Squalene decreased fasting blood glucose level of type II diabetic rats

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Abstract. Squalene is a chemical compound that has been reported to have antidiabetic activity. The present study aimed to investigate the effect of squalene on fasting blood glucose level (FBGL) in type 2 diabetic rats. Diabetes type II in rats was obtained by giving nicotinamide (120 mg/kg) before high dose streptozotocin (60 mg/kg) intraperitoneally. A total of 18 diabetic rats were divided into 3 groups and served once daily for 12 days as follows; Group I (Aquades (Diabetic Control) 10 ml/kg), Group II (Metformin 45 mg/kg) and Group III (Squalene 160 mg/kg). FBGL was measured at day 0, day 6 and day 12. The results showed that FBGL in both Squalene- (194.67 ± 28.32 mg/dL) and Metformin- (178.50 ± 34.27 mg/dL) were significantly decreased after 12 days intervention compared to Diabetic Control-treated groups (438.33 ± 65.79 µmol/L) with p<0.001. This study concluded that squalene was able to decrease FBGL in type II diabetic rats.

1. Introduction
One of the challenging health problems that poses a serious economic burden to individuals and nations is type 2 diabetes mellitus (DM) [1,2]. Type 2 diabetes mellitus (T2DM) individuals are at high risk for both microvascular complications (including retinopathy, nephropathy and neuropathy) and macrovascular complications (such as cardiovascular comorbidities), owing to hyperglycaemia and individual components of the insulin resistance (metabolic) [2].

Squalene, an intermediate in the cholesterol biosynthesis pathway [3], has been reported to have several activities including antidiabetic [4]. Therefore, the present aim of this study was to carry out the effect of squalene in type 2 diabetes mellitus rats.

2. Materials and Methods
The study was conducted from Mei to July 2021 and has been approved by Animal Research Ethics Committees FMIPA, Universitas Sumatera Utara, Medan, Indonesia.

2.1. Chemical
Streptozotocin, squalene and tween 80 (Sigma Aldrich (St. Lous, MAU, USA). The Conventional drug, Metformin tablet was used as a positive control.
2.2. Animals
Male Wistar rats, healthy condition with bodyweight range 180-250 g, were obtained from animal house of Universitas Sumatera Utara. Before being used for the experimentation, the rats were acclimatized at room temperature and a 12-h dark/light cycle, and were allowed to access food and water ad libitum.

2.3. Diabetes Induction
To obtain type 2 diabetic models, the rats were injected with nicotinamide (120 mg/kg) 15 minutes before streptozotocin (60 mg/kg) induction. Both chemicals were administered intraperitoneally. The rats that had fasting blood glucose levels (FBGL) above 200 mg/dl were confirmed as diabetes and were included for the study. FBGL were determined using glucometer (®Easy touch), 3x24 hours after injection.

2.4. Experimental Set up
Type II diabetic rats were divided randomly into three groups (n=6) and treated as follows: Group I (Diabetic Control) was treated aquades (10 ml/kg) and served as the negative control; Group II (Metformin) was given metformin (45 mg/kg) to serve as the positive control; Group III (Squalene) was treated squalene 160 mg/kg. All treatments were dissolved in aquades and tween 80 5%. The FBGL were measured on day 6 and day 12.

2.5. Data Analysis
Data were analyzed with dependent T-test; One way Anova followed by Bonferoni; Kruskall-Wallis followed by Mann-Whitney test as post hoc test using IBM SPSS Statistic 22.

3. Results
As shown in Table 1, fasting blood glucose level (FBGL) of rats increased significantly after induction of streptozotocin and nicotinamide (p< 0.001). Before induction, the FBGL were 92.72 ± 11.79 mg/dL then increase to 453.55 ± 86.00 mg/dL.

Table 1. Effect of Streptozotocin-nicotinamide on Fasting Blood Glucose Level in Rats

|                         | Fasting Blood Glucose Level (mg/dL) |
|-------------------------|-------------------------------------|
|                         | Before Induction | After Induction | p      |
|                         | 92.72 ± 11.79    | 453.55 ± 86.00 | 0.000  |

The FBGL of type II diabetic rats as depicted in Table 2 decrease on day 6 and day 12 observation in both Metformin- and Squalene-treated groups. While in Diabetic Control group the FBGL showed no different value. FBGL of Squalene- and Metformin-treated groups were decreased significantly (p<0.01).

Table 2. Effect of Squalene on Fasting Blood Glucose Level in Type II Diabetic Rats

|                         | Fasting Blood Glucose Level (mg/dL) Mean ± SD |
|-------------------------|-----------------------------------------------|
|                         | Day 0         | Day 6         | Day 12           |
| Diabetic Control        | 440.00 ± 68.86 | 350.50 ± 58.89 | 438.33 ± 65.79  |
| Metformin               | 523.83 ± 51.31* | 351.50 ± 47.26 | 178.50 ± 34.27** |
| Squalene                | 396.83 ± 88.14 | 300.83 ± 51.67 | 194.67 ± 28.32** |
| p                       | 0.022*        | 0.198         | 0.003**          |
| *p<0.05 One way Anova-Bonferoni; **p<0.01 Kruskall Wallis-Mann Whitney
4. Discussion
Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia and one of the five leading causes of death in the world [1,2]. This metabolic syndrome is caused by dysfunction of insulin secretion and or function. The present study showed that giving nicotinamide before streptozotocin may develop diabetic condition in rats. The FBGL of rats increased after induction which proof that both nicotinamide and streptozotocin may able to create type 2 diabetic rats. Squalene has been known to several pharmacological properties including antihiperlipidemia, hepatoprotective, cardioprotective and antioxidant. This bioactive substance has low toxicity and, in therapeutic doses, does not produce any damaging action on the human organism [6]. The present study showed that it can decrease the high of FBGL from day 6 to day 12. This results supported previous study that showed squalene decreased FBGL and protected the islet of Langerhans [4].

Previously, we used streptozotocin only to induced diabetic rats, while at this present study we used nicotinamide before streptozotocin induction. Streptozotocin, an antibiotic, causes pancreatic islet β-cell destruction and is widely used experimentally to develop a model of type 1 diabetes mellitus (T1DM) [7]. By giving nicotinamide previously, it partially protected insulin-secreting cells against streptozotocin [8].

5. Conclusion
Squalene (160 mg/kg) able to decrease fasting blood glucose level of type II diabetic rats.

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