Squalene decreased malondialdehyde level of diabetic rats

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Abstract. Squalene, a precursor for the secondary metabolite synthesis, is synthesized in animals, bacteria, fungi and plants. It has been reported to have some biological activities, one of which is as an antioxidant. This study aimed to evaluate the effect of squalene on malondialdehyde (MDA) level, an antioxidant activity marker, in diabetic rats. Diabetes type II was induced by a high-fat diet with low dose streptozotocin (30 mg/kg) intraperitoneally. A total of 12 diabetic rats were divided into 3 groups and served once daily for 14 days as follows; Group I (Squalene (S) 160 mg/kg), Group II (Metformin (M) 45 mg/kg) and Group III Aquades (DC) 10 ml/kg). MDA level were measured using Elisa method. Data were analyzed using Kruskall Wallis and Mann-Whitney as post hoc test. The results showed that MDA level in both S- (8.50 ± 1.40 µmol/L) and M- (7.74 ± 1.63 µmol/L) were lower than DC-treated groups (12.82± 2.86 µmol/L). Statistically, significant different were found between S- and DC- as well as S- and M-treated groups (p< 0.05). This study suggested that squalene able to decrease MDA level in type II diabetic rats.

1. Introduction
One of the key mechanisms of insulin resistance is oxidative stress [1] which can progress to type 2 diabetes mellitus (DM)[2]. Oxidative stress oxidizes the membrane lipids that cause damage to cell membrane and cellular organelles membranes [3]. One of biomarkers to evaluate the lipid peroxidation ie. malondialdehyde (MDA). It has been reported to be significantly raised in diabetes [4].

Squalene, a bioactive compound that is useful in the biosynthesis of cholesterol [5]. This compound has been reported to have effect to alleviate the hyperlipidemia, hyperglycemia, to protect liver and heart [6,7] and to fight free radicals as an antioxidant [6]. This triterpene compound is distributed in natural source widely, which found in rice bran, palm, olive, wheat-germ, and amaranth [8,9]. In addition, this compound was also reported to be identified in leaf plants such as Syzygium polyanthum Wight [10]. Previous study showed the antioxidant properties of squalene that found in part of plant [8]. Another study by Gabás-Rivera [11] showed that dietary squalene (1 g/kg) during 11 weeks improved lipid profile and decreased MDA level in mice.

As the explanation above, in diabetic condition, the MDA levels are known to be elevated. However, there have been no reports antioxidant properties of squalene in streptozotocin-induced diabetic rats. Therefore, this current study was conducted to investigate the squalene effect in type 2 diabetes mellitus rat with MDA as the biomarker.
2. Material and Methods
The research was conducted during April to September 2020 and has been approved by Animal Research Ethics Committees FMIPA, Universitas Sumatera Utara, Medan, Indonesia: No.00521/KEPH-FMIPA/2020.

2.1. Chemical
Squalene, streptozotocin and tween 80 were obtained from Sigma Aldrich (St. Louis, MO, USA). Metformin tablet was used as oral antidiabetic standard as positive control.

2.2. Animals
Healthy rats, male Wistar species, 180-250 g, were purchased from Universitas Sumatera Utara animal house. The animals were acclimatized for 7 days before being used for study.

2.3. Induction of Diabetes
To obtain type 2 diabetic rats model, the rats that have been fed a high-fat diet (HFD) for 14 days were induced with streptozotocin (30 mg/kg) intraperitoneally. Diabetes was confirmed by determining the blood glucose level (BGL) using glucometer (Easy touch), after 72 hours of STZ injection. The rats were included in the study if the BGL more than 200 mg/dl.

2.4. Experimental Set up
Diabetic rats were grouped randomly into three (n=4 respectively). Group I (S): Squalene (160 mg/kg); Group II (M): metformin (500 mg/kg) as the positive control; Group III (DC): aquades (10 ml/kg) as the negative control, per oral, once daily. All treatments were dissolved in aquades and tween 80 5%. After 14 days the rats were sacrificed to obtain their serum. The MDA level was measured using MDA ELISA Kit for rats.

2.5. Data Analysis
Data were analysed with Kruskall-Wallis followed by Mann-Whitney test as post hoc test using IBM SPSS Statistic 22.

3. Results
As shown in Table 1, MDA level of both S- (8.50 ± 1.40 µmol/L) and M- (7.74 ± 1.63 µmol/L) were lower than DC- (12.82 ± 2.86 µmol/L) treated groups. Statistically, there were significant level different among groups (p <0.01). Interestingly, no significant different of MDA level between S- and M-treated groups (p>0.05).

Table 1. Squalene Effect on Malondialdehyde Level in Type II Diabetic Rats

| Group | MDA (µmol/L) (mean ± SD) |
|-------|--------------------------|
| S     | 8.50 ± 1.40**            |
| M     | 7.74 ± 1.63 **           |
| DC    | 12.82 ± 2.86             |

p = 0.009

**p<0.01 compared to DC

4. Discussion
Diabetes mellitus (DM) is a major non-communicable disease that requires an effort to address its prevalence and associated complications [12]. This chronic metabolic disease caused by defect in insulin
secretion from beta pancreas and/or insulin action [13,14]. The correlation between diabetes and oxidative stress through measurement of oxidative stress biomarkers in both human and animal has been extensively investigated [12]. Thus, studies showed that obesity is a principal cause of insulin resistance combined with metabolic dysregulation to the progression of type 2 DM. In the present study we developed obesity rats by giving high-fat diets (HFD) before streptozotocin induction to obtain type 2 diabetic rats model. The results showed (data not shown) that the HFD increased bodyweight of rats and afterwards, low dose of streptozotocin (30 mg/kg) injection intraperitoneally increased fasting BGL.

Malondialdehyde (MDA) is the most frequently used biomarker for lipid peroxidation and oxidative stress [4]. Oxidative stress is an imbalance state between biological system and oxygen reactive species (ROS), which means the ability of a biological system to detoxify ROS is not balanced with their production in cells [15,16]. Peroxidation of lipid is a chain reaction that occured during oxidative stress leading to the production of various active compounds resulting in the cellular defect. Polyunsaturated fatty acids, the sources of unstable lipid peroxides. These lipid readily decompose to generate a complex series of compounds including malondialdehyde (MDA). Higher the level of MDA related to higher oxidative stress [4]. ROS and nitrogen species (ROS/RNS) are essential for detoxifications, immunity and chemical signalization [17]. The increasing of ROS/RNS can be reduced by the consumption of foods rich in phytocheimicals such as flavonoids and phenolics. These compounds were known to have antioxidant properties [18]. The present study demonstrated that both squalene and oral antidiabetic metformin decreased the MDA level of diabetic rats. Therefore, the present results suggest that squalene has potential to be developed for its antioxidant properties. This bioactivity may support to prevent the progression of DM state.

5. Conclusion
Squalene (160 mg/kg) able to decrease malondialdehyde level of streptozotocin-induced high fat diet diabetic rats.

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