induction in diabetic animal models showed that lipid profiles were not significantly different between the DM group and normal controls. In the SCAP treatment group, the lipid profile tended to be the same compared to normal controls. In general, the administration SCAP extracts did not show improvement in lipid profiles in diabetic animal models. The administration of SCAP at a dose of 100 mg/kg BW has effects in lowering TC levels in diabetic rats. The administration of SCAP extract at a dose of 100 and 200 mg/kg BW showed side effects in increasing TG levels in diabetic rats compared to untreated DM rats. There is a dose response relationship in administering a SCAP extract so that when given in small amounts, the effect of a SCAP extract will decrease TG levels. In larger doses, the SCAP extract will increasing the TG level. This dose-response effect of lowering TG levels was also observed in studies using obese rats.[19] The effect of SC extract alone at higher doses on hypertriglyceridemia is influenced by the level of flavonoids, especially myricetin which has an effect on increasing PPARγ protein expression, allowing the storage and mobilization of lipids, in glucose metabolism.[20] There is a need further study to analyse the interaction between AP and SC in increasing the TG level.

Animal models of diabetes-induced with STZ showed a decrease in the number of β-pancreatic cells without causing death. This decrease in cell numbers is caused by STZ which damages some β-pancreatic cells.[21] Metformin administration in diabetic rats showed an increase in the number of β-pancreatic cells. The administration of SCAP at a dose of 200 mg/kg BW gave the effect of increasing the number of cells that was higher than that of the combination of SCAP extract at a dose of 100 mg/kg BW. There is dose related effect on SCAP extract to have a proliferative effect on increasing the number of β-pancreatic cells. The combination of SCAP in this study is also useful as a barrier to the rate of deterioration of β-pancreatic cell damage in diabetic animal models.

The combination of SCAP contain of many phytochemical constituents, such as flavonoids, alkaloids, triterpenoids, and saponins.[11,12] The anti-glycemic effects of SCAP may be due to single or combination of phytochemical constituents that affects blood glucose levels. High levels of flavonoids and triterpenes, which could act synergistically and/or independently to stimulate insulin production followed by pancreatic β-cell repair or inhibition of glucose absorption by the intestinal system.[22]

CONCLUSION

The combination of SCAP doses of 100 and 200 mg/kg BW did not show superiority over the use of AP or SC single extracts on the antihyperglycemic effect. The combination of SCAP and single extract of AP or SC, has the potential for antihyperglycemic although it is lower than that of metformin. The administration of a single 100 mg/kg BW AP extract showed a better pancreatic beta cell repair effect than metformin. The single extract of AP, SC and the combination of SCAP tends to increase serum lipid levels and increase the number of adipose fat cells.

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CONFLICTS OF INTEREST

The authors state that they do not have any conflicts of interest.

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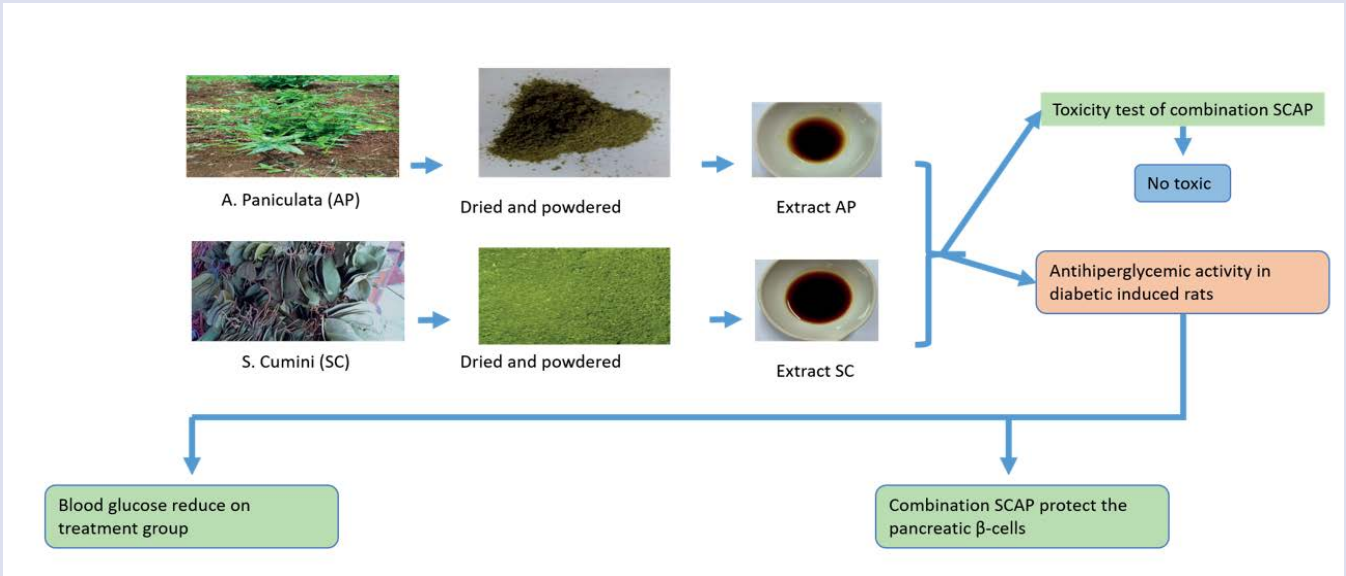
GRAPHICAL ABSTRACT



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