JURNAL ILMU KEFARMASIAN INDONESIA, April 2023, page 9-16 Vol. 21, No. 1
ISSN 1693-1831

The Effects of Kaliputih Traditional Herbs on Fasting Blood Glucose Levels, SOD, HbA1c, Histopathology Pancreatic in Streptozotocin-Induced Diabetic Rats

(Efek Ramuan Tradisional Kaliputih terhadap Kadar Glukosa Darah Puasa, SOD, HbA1c, dan Histopatologi Pankreas pada Tikus Diabetes yang Diinduksi Streptozotocin)

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Submitted 01 December 2021, Accepted 18 February 2023

Abstract: Prevention of DM by controlling sugar and lipid levels is the main therapy. The traditional medicine diabetes herb from Kaliputih Batur, Banjarnegara, Central Java consists of 11 kinds of medicinal plants that are empirically proven to be efficacious for diabetes mellitus. The potion is prepared by the infundation method. Three control groups (normal, positive, and negative) and three test groups with different doses (18, 36, and 54 mL/kg BW) that had previously been induced by streptozotocin were created using Sprague Dawley (SD) albino rats. Using HE staining, observations were obtained on fasting blood glucose (FBG), superoxide dismutase (SOD), HbA1c, and pancreatic organs. At doses of 36 and 54 mL/kg bw, results analysis of biochemical parameters of FBG levels revealed a substantial decline ($P<0.05$). At all dosages of the herb, there was a significant change in SOD activity ($P<0.05$), and at a dose of 18 mL/kg BW compared to negative control, there was a significant difference in HbA1c levels ($P<0.05$). HE staining results revealed no distinct pathological alterations in pancreatic organ at the test dose compared to negative control with necrosis in the Langerhans insula. Herbal medicine for diabetes traditional medicine from Kaliputih Batur, Banjarnegara, Central Java has decreased blood glucose.

Keywords: Diabetes mellitus, HbA1c, pancreatic histopatology, SOD

Abstrak: Pencegahan diabetes mellitus (DM) dengan mengontrol kadar gula dan lipid adalah terapi utama. Ramuan tradisional dari Kaliputih Batur, Banjarnegara, Jawa Tengah terdiri dari 11 macam tanaman obat terbukti secara empiris berkhasiat untuk penyakit diabetes melitus. Tujuan penelitian untuk menentukan efek ramuan tradisional Kaliputih pada kadar gula darah, SOD, HbA1c, dan gambaran histopatologi pankreas pada tikus. Ramuan disiapkan dengan metode infundasi. Tikus jantan albino Sprague Dawley (SD) dibagi menjadi 3 kelompok kontrol (normal, positif dan negatif) dan 3 kelompok uji dengan 3 dosis berbeda (18, 36, dan 54 mL/kg BB) yang sebelumnya diinduksi streptozotocin. Pengamatan dilakukan terhadap kadar glukosa darah puasa (GDP), superoksida dismutase (SOD), HbA1c, dan organ pankreas secara histopatologi dengan pewarnaan HE. Hasil pemeriksaan parameter biokimia kadar GDP menunjukkan adanya penurunan yang bermakna ($P<0,05$) pada dosis 36 dan 54 mL/kg BB. Aktivitas SOD terdapat perbedaan yang bermakna ($P<0,05$) pada semua dosis ramuan, dan kadar HbA1c menunjukan perbedaan bermakna ($P<0,05$) pada dosis 18 mL/kg BB dibandingkan kontrol negatif. Hasil pewarnaan HE menunjukkan tidak ada perubahan patologi yang spesifik pada organ pankreas dosis uji dibandingkan dengan kontrol negatif yang mengalami nekrosis di insula Langerhans. Ramuan tradisional untuk diabetes dari Kaliputih Batur, Banjarnegara, Jawa Tengah mampu menurunkan kadar gula darah tikus diabetes.

Kata kunci: Diabetes mellitus, HbA1c, histopatologi pankreas, SOD

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INTRODUCTION

DIABETES mellitus (DM) is a chronic metabolic disease characterized by insulin deficiency (pancreatic cell dysfunction) and insulin resistance. There are three main forms of DM that are widely accepted, including gestational DM, diabetes mellitus type 1 (DM1), and diabetes mellitus type 2 (DM2), which accounts for approximately 90% of all DM cases[1]. According to the basic health research (Riskesdas) from 2018, 2% of Indonesians aged 15 or older have a medical diagnosis of diabetes mellitus[2]. According to the 2013 basic health research, this number increased by 1.5% when compared to the prevalence of diabetes mellitus among residents aged 15 or older[3]. Even though the prevalence of diabetes climbed from 6.9% in 2013 to 8.5% in 2018, only around 25% of diabetics reportedly have the disease, according to the results of blood glucose tests[4]. It is believed that oxidative stress is a significant factor in the vascular problems of diabetes, in particular (DM2). Increased production of the antioxidants catalase (CAT/non-enzymatic enzymes), superoxide dismutase (SOD), and glutathione peroxidase may contribute to higher levels of reactive oxygen species (ROS) in diabetes (GSH-Px)[5]. Changes in these enzymes' concentrations increase tissues' susceptibility to oxidative stress, which promotes the emergence of diabetes complications[5]. Oxidative stress is caused by an imbalance between the systems that produce and remove free radicals, which either results in an increase in free radical generation or a decrease in antioxidant protective activity, or both[6]. The impact of oxidative stress in the pathogenesis of diabetes is not only due to the formation of free oxygen radicals, but also non-enzymatic protein glycosylation, glucose auto-oxidants, impaired glutathione metabolism, changes in antioxidant enzymes, formation of lipid peroxide and decreased concentrations of ascorbic acid[6].

The basic treatment for preventing diabetes mellitus is to regulate blood lipid and glucose levels. The amount of glycated hemoglobin (HbA1c) is a measurement of a diabetic patient's glycemic control during the past two to three months[7]. Cells include enzymes that detoxify ROS, such as SOD, which changes superoxide (O_2) to hydrogen peroxide (H_2O_2), CAT, and GSH-Px, which act in the order of converting H_2O_2 to water, to prevent oxidative damage[8]. However, abnormalities in these natural enzymatic systems have been identified in pathological circumstances, such as DM, where there is an excessive production of ROS, and may be a factor in the increased protein and cell membrane oxidation[9].

There is now no anti-diabetic medication that can stop diabetes complications, including nephropathy. As a result, this condition requires serious treatment, one of which is the investigation of drug discovery and therapeutic chemicals. The World Health Organization's (WHO) member nations were given a directive by the 56th World Health Assembly (WHA) to conduct more research on traditional medicines and to establish standards for traditional medicine in order to guarantee the efficacy, quality, and safety of herbal remedies[10].

In Kaliputih, Batur, Banjarnegara Regency, Central Java, the 2015 Study of Medicinal Plants and Herbs (Ristoja) in the Banyumasan ethnic group was successful in discovering native herbs and the production of traditional medicines by traditional healers (batra)[11]. Based on materials obtained from Ristoja 2015, batra has anti-diabetic ingredients from a mixture of 11 herbs which function as; *Strobilanthes crispus* leaves as antidiabetic and antioxidant. Test results of *Strobilanthes crispus* leaf extract doses of 1, 1.5 and 2 mL/kg BW for 30 days in STZ-induced diabetic male Sprague Dawley (SD) rats showed significant reductions in blood glucose, cholesterol, triglyceride and LDL blood levels[12]. When tested on STZ-induced SD rats, the chloroform fraction of *Orthosiphon stamineus* at a concentration of 1 g/kg BW was able to lower blood glucose levels[13]. In tests, *Coffea arabica* water extract at a concentration of 1000 mg/kg BW was able to lower blood sugar and lipid levels in Webster rats that had STZ-induced high levels in those substances[14]. Blood sugar levels in STZ-reared mice can be lowered by feeding them a diet combination containing up to 5% *Bambusa vulgaris* shoots[15]. Results of an in vitro study using *Saccharum officinarum* extract on Caco-2 cells can improve pancreatic cells that have decreased insulin production and aid with the uptake of glucose and fructose in the gut[16]. *Canna edulis* bulb lignin was used in in vitro tests to demonstrate inhibition of the α-d-glucosidase enzyme[17]. Test results of a 400 mg/kg dosage of *Curcuma mangga* rhizome extract revealed a reduction in blood glucose levels in alloxan-induced diabetic mice[18]. The rhizome of *Curcuma domestica* is anti-diabetic. In diabetic SD rats, the findings of the nano curcumin test using *Curcuma domestica* at a dose of 50 mg/kg BW were able to lower blood glucose levels and raise insulin levels[19]. In STZ-induced diabetic rats, the *Curcuma xanthorrhiza* rhizome has anti-diabetic properties and can enhance pancreatic morphology[20]. *Averrhoa bilimbi* fruit as a diabetes preventative[21] as well as *Boesenbergia panurata* as an antioxidant and anti-inflammatory[22]. The aim of this study was to evaluate the efficacy of the mixture

in relation to blood sugar levels, SOD, HbA1c, and histological characteristics in the rat pancreas at various doses, in order to determine whether it might be used as a substitute for existing diabetes treatments.

MATERIALS AND METHODS

MATERIALS. GOD-PAP kit (DiaSys, Conneticut, United States), SOD kit (Biovision-980, CA, USA), HbA1c kit (Fine Test, Hubei, China), Streptozotocin (Nacalai Tesque Inc., Kyoto, Japan), buffer citrate pH 4.5, ethanol, pioglitazone (Deculin® @30 Dexa Medika, Jakarta, Indonesia).

Tools. Electric scales (Precisa 40SM-200A, Swiss), Centrifuge Kubota KR-20000T (Kubota Seisakusho Co., Ltd., Japan), Spectrophotometer UV-vis (Shimadzu, Japan), ELISA Reader (MCL-2100C, China).

METHODS. Plant Determination. Plants were determined at the Universitas Ahmad Dahlan Biology Laboratory No.: 092/Lab.Bio/B/VII/2018 use literature Flora of Java[23,24].

Potion Preparation. According to the Kaliputih batra's instructions, a blend of 11 herbal plants that was gathered directly from the batra home was used as the testing substance. The first step involved quantifying each ingredient, which converted traditional dosages into grams in accordance with the Formularium Ramuan Obat Tradisional Indonesia[25]. All measured components were cleaned, ground, and cooked in 2 liters of water until they are reduced by half. Table 1 contains a list of the doses for the prepared substances. When the ingredients were done, rats were used to convert the substances (conversion factor 0.018).

Ethical Approval. This research was conducted with ethical feasibility issued by the Health Research Ethics Committee of the Universitas Ahmad Dahlan University Faculty of Pharmacy Number: 011609136.

STD Induction. 8-week-old white rat (Sprague Dawley) purchased from BPOM weighing 200–250 g. Streptozotocin (40 mg/kg BW) dissolved in citrate buffer pH 4.5 was administered intraperitoneally (IP) to mice to produce anesthesia. After one week, the induced mice were monitored to gather data on the rise in blood sugar levels.

Kaliputih Traditional Herbs Activity Test. The experiment used mice with blood sugar levels greater than 350 mg/dL. Six groups of normal rats and rats with diabetes were created; Table 2 shows the treatment groups. Batra reported that the mixture was administered for 15 days. On the fifteenth day following the potion's administration, fasting blood sugar levels were measured.

Checking Blood Sugar Levels. Blood drawn from the plexus retroorbitalis using a capillary tube and placed in an Eppendorf tube for the purpose of measuring blood sugar levels. To avoid hemolysis, blood flows through the tube wall. The serum was then extracted by centrifuging the sample at 8000 rpm for 10 minutes at 4°C. The GOD-PAP method was used to create a total of 10 µL of serum using the reagent kit's instructions: photometric enzymatic test.

Table 1. The composition of traditional Kaliputih ingredients.

Local name	Latin name	Used part	Composition	Equalization
Keji beling	*Strobilanthes crispus*	Leaf	1 handful	80 g
Kumis kucing	*Orthosiphon stamineus*	Leaf	2 handful	160 g
Buah kopi arabika	*Coffea arabica*	Fruit	1 handful	80 g
Bung pring gading	*Bambusa vulgaris*	Bamboo shoots	4 fingers	40 g
Cokol tebu ireng	*Saccharum officinarum*	Stem	4 fingers	40 g
Ganyong merah	*Canna edulis*	Rhizome	0.5 kg	500 g
Temulawak	*Curcuma xanthorrhiza*	Rhizome	half handful	40 g
Temu mangga	*Curcuma mangga*	Rhizome	half handful	40 g
Kunir	*Curcuma domestica*	Rhizome	half handful	40 g
Belimbing wuluh	*Averrhoa bilimbi*	Fruit	5 pcs	100 g
Srintil hutan	*Boesenbergia panurata*	Rhizome	2 fingers	20 g

Checking the Levels of SOD and HbA1c. SOD and HbA1c measurements utilizing the same serum. The colorimetric approach was used to examine SOD activity in accordance with the kit's instructions. The sandwich elisa method was used to examine the HbA1c activity in accordance with the kit's instructions.

Histopathological Analysis of the Pancreas. The rats were slaughtered after blood was obtained in order to remove their pancreas. At the Faculty of Veterinary Medicine, Universitas Gadjah Mada University, hematoxylin-eosin (HE) staining was used for a histopathological evaluation.

Data Analysis. Blood sugar levels, SOD levels, and HbA1c are only a few examples of the quantitative and qualitative data collected (pancreatic histopathology). Mean±SD is the way quantitative data are expressed. Levine's test for homogeneity and Kolmogorov-test Smirnov's for normality were used to pre-test

Table 2. Kaliputih traditional herb treatment group.

Group	Mouse state	Treatment
Normal control	Normal	NaCl solution (PO)
Negative control	Diabetes	NaCl solution (PO)
Positive control	Diabetes	Pioglitazone 2.4 mg/kg BW (PO)
Dose 1	Diabetes	Traditional herb 18 mL/kg BW (PO)
Dose 2	Diabetes	Traditional herb 36 mL/kg BW (PO)
Dose 3	Diabetes	Traditional herb 54 mL/kg BW (PO)

quantitative data. ANOVA and a T-test were used to analyze parametric data. When non-parametric data passed the Kruskal-Wallis test, the Mann-Whitney test was performed with a 95% confidence level. In the meantime, descriptive analysis was done on the qualitative data.

RESULTS AND DISCUSSION

STZ Induction Results. Table 3 displays the findings of tests to determine if STZ-treated test animals had elevated levels of FBG. Using a paired sample t test, the findings of STZ induction in test animals revealed a significant difference in the average FBG levels between pre-induction and post-STZ rats for a period of seven days ($p<0.05$). This demonstrates how the rat has evolved into a DM rat model. Since STZ can enter Langerhans cells via the glucose transporter, it was chosen for the induction of test animals. Pancreatic cells are harmed by STZ's alkylation of DNA through nitrosourea groups. Furthermore, STZ has been successfully used to cause diabetes in mice models by increasing ROS, a compound that plays a significant role in pancreatic -cell destruction[26].

Kaliputih Traditional Herbs Activity Test Results. Results of the traditional Kaliputih herb's activity test in the group of rats that had been fed the herb for 15 straight days after being induced and seeing an elevation in FBG levels. The results of the paired sample t test show that rats post-STZ induction and post-administration of diabetic mixtures for 15 days had significantly different average FBG levels ($p<0.05$). These findings are shown in Table 4. This demonstrates that the traditional Kaliputih components alter the levels of FBG.

Table 5 displays the findings of analyzing FBG levels following administration of traditional Kaliputih constituents. Compared to the negative control (393.02±115.46 mg/dL), it significantly decreased ($p<0.05$) in mice fed the traditional Kaliputih herb at doses 2 (83.15±30.79 mg/dL) and 3 (95.71±52.10 mg/dL). Rats receiving doses 2 and 3 showed a smaller drop in FBG than the positive control (125±52.82 mg/dL), but there was no statistically significant difference between the two. Despite having different doses, the decrease in FBG at doses 2 and 3 essentially produced the same effects. Typically, raising the dose

Table 3. The average difference between pre-induction and post-induction FBG.

	Delivery time	n	FBG levels	p
STZ induction	Pre-induction	36	66.96±4.14	0.000
	Post-induction (7 days)	36	282.79±13.72	

Paired sample t test: pre-induction vs post-induction p= 0.000.

Table 4. Differences in average FBG post-induction and after treatment.

	Delivery time	n	FBG levels	p
STZ induction	Post-induction	36	282.79±82.32	0.005
	After treatment	36	200.72±149.45	

Paired sample t test: post-induction vs after treatment p= 0.005.

Table 5. Changes in average FBG levels of rats on day 0 pre-induction, day 7 after STZ induction, and day 15 after being given traditional Kaliputih ingredients for 15 days.

Group	FBG levels pre-induction (mg/dL)	FBG levels after induction (mg/dL)	FBG levels after being given traditional herb (mg/dL)
Normal control	56.72±15.26	88.74±15.44	108.62±8.58
Negative control	64.72±22.45	292.09±112.45	393.02±115.46
Positive control	55.12±7.92	254.51±75.43	125.00±52.82
Dose 1	72.10±30.33	304.49±12.73*	402.41±80.18
Dose 2	96.34±29.34	282.46±100.46*	83.15±30.79*
Dose 3	62.77±18.96	322±73.14*	95.71±52.10*

The average value for 6 groups of 6 rats in each group. Significantly different value*$p<0.05$ to negative control.

will improve the patient's reaction to the therapeutic effects. However, as the dose is increased, the impact will plateau once the dose has been reached, preventing a rise in the reaction[27]. In pharmacological activity tests employing natural substances, this sort of event frequently occurs. Natural materials have a wide variety of phytochemical elements, where the compounds interact to produce a result. However, as the dose is increased, the phytochemical compound's number of components also increases, leading to non-linear effects that lessen the intended benefit. Because not all phytochemical components can attach to the receptors, the few available receptors also limit the effects. Because of this, an increase in dose is not always directly proportionate to the outcome[28]. The decrease in blood sugar levels is due to the content of phytochemicals such as quercetin from *Averrhoa bilimbi*[21], curcumin from *Curcuma domestica*[29] dan saccharant from *Saccharum officinarum*[15] with antidiabetic effects.

Results of Determination of Blood SOD Levels. Table 6 shows the results of the colorimetric approach used to determine SOD levels. Increased SOD activity in the blood is evident from the data. All dose treatments, including dose 1 (101.48±28.04 U/mL), dose 2 (85.19±32.29 U/mL), and dose 3 (103.33±18.86 U/mL), significantly increased ($p<0.05$) when compared to the negative control (17.90±13.90 U/mL). The outcomes demonstrated that STZ-induced rats had lower SOD levels. Between treatment groups, ingestion of the traditional Kaliputih herb raised SOD levels in white rats with diabetes. SOD levels increased in tandem with dose, with the dose group 3 having the highest value in comparison to the dose groups 1 and 2. This is consistent with other research that shown that STZ induction can impede ATP synthesis in mitochondria, leading to the formation of ROS and oxidative stress, which is what caused the drop in serum SOD levels in negative controls induced by STZ[30] and brings about a dysfunctional glucose metabolism. Increased ROS production is associated with impaired glucose metabolism and hyperglycemia[31]. The body possesses defense mechanisms like the SOD enzyme to deal with the generation of free radicals or to destroy ROS under normal conditions[32]. In all dose treatments, the rise in antioxidant levels coincided with the fall in blood glucose levels. Antioxidants that increase pancreatic cell mass, allowing them to produce more insulin to lower blood glucose levels, is one of the ways for raising SOD antioxidant levels[31]. The traditional Kaliputih herb from Batra includes panduratin-A, a flavonoid found in *Boesenbergia panurata*, among other phytochemicals in plants that have antioxidant action[22]. Phenolic chemicals known as flavonoids have antioxidant properties. In addition, there is curcumin, which originates from *Curcuma domestica* and *Curcuma xanthorrhiza*[20,29], Intersection *Curcuma mangga* flavonoids[18], and the *Coffea arabica* beans' tocopherols, which have antioxidant properties[33].

HbA1c Blood Levels. Table 7 displays data on HbA1c activity in diabetic rats. Rats receiving the traditional Kaliputih herb at dose 1 showed a substantial reduction in HbA1c levels (17.96±2.53%) compared to negative controls (27.04±4.29%). The results at doses 2 and 3 (30.50±7.48) and 29.50±6.77) did not differ substantially ($p>0.05$) from the negative control (19.97±1.83) or the normal control (27.04±4.29), although they did considerably ($p<0.05$) from each other. This may be brought on by the phytochemical interactions between several plant species included in traditional Kaliputih components at specific concentrations. A plant can have several different pharmacological effects since it produces a large number of secondary metabolites. Both antagonistic and synergistic effects may result from these effects. It's known as significant side effect elimination subtanced (SEES) when some metabolite antidote mechanisms can counteract the effects of other metabolites[34].

Table 6. The average results of measuring the levels of SOD in rats.

Group	SOD (U/mL)
Normal control	92.22±27.04*
Negative control	17.90±13.90#
Positive control	84.07±26.11*
Dose 1	101.48±28.04*
Dose 2	85.19±32.29*
Dose 3	103.33±18.86*

Data stated ± SD (n= 5) significance
*p <0.05 to Negative control.
#p<0.05 against Normal control.

One of the mechanisms underlying hyperglycemia and the difficulties of DM is an increase in non-enzymatic glycolation. HbA1c is produced when hemoglobin and extra glucose in the blood react under DM circumstances[35]. The negative control group's average HbA1c was significantly higher than that of the normal group, at 27.04±4.29%. This is a result of the pancreatic cells' necrosis brought on by STZ induction, which leads to hyperglycemia and has a diabetogenic effect[36].

According to various earlier investigations, there was a substantial decrease between the traditional Kaliputih herb dose 1 and the negative control. Study results on STZ-induced diabetic mice revealed that 30 g/L of *Coffea arabica* seed water extract reduced

HbA1c levels[35]. There are no other investigations on the other components of the traditional Kaliputih herb's ability to lower HbA1c levels in vivo. HbA1c reduction correlated with FBG reduction[37]. As a result, there is a connection between the activity of lowering FBG levels and HbA1c, and various plant species found in the traditional Kaliputih herb have been linked to anti-diabetic properties.

Table 7. The average results of measuring rat HbA1c levels.

Group	HbA1c (%)
Normal control	19.97±1.83*
Negative control	27.04±4.29#
Positive control	21.79±1.59
Dose 1	17.96±2.53*#
Dose 2	30.50±7.48#
Dose 3	29.50±6.77#

Data stated ± SD (n = 5) significance
*p <0.05 to Negative control
#p<0.05 against Normal control

Pancreatic Histopathology Results. For the morphology of the pancreatic tissue, the results of staining (HE) on the pancreatic tissue slices of all groups were studied. The islets of Langerhans, which are endocrine, are lighter (pink) than the exocrine region on HE staining, and they include capillaries. By examining the morphology of the pancreatic tissue structure of mice stained with HE staining, histopathological observation of the pancreas was carried out. Figure 1 displays the rat pancreas' histopathology.

In pancreatic histology, Langerhans insular cell necrosis, a sign of pancreatic injury, was present in the negative control. Necrosis is characterized by the presence of cell death, which gives the area a black hue, and by the presence of empty spaces in the center of the Langerhans islets. Necrosis is a form of cell death brought on by lethal injury that results in harm to the structure and function of all cells, followed by tissue inflammation. A disruption in insulin metabolism in the pancreas is indicated by a decrease in pancreatic cells[38]. Due to the administration of the traditional Kaliputih herb, there were no histological alterations in the three test doses' HE results, and there was substantial necrosis. *Boesenbergia panurata*,

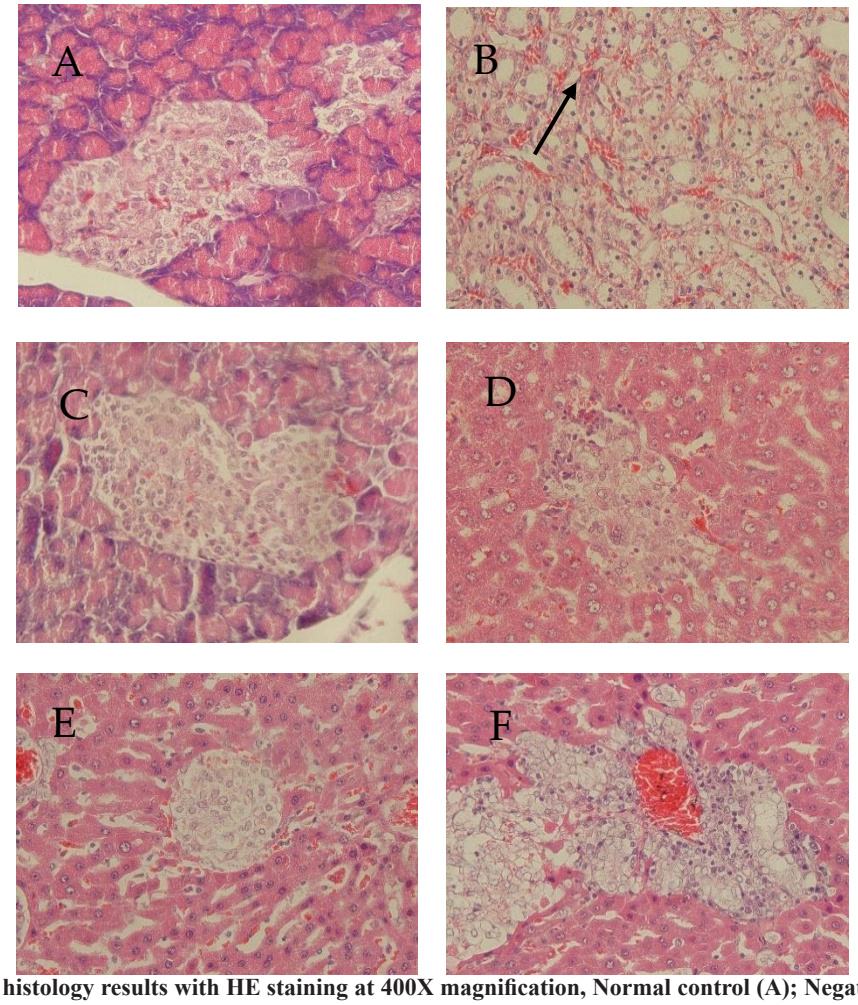

Figure 1. Pancreatic histology results with HE staining at 400X magnification, Normal control (A); Negative control (B); Positive control (C); dose 1 (D); dose 2 (E); dose 3 (F). Figure A, C, D, E, and F: Langerhans insula and aciner cells did not show specific pathological changes; figure B: presence of insula Langerhans necrosis; (→): location of necrosis.

Curcuma xanthorrhiza, *Curcuma domestica*, *Curcuma mangga*, and *Coffea arabica* are just a few of the 11 plant species that make up the traditional Kaliputih herb from Batra. These plants all have antioxidant properties. Antioxidants are substances that the body requires to balance out free radicals by supplying the electron deficit that free radicals have[39].

CONCLUSION

The traditional Kaliputih tonic considerably decreased FBG levels in the test animals at doses of 36 and 54 mL/kg BW, dramatically increased SOD levels in the test animals at all doses, and significantly decreased HbA1c levels in the test animals at a dose of 18 mL/kg BW.

ACKNOWLEDGEMENTS

The author would like to thank Mr. Bohardi as the resource person and for allowing and helping to collect materials from the forests and gardens around Kaliputih. Researchers also thank the Ministry of Health of the Republic of Indonesia for funding support with SK Number: HK.03.05/VI.I/1491/2016.

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