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Antidiabetic Effect of the Aqueous Extract Mixture of *Andrographis paniculata* and *Syzygium polyanthum* Leaf

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Authors' contributions

This work was carried out in collaboration between all authors. Author ECW designed the study in general, performed the statistical analysis, wrote the protocol and managed the literature searches. Author RMW designed the study of oral glucose tolerance, performed the laboratory analysis for the oral glucose tolerance test and wrote the first draft of manuscript. Authors WDT and F designed the study of the antidiabetic effect and the toxicity test, performed the laboratory analyses for the test. Author ISH designed the study of histopathology. Author LH prepared the plant material and performed laboratory analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the oral glucose tolerance effects on normal rats and the antidiabetic activity of extract mixture of *Andrographis paniculata* and *Syzygium polyanthum* on alloxan-induced diabetic rats.

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Study Design: Seventy-two normal male rats were randomly distributed into six groups for the oral glucose tolerance test and six groups for the antidiabetic test. Group 1 to 3 were control groups, Group 4 and 5 were treated with the single extracts of *Andrographis paniculata* and *Syzygium polyanthum*, respectively, at dose of 200 mg/kg b.w. Group 6 was administered with the extract mixture at dose of 200 mg/kg b.w.
Place and Duration of Study: This work is carried out in Faculty of Pharmacy and Research Center for Traditional Medicine, Widya Mandala Catholic University, Surabaya, between March 2011 and February 2012.
Methodology: Oral glucose tolerance test was performed by collecting blood sample for the tail of each animal at 0 to 150 mins and the blood glucose levels were estimated. The antidiabetic effect of the extract mixture was studied on diabetic rats. Blood samples were collected and the fasting blood glucose levels were estimated. The pancreas were excised and fixed in formalin for histopathology.
Results: The effects of the extract mixture on glucose tolerance tests were significant ($p \leq 0.05$) and the hypoglycemic effects were not observed from 30 to 120 min after treatment. The treatment of alloxan-induced diabetic rats with the extract mixture (200 mg/kg) for 14 days also produced significantly lower blood glucose levels compared to thoses of the groups treated the single extracts. Moreover, the extract feeding showed definite improvement in the histopathology of islets and the plant extracts have not produced any toxic sympton within the treated animals.
Conclusion: The results suggest the extract mixture to be beneficial for treatment of type 2 diabetes without toxicity.

Keywords: Alloxan-induced diabetic; oral glucose tolerance test; diabetes; Andrographis paniculata; Syzygium polyanthum; extract mixture.

1. INTRODUCTION

Despite all the advances in therapeutics, diabetes still remains a leading cause of morbidity and mortality in the world. Diabetes mellitus is potential to be the cause of kidney, liver and heart disease, where coronary heart disease and vascular damage increase twice to four times due to diabetes. These diseases are responsible of 50-80% of the diabetes patient mortality [1-4]. In Indonesia, the incidence of diabetes keeps increasing annually and diabetes mellitus is a chronic disease with high prevalence [5]. As a country rich in biodiversity, Indonesia offers many plants with antidiabetic potential that have great importance both professional and economical point of view. One of these plants is *Andrographis paniculata* that has long been used in *jamu pahitan* (Indonesian traditional medicine) used for years to maintain health and to treat many health problems such as itching and diabetes, lack of appetite, eliminating of body odor, reducing cholecterol, abdominal bloating, acne and dizziness [6,7].

In the recent years, the various pharmacology activities of *Andrographis paniculata* either alone or in combination with other medicinal plants have been examined preclinically, and the results supported the clinical trial in human [8-16]. Many bioactive compounds of *Andrographis paniculata* that have been isolated and identified indicated beneficial health effect for complex disease such as diabetes [16-21]. Its major constituents are lactones, diterpenoids, diterpene glycosides, flavonoid, and flavonoid glycosides [9,18,19]. Among those, diterpene lactone andographolide was reported to posseess antidiabetic activity and showed α-glucosidase inhibitory effects in a concentration manner [22-24]. Beside lowering blood glucose, *Andrographis paniculata* can preserve pancreatic beta cells at the same time [25]. While antidiabetic potential of *Andrographis paniculata* has been investigated widely in streptozocin- or alloxan-induced diabetic animals, the antidiabetic potential of *Syzygium polyanthum* (Sp) or Indonesian bay leaf has not been studied intensively [26,27].

Syzygium polyanthum herb empirically was said to have cleanse heat, to detoxify toxins, to dry moist, especially in fever therapy, headache, cough due to hot lungs, inflammation / pain of throat, dysentry, pain or heat sensation when urinating, eczema, etc [28]. In Indonesia, *Syzygium polyanthum* is used as anti-inflammatory medicine, antipiretic, and to detoxify toxins. Meanwhile, root and leaves are used to cure the bite of snake and insects in India [29].

Syzygium polyanthum consists of essential oil (citral, eugenol), tannin and flavonoid [26,27]. It is proven to cure diarrhea in mice, which was observed in amount, consistency of the feces, and the duration of the diarrhea [30]. The water extract also lowers cholesterol in rats heart cell culture [31]. Pidrayanti also found the anticholesterol effect in Wistar rats [32]. Both of the researches indicated significant effect of the *Syzygium polyanthum* extract compared to negative control. Another pharmacological effect of *Syzygium polyanthum* infuse is lowering uric acid in male mice induced by potassium [33,34]. Although the effect is not similar to allopurinol as positive control, the infuse lowered uric acid level compared to the negative control. Antioxidant activity of *Syzygium polyanthum* is found to be highest when extracted with combination of methanol-water. Antihyperglycemia activity test of *Syzygium polyanthum* has been conducted in alloxan-induced mice. Extract administration for 7 days significantly lowered blood glucose level the most compared to control [35]. Then, the hypoglycemia activity of the ethanolic extract was also tested in rabbits by Glucose Tolerance Test (GTT) [36]. Besides showing significant hypoglycemic effect, it was suggested that the mechanism of the blood glucose decrease was different from *Andrographis paniculata*. The evaluation of *Syzygium polyanthum* leaves absorption was observed by analyzing the metabolites in feces, blood and urine [37].

Realizing the beneficial mechanisms of action and effects, this opens up the chance to combine both of the plants to have a synergistic effect. The main objectives of the present investigation were to evaluate the antidiabetic effect of mixture of aqueous extract of *Andrographis paniculata* and *Syzygium polyanthum* leaves on oral glucose tolerance test and on alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All the chemicals and reagents used for this study were of analytical grade. To check the blood glucose level, Accu Check Advantage glucometer and Accu Check Advantage stick from Roche was used.

2.2 Plant Materials

Andrographis paniculata and *Syzygium polyanthum* leaf were collected in the area of Pandaan, East Java, Indonesian in the summer 2010. The plant was identified and autheticated by a Taxonomist of PT Natura Laboratoria Prima, Pandaan, Indonesia. Voucher specimen was prepared and deposited in the Herbarium with voucher No: P05SR014.01 and P04SR072.00 for *Andrographis paniculata* and *Syzygium polyanthum* respectively.

2.3 Preparation of Aqueous Extract of *Andrographis paniculata* and *Syzygium polyanthum*

The collected *Andrographis paniculata* and *Syzygium polyanthum* leaf were washed, rinsed, blotted, sliced and ground. The extraction process was carried out at 80°C for 4 hours in ratio of plant to water 1:4. The extract was filtered, concentrated, evaporated *in vacuo* (50-60 mmHg), and mix with maltodextrin. The drying process was performed using spray drying technology in PT. Natura Laboratoria Prima, Pandaan, East Java, Indonesia. The powder yield of aqueous extract was approximately 10% of its dried weight.

2.4 Animals

Eighty-four healthy male Wistar rats, weighing between 100 and 150 gram were obtained from UD Wistar, Yogyakarta and certified by drh. Slamet Raharjo, MP from Gajah Mada University. Animals were maintained under standar animal house condition, fed with a standard diet and allowed water *ad libitum*. The study was approved by ACUC (Animal Care and Use Committee) of Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia with approval number 084-KE.

2.5 Oral Glucose Tolerance Test

The oral glucose tolerance test was performed on thirty-six overnight (16 h) fasted rats that were divided into six groups (n = 6). Group 1 (control) received drinking water. Group 2 and 3 received the standard drug glibenclamide (0.45 mg/kg) and metformin (63 mg/kg), respectively. Group 4 and 5 received the single extracts of *Andrographis paniculata* and *Syzygium polyanthum* respectively. Group 6 received the extract mixture of *Andrographis paniculata* and *Syzygium polyanthum* at dose of 200 mg/kg b.w. in ratio of 6:1. This dose and ratio of extract mixture were determined from a preliminary dose–response study evaluating the effects of 100, 200 and 400 mg doses of the single extract

of *Andrographis paniculata* on oral glucose tolerance test in Wistar rats. Then, the optimum effective dose of 200 mg/kg was used for a ratio-response study evaluating the effect of 6:1, 2:1, 1:2, 1:6 ratio of the extract mixture of *Andrographis paniculata* and *Syzygium polyanthum* on oral glucose tolerance test and on fasting glucose level in diabetic Wistar rats (data not shown). Glucose (2 g/kg) was fed 30 min after the administration of the samples, and then blood was collected by tail snipping at 30, 60, 90, 120, and 150 minutes after glucose load and the blood glucose levels were estimated. The glucose level data was used to compare glucose tolerance of each group.

2.6 Induction of Diabetes

Diabetes was induced by a single intravenous injection of 150 mg/kg of alloxan monohydrate (dissolved in 0.9% NaCl) to thirty-six overnight fasted rats. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 10 percent glucose solution orally after seven hours of alloxan induction to the next three days. The animals were fed standard pellets after the injection. The fasting blood glucose level was measured after 24 hr of injection. The threshold value of fasting blood glucose to diagnose diabetes was taken as > 126 mg/dl.

2.7 Experimental Procedure for Antidiabetic Activity

The alloxan diabetic rats were divided into six groups of six rats each. Feed and water were provided *ad libitum* to the animals. The first untreated healty (control) and second group (diabetic rats) received drinking water. The third group was administered daily metformin (63 mg/kg orally) which served as the positve control. Group four to six received 200 mg/kg/day a single extract *Andrographis paniculata, Syzygium polyanthum* and the extract mixture in ratio of 6:1 respectively. Blood samples were collected by cutting the tail-tip of the rats, for blood glucose determination measured on days 0, 7 and 14 following the treatments and rats were weighed. The whole pancreas from each animal was removed after killing the animals on the 14th day, was placed in 10% formaline solution, and processed by the paraffin technique. Sections of 5 µm thickness were cut and stained by haematoxylin and eosin (H&E) for histological examination.

2.8 Acute Toxicity Study

The extract mixture was studied for oral toxicity in twelve Wistar male rats at a single dose of 2000 mg/kg b.w. The animals were starved overnight and divided into two groups (n = 6). Group 1 served as the untreated healthy control group. Group 2 was fed orally with the extract mixture 6:1 at dose level of 2000 mg/kg. The animals were observed individually after dosing once during the first 30 min, 1h, 2h, 4h and 24 h for behavioral profile (restlessness, dullness, agitation, irritability), neurological profile (spontaneous activity, reactivity, touches response, pain response, and gait) and autonomic profile (defecation and urination). The surviving animals were monitored daily for 2 weeks for signs of acute toxicity. Recovery and weight gain were seen as indications of having survived the acute toxicity. At the end of 14 days, all surviving animals were sacrificed, the blood was collected via cardiac puncture, and the internal organs examined macroscopically for pathological changes compared to the control group.

2.9 Statistical Analysis

Results was expressed as Mean ± SEM. The dose response and toxicological data were analyzed by ANOVA followed by post hoc Tukey HSD. Results were considered to be significant at the level of $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Oral Glucose Tolerance Test

The effect of single extracts and extract mixture of *Andrographis paniculata* dan *Syzygium polyanthum* on oral glucose tolerance test are shown in Fig. 1. The mean blood glucose levels of control group were significantly higher at all time of glucose challenge i.e. at 30, 60, 120, and 150 minutes compared to all other groups. The highest increase of blood glucose levels at 30 minutes due to an oral glucose administration of a glucose load was on control group (91%) and the lowest on extract mixture treated group (12%). All the treated groups showed significantly lowered blood glucose levels at 30 minutes. A significant decrease in blood glucose levels for control group was observed after 90 minutes and even at 150 minutes its blood glucose levels were still significantly higher compared to the starting blood glucose levels at 0 minute, whereas the blood glucose levels of the other groups decreased significantly sooner

i.e. after 60 minutes. The blood glucose levels in glibenclamide treated group went down the normal after 90 minutes. Previous studies indicated that glibenclamide known as one of the leading drugs from sulphonyl ureas category is commonly associated with hypoglycemic side effects [38,39].

Andrographis paniculata extract showed antidiabetic effect, similar to metformin. The previous *in vivo* and *in vitro* studies indicated that alpha-glucosidase inhibition may possibly be one of the mechanisms for the Ap extract to exert antidiabetic activity [23,40]. Nevertheless, the extract of *Syzygium polyanthum* and the extract mixture demonstrated significantly ($p<0.01$) more effective lowering effect on blood glucose level at 30 min the single extract of *Andrographis paniculata*. Moreover, the extract mixture that showed the most effective blood glucose controlling reduced significantly the increase in blood glucose levels at 30 minutes without hypoglycemic effect.

3.2 Alloxan-induced Study

The antidiabetic effects of single extracts and extract mixture of *Andrographis paniculata* dan *Syzygium polyanthum* on fasting blood glucose levels of alloxan-induced diabetic rats are shown in Fig. 2. Fasting blood glucose levels were elevated significantly in alloxan-induced rats compared to control normal animals due to single intervenous administration of alloxan. The elevated fasting glucose levels in the diabetic animals were used in the range of 300–370 mg/dl with considerable pancreas damage. This 3-fold elevation was maintained for period of 2 weeks shown by the untreated group. Treatment of alloxan-induced diabetic rats with the extracts in the dose of 200 mg/kg, oral for 14 days resulted in a significant decrease in fasting glucose level, whereas metformin also reduced blood glucose significantly in comparison with untreated diabetic rats. Metformin, an oral antidiabetic drug in the biguanide class, is commonly used as a first line treatment for type 2 diabetes and exerts beneficial glucose lowering effects without causing clinical hypoglycaemia [41,42]. It is claimed to have a multifactorial action with main effects on insulin sensitivity and preventing the liver from producing glucose [43]. Daily oral administration of metformin led to a fall in 48% which showed no significant difference with the single extract of *Syzygium polyanthum* (45%) and the extract mixture (53%). However, the fasting glucose levels of the group treated with extract mixture displayed the largest decrease and were significantly lower compared to those of the groups treated with the single extracts. Our results also indicate that the effect of antidiabetic of single extract of *Andrographis paniculate* was weaker (37%) than that of metformin. The exact chemical constituent responsible for observed antidiabetic effect of *Andrographis paniculata* is andrographolide [22-24].

On the other side, *Syzygium polyanthum* has been reported to contain chemical constituents like essential oil (citral, eugenol), tannin and flavonoid [26,27], but its active compound responsible for the antidiabetic effect is still unknown. According to previous investigations, the antidiabetic effect of *Syzygium polyanthum* may be due to the presence of the phenolic compounds i.e. tannin and flavonoid, which could act synergistically with andrographolide in enhancing the activity of glycolytic and gluconeogenic enzymes [44,45].

During the 14 days experimental period the normal control rats gained approximately 10% in the b.w. (Fig. 3). On the other hand, untreated diabetic control showed no significant gain in the body weight over the same period of time, but caused significant weight reduction of 7.6%. No significant difference was observed between percentage of body weight gain for all treated group.

Moreover, the histopathology of pancreatic tissue of normal control, untreated diabetic animals, and diabetic animals treated with the metformin, single extracts separately, and extract mixture is shown in Fig. 4. The endocrine portion of the pancreas takes the form of many small clusters of cells called islets of Langerhans (shown by the thick arrows). One of three major cell types of pancreatic islets, β-cells, produces insulin and the most abundant of islet cell. The normal pancreatic tissue (A) demonstrated normal morphological features of beta cells. The islets of alloxan-induced diabetic rats of the untreated group (B) showed extensive necrotic changes and signficant lession on β-cells followed by fibrosis, and atrophy. the pancreas of alloxan-induced animals treated with metformin (C) showed marked necrotic changes on the β-cells with few survivor cells interspacing. The pancreatic tissue of diabetic animals treated with the extract mixture (F) equally showed marked necrotic changes but with more survivor β-cells compared to treatment with single extracts (D

and E). Extract mixture of *Andrographis paniculata* and *Syzygium polyanthum* demonstrated to provide better restoration of pancreatic islet cells.

3.3 Acute Toxicity Study

Acute toxicity studies revealed the non-toxic nature of the extract mixture of *Andrographis paniculata* and *Syzygium polyanthum* on normal healthy rats at dose of 2000 mg/kg b.w. There were no changes in behavioral activities such as restlessness, respiratory distress, diarrhea, convulsions, and coma of the animals which received extract mixture in two different doses. Futhermore, no mortality was observed in the extract mixture-treated rats and behaviour of all the experimental groups showed no toxic sign and appeared the same and normal up to day 14.

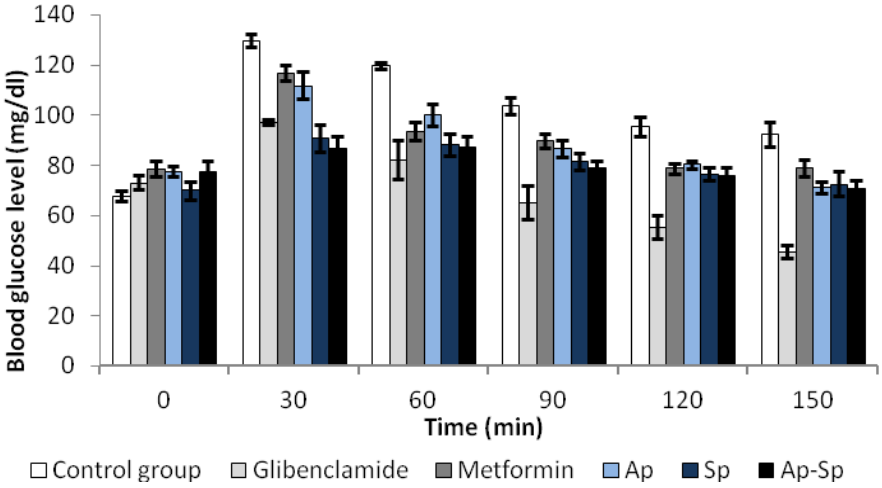

Fig. 1. Comparative effect of single extracts and extract mixture of *Andrograpis paniculata* and *Syzygium polyanthum* on oral glucose tolerance test. Samples and glucose were orally administered at 0 and 30 min respectively. Blood glucose was measured in 30 min interval

Each bar/point represents Mean ± SEM of 6 animals. Abbreviations: Ap, Andrographis paniculata; Sp, Syzygium polyanthum; Ap-Sp, extract mixture of Andrographis paniculata and Syzygium polyanthum

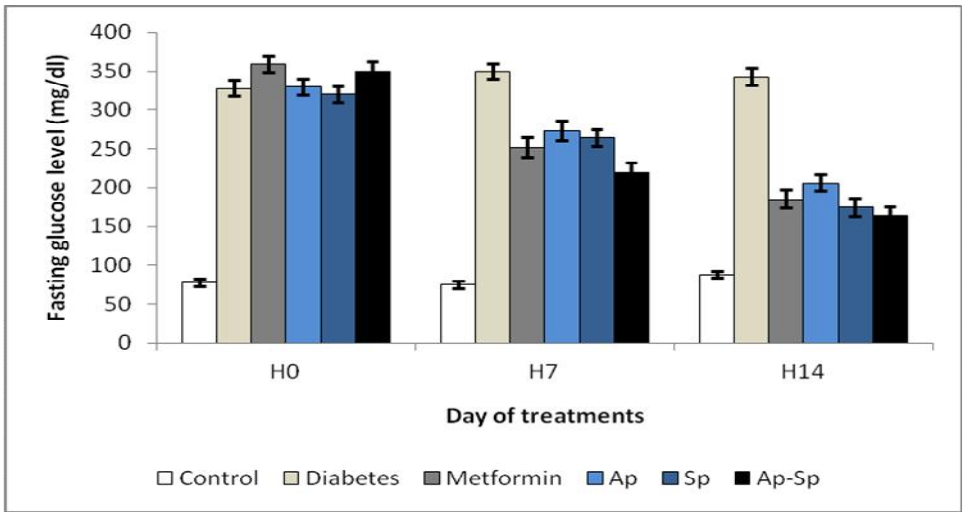

Fig. 2. Comparative effect of 14 days administration of single extracts and mixture extract of *Andrograpis paniculata* and *Syzygium polyanthum* on fasting glucose level in alloxan-induced diabetic rats

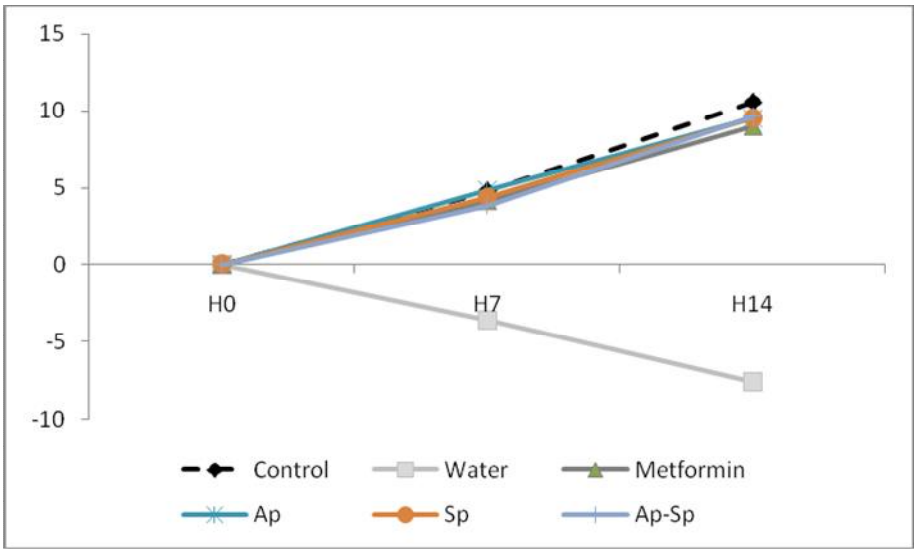

Fig. 3. Percentage increase/decrease in weight of the alloxan-induced diabetic rats untreated and treated compared to control normal rats

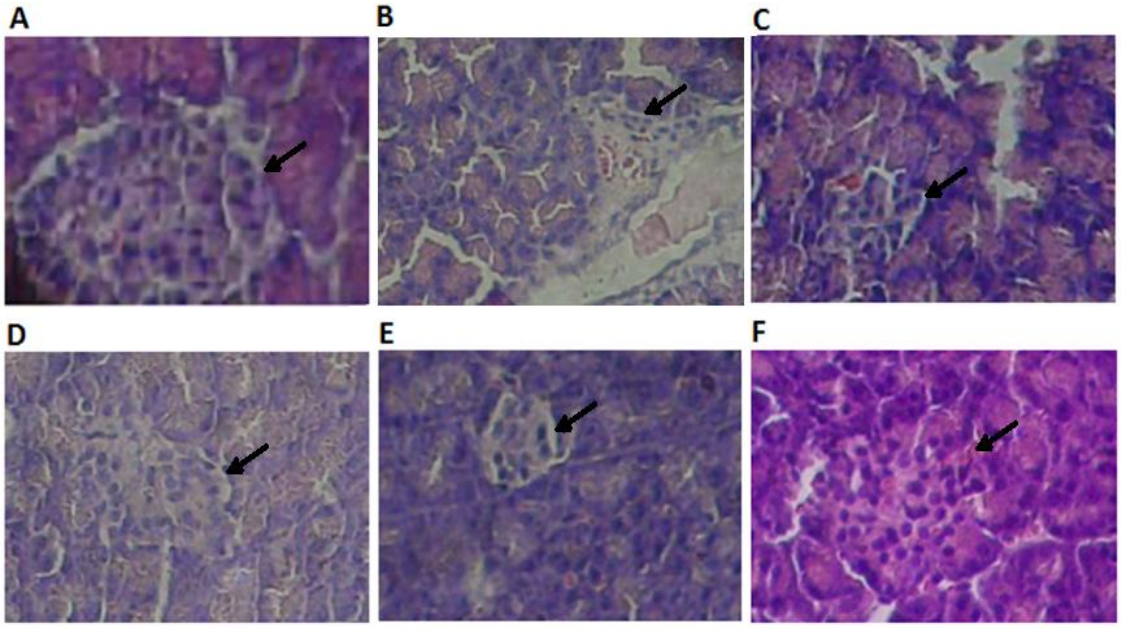

Fig. 4. Histopathological studies of pancreas (A) Normal, (B) Untreated, (C) Metformin, (D) *Andrographis paniculata*, (C) *Syzygium polyanthum*, (D) extract mixture

The thick arrows show islets of Langerhans in pancreas of rats

4. CONCLUSION

The present study showed that both the single herbal extracts and the extract mixture had good oral glucose lowering. The datas also suggest that oral administration of the extract mixture resulted to a significant decrease on the levels of blood glucose in alloxan-induced diabetic Wistar rats compared to groups treated with single extracts. The histopathological studies carried out indicated that the extract mixture of *Andrographis paniculata* and *Syzygium polyanthum* preserved pancreatic islet cells in alloxan-induced diabetic male rats. Acute toxicity study revealed that the extract mixture was safe at dose upto 2000 mg/kg body weight and did not

provoke toxic effects to the animals' activities, indicating that the LD_{50} more than 2000 mg/kg body weight. Other tests for toxicity reported previously suggest that extract of Andrographis paniculate was practically safe and non-toxic [19,46]. Besides, the leaves of *Syzygium polyanthum* (Indonesian bay leaf) are widely used as spice in South-East Asian culture, and the previous study shows that methanolic extract of *Syzygium polyanthum* leaf was non-cytotoxic to normal mammalian cell lines [47]. Further study in humans type II diabetes management should be carried out in future.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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