Antihyperglycemic and Pancreatic Protective Effect of Squalene in Streptozotocin-induced Diabetic Rat

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Keywords: antihyperglycemic, pancreatic islets of Langerhans, squalene, diabetic rat

Abstract: Squalene (Sq), is one compound that reported found in antidiabetic plant (*Syzygium polyanthum* Wight Walp leaf). The aim of this study was to evaluate its antihyperglycemic activity and potential pancreactic protective effect in streptozotocin-induced diabetic rats (SDR). Twenty Wistar rats (male, 180-230 g) were divided into four groups (n=5) and treated once daily for 14 days p.o. Group I (Sq 160 mg/kg), Group II (metformin (M) 500 mg/kg), Group IV (diabetic control (DC) distilled water 10 ml/kg) and Group III (normal control (NC) distilled water 10 ml/kg). Blood glucose level (BGL) and a histological study of the pancreas were performed. As compared to DC, SQ showed significant reduction ($p<0.05$-0.01) at day6-day14. Interestingly, histopathological assessment showed the restoration of the STZ-induced pancreatic islet cells damage. The present study concluded that Sq have antihyperglycemic activity and pancreatic protective effect.

1 INTRODUCTION

Diabetes is a major public health concern (Ramadan *et al*., 2017) that being the number one killer among all chronic diseases in the world (Widyawati *et al*., 2015a).

In spite of continuous new drug development to treat diabetes, medicinal plants remain a potential alternative therapy as antidiabetic agent (Ramadhan *et al*., 2017). The potential of antidiabetic plant is not only for its antihyperglycemic activity but also for identification of the responsible active compounds. Previous study (Widyawati *et al*, 2015b) identified squalene (Sq) as one of compounds in antidiabetic plant ie. *Syzigium polyanthum* leaf. Squalene, a triterpene, that has been implicated in several studies as a compound that contributes to the antihyperglycemic activities of plants (Baskar *et al*., 2011; Jananie *et al*., 2011; Widyawati, 2015a) Considering that diabetes is associated with impaired pancreatic function, in this study we investigated the effect of Squalene (Sq) on blood glucose level and pancreatic structure in streptozotocin-induced diabetic rats.

2 MATERIAL AND METHODS

2.1 Chemical

Squalene, streptozotocin and tween 80 were purchased from Sigma Aldrich (St. Lous, MAU, USA).

2.2 Animals

Healthy male Wistar rats weighing between 180-250 g were obtained from animal house of Universitas Sumatera Utara. The animals were acclimatized at room temperature and a 12-h dark/light cycle, and were allowed to access food and water ad libitum for one week before being used for experimentation. The study was performed after approved by Animal Research Ethics Committees (AREEC), Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Sumatera Utara (No. EC: 115/KEPH-FMIPA/2017).

Widyawati, T., Syarifah, S., Ichwan, M., Anggraini, D. and Wahyuni, A.
Antihyperglycemic and Pancreatic Protective Effect of Squalene in Streptozotocin-induced Diabetic Rat.
DOI: 10.5220/0010045204830486
In *Proceedings of the 3rd International Conference of Computer, Environment, Agriculture, Social Science, Health Science, Engineering and Technology (ICEST 2018)*, pages 483-486
ISBN: 978-989-758-496-1

2.3 Diabetes Induction in Experimental Diabetic Rats

STZ (55 mg/kg, prepared in a 0.9% NaCl solution) was injected intra-peritoneally to 16-hrs-fasted rats. , administered at single dose of 120 mg/kg body weight intraperitoneally. Diabetes was confirmed by determining the blood glucose concentration using glucometer (®Accu Check), after 72 hours of STZ injection. The rats that had BGL above 200 mg/dl were included for the study (Yusoff *et al*, 2017).

2.4 Experimental Set up

Diabetic rats were divided randomly into three groups (n=5). The first group (Sq) was administered Sq (160 mg/kg). The second group (M) was given metformin (500 mg/kg) to serve as the positive control. The third group (DC) was treated with normal saline (10 ml/kg) and served as the negative control. Normal control (NC) rats were included to the study that received normal saline (10 ml/kg). All treatments were dissolved in NaCL 0.9% and tween 80 5% before administration.

2.5 Preparation Pancreatic for Histopathological Analysis

The 14-days treated diabetic rats were sacrified with the carbogen gas (95% O2 and 5% O2) and the pancreas was excised for histological studies. The pancreas was fixed in 10% buffered formaldehyde for 24 hours, followed by dehydration using 70% alcohol (60 min), 96% alcohol (45 min), and absolute alcohol (2 h). The clearing phase of the samples was made by repeated xylene immersions, followed by paraffin wax infiltrations.

The samples were automatically processed with tissue processor Thermo Scientific STP 120-3 and paraffin embedding was prepared using modular tissue embedding center Thermo Scientific Microm EC 350-1. The parafffin-embedded tissues were sectioned into 5 µm using the Leica RM 125RTS microtome and mounted on a microscope slides. The mounted slides were stained with hematoxylline (H) and eosin (E) according to H&E staining technique. The stained sections were then mounted in DPX mounting medium with cover slide.

2.6 Photomicrography and Image Analysis

Records of the histopathological results were obtained by photomicrography using digital photomicrographic microscope (®Olympus BX 41 and ® Olympus DP25 video camera) at the Anatomic Pathology Laboratory, Department of Anatomic Pathology, Universitas Sumatera Utara.

2.7 Statistical Analysis

Data was expressed as mean ± standard error of the mean (S.E.M). The results were analysed using Kruskall Wallis followed by Mann-Whitney. $p < 0.5$ was considered significant.

3 RESULT AND DISCUSSIONS

3.1 Blood Gucose Level (BGL)

The effect of once daily oral administration of Sq on the BGL is presented in Table 1. Administration of Sq (160 mg/kg) showed significant blood glucose reduction at day6 to day14 ($p<0.05$-0.01) compared to DC-treated group. Metformin as the positive control decreased the BGL significantly at day6 to day14 as well ($p<0.01$). However, only NC-treated groups showed significant different from day3-day14 ($p<0.01$).

Table 1. Effect of 14 days daily oral administration of Squalene on the blood glucose level of SDR

BGL (mg/dL)	Group			
	Sq	M	NC	DC
BL	67.2 ± 1.4*	75 ± 2*	80 ± 4.8	80.8 ± 2.2
Day0	327 ± 9**	354.8 ± 24**	79 ± 5.1**	249.6 ± 14.6
Day3	280.4 ± 10.8	271.8 ± 15.9	81.2 ± 3.7**	284.4 ± 8.5
Day6	241.4 ± 16.8*	181.8 ± 24.7**	81.4 ± 3.9**	293.6 ± 17.6
Day9	184 ± 11.7*	144.2 ± 14.2**	79.4 ± 3.4**	300.2 ± 10.9
Day12	136.2 ± 9.2*	108.4 ± 8.2**	81 ± 4.1**	307 ± 20
Day14	85.2 ± 1.6**	80.4 ± 5.7**	78.4 ± 4**	318.4 ± 26

Data is expressed as mean ± S.E.M, n=5.*p<0.05, **p<0.01, ***p<0.001versus DC.

Sq has been reported in several studies as one of compounds found in the plants that have antihyperglycemic activity (Baskar *et al*, 2011; Jananie *et al*, 2011; Widyawati, 2015b; Ragasa *et al*, 2014). The present study support the previous study that showed dose-dependently antihyperglycemic activity of Sq (Widyawati, 2015b). However, the present study evaluate the effect after 14 days treatment that longer than the previous study.

3.2 Evaluation of the islets of Langerhans (iL)

Figure 1 shows a digital visualization of H & E staining of normal pancreas rat. The islet of Langerhans (IL) was clearly distinguished from the surrounding exocrine tissue by a continuous

conncetive tissue capsule. Exocrine area is the area outside of the IL. The islet cells were appeared rounded with prominent blue-black nuclei and various light pink cytoplasma. The figure 1 also shows the IL with granulated cytoplasm of islet cell with small, dark nuclei on the peripheral (alpha-cells), and with light and large nuclei (beta-cells), and pancreatic acinus (PA).

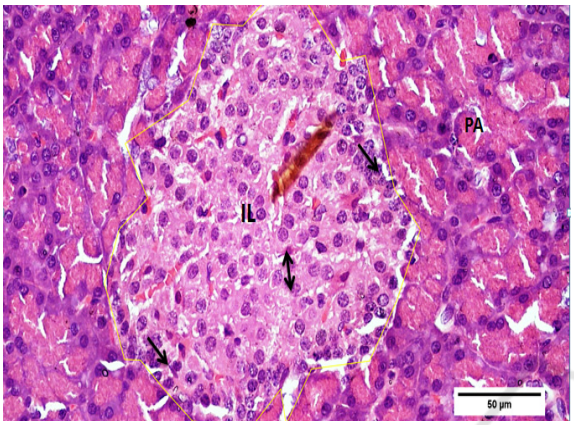

Figure 1. Histological appearance with H&E staining of the islet of Langerhans of normal rats (40x10 magnification).
*alpha-cells (arrow), beta-cells (double arrow), pancreatic acinus (PA)

Figure 2 shows a digital visualization of H & E staining of DC-treated pancreas rat. The figure shows that the size of IL of a diabetic rat decreased of size and the outline was irregular and shrinked.

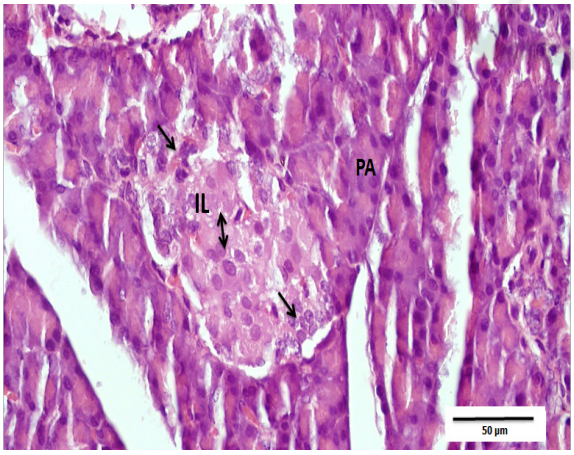

Figure 2. Histological appearance with H & E staining of the islet of Langerhans of diabetic control rats (40x10 magnification).
*alpha-cells (arrow), beta-cells (double arrow), pancreatic acinus (PA)

Figure 3 shows a digital visualization of H & E staining of Sq-treated pancreas rat. The figure shows the nearly regular outline of an IL with apparently normal appearance of most cells.

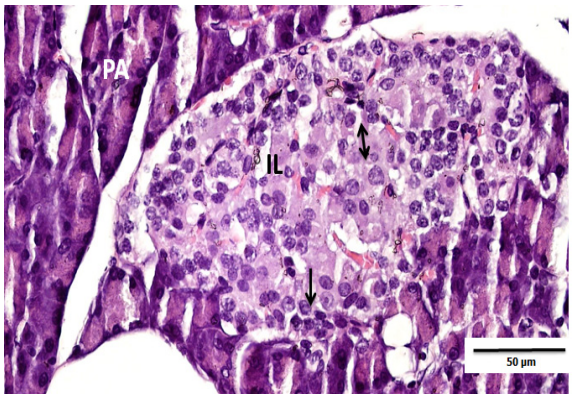

Figure 3. Histological appearance with H&E staining of the islet of Langerhans of SQ-treated rats (40x10 magnification).
*alpha-cells (arrow), beta-cells (double arrow), pancreatic acinus (PA)

H & E staining at the present study revealed significant changes in the general histological organisation of the pancreatic tissue between NC and DC (Figure 1 & 2). The STZ-demonstrated its ability to destroy the structure of pancreatic IL following STZ administration. The IL clearly seen in both normal and diabetic rats. It was due to the surrounding exocrine cells that were not affected by the induction (Razak *et al*., 2010). In NC, the IL featured circular shape with regular cell lining and no degenerated cells were observed. This appearance support the previous reported studies by Juarez *et al*., (2012) and Andrade-Cetto *et al*., (2008). On the other hand, DC showed a different histological changes of IL. The IL showed a shrunk with degeneration of islet cells. Treatment with Sq at the dose of 160 mg/kg restored the histological appearance of the IL as the outline and most of cells appeared nearly regular and normal.

4 CONCLUSIONS

Squalene have anti-hyperglycemic activity and pancreatic protective effect in STZ-induced diabetic rats.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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