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# Extracts of okra (*Abelmoschus esculentus* L.) improves dyslipidemia by ameliorating lipid profile while not affecting hs-CRP levels in streptozotocin-induced rats

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**Abstract.** Okra (*Abelmoschus esculentus* L.) is a tropical vegetable which has been utilised for many years as panacea for various ailments, including diabetes and its associated metabolic derangements such as hyperlipidemia. Quercetin, a major flavonoid compound found in okra, has previously been reported to ameliorate lipid profile and inflammatory status. This study was carried out to investigate the effect of okra extracts on lipid profile and hs-CRP levels in animal model. Eight-week-old male *Sprague Dawley* rats were injected with streptozotocin (50 mg/kg) to induce type 2 diabetes. Quercetin (5 and 10 mg/kg BW) from methanolic extract of green and purple okra were subsequently administered for two consecutive weeks. Results showed that the raised concentration of total cholesterol (TC), triglycerides (TG), and LDL-cholesterol (LDL-C) were reduced in diabetic rats receiving either green or purple okra extracts compared to diabetic control group. Based on this study, it can be suggested that okra has the potential to act as a lipid-lowering agent in treating hyperlipidemia.

## 1. Introduction

Type 2 diabetes is a syndrome characterized by chronic hyperglycemia due to insulin resistance, causing abnormal lipid metabolism or hyperlipidemia that is defined as an increase in total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and/or a decrease in high-density lipoprotein cholesterol (HDL-C) levels in the blood. Hyperlipidemia is a contributing factor which increases the risk of cardiovascular disease and metabolic disorders [1]. The number of diabetic patients along with its mortality and morbidity have been escalated at an alarming rate, and there is a growing evidence indicating a strong relationship between inflammatory processes and the development and progression of diabetic complications [2]. Both insulin deficiency and insulin resistance were associated with increased production of c-reactive protein (CRP), a marker of systemic inflammation, as its correlation have been reported in both type 1 and type 2 diabetic patients [3, 4].

Phytochemicals identified from traditional plants are presenting a promising opportunity and concurrently accelerating global efforts to develop diet-based therapeutics in combating hyperlipidemia. Okra (*Abelmoschus esculentus* L.), commonly known as lady's finger, is a multipurpose crop belongs to Malvaceae family, originated in African countries and is now widely distributed to Asia, including Indonesia. Okra has long been consumed as a vegetable (eaten both raw and cooked) and is used extensively as a traditional remedy to cure health problems such as constipation, diarrhea, jaundice, urinary calculi, and diabetes [5]. In addition, qualitative and quantitative evidence has accumulated that okra is abundant in polyphenolic compounds namely



quercetin and its derivatives [6, 7]. Scientific studies have also reported that okra possessed a myriad of beneficial effects due to the quercetin content such as antioxidant [8], lipid peroxidation inhibitor [9], antiinflammatory, antidiabetic, and antihyperlipidemic [10, 11].

However, the hypolipidemic activity of purple okra, a variety of okra developed from seed-crossing of quality okra, has not yet been elucidated. This research therefore aimed to evaluate the proposed protective role of okra against hyperlipidemia by examining the effects of green and purple okra extracts in diabetic rats.

## 2. Methods

### 2.1. Plant material and preparation of extract

Fresh okra pods were collected from okra field in Bogor, West Java. Green okra was of *Naila* variety whereas purple okra was of *Zahira*. All pods were washed and weighed thoroughly before extracted using methanol extraction method, and later freeze-stored before used for treatment.

### 2.2. Phytochemical screening

Antioxidant activity of okra extracts were analysed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Flavonoid content were observed qualitatively and analysis of total phenolics was performed by spectrophotometric method.

### 2.3. Animals and experimental protocols

A total of 24 eight-week-old male *Sprague Dawley* rats weighing 180-240 gram were obtained from The National Agency of Drug and Food Control of Republic of Indonesia (NADFC) and housed individually in cages at controlled temperature (22-25°C) and under a 12-hour light/dark cycle. After acclimation period, rats were randomly distributed into six groups of four rats each: NC (normal control, untreated); DC (diabetic control, untreated); T1 (Diabetic, treated with 5 mg/kg BW of quercetin from green okra extract (GOE I)); T2 (Diabetic, treated with 10 mg/kg BW of quercetin from green okra extract (GOE II)); T3 (Diabetic, treated with 5 mg/kgBW of quercetin from purple okra extract (POE I)); T4 (Diabetic, treated with 10 mg/kg BW of quercetin from purple okra extract (POE II)). Dosage of quercetin used in this study was determined according to Gomes et al. [12] in which 5 mg quercetin/BW administered in rats is similar to 263 gram (2.6 portion) of fresh okra for human weighed 70 kilogram whereas 10 mg quercetin/BW is similar to 526 gram (5.2 portion). Rats were rendered diabetic by a single intraperitoneal injection of streptozotocin (STZ, 50 mg/kg) and type 2 diabetes was later confirmed by detecting fasting blood glucose (FBG) level (>126 mg/dl known as hyperglycaemia) with the help of glucometer. The okra extracts were administered orally for 14 consecutive days. Throughout the experimental study, rats were provided with the standard chow diet and water *ad libitum*. This experimental study complied with all protocols and policies outlined by Animal Ethics Committee at Bogor Agricultural University, Indonesia.

### 2.4. Blood collection and biochemical analysis

At the end of the experimental period, rats were sacrificed under ketamine/xylazine anesthesia after an overnight fast. Blood collected by intracardiac puncture were immediately centrifuged at 3000 rpm for 10 minutes to acquire the serum for determination of lipid profile and hs-CRP levels. The serum TC, TG, and HDL-C levels were measured enzymatically using commercial kits (DiaSys®) while LDL-C levels was indirectly calculated by Friedewald equation. The concentration of hs-CRP was assessed by latex agglutination method using automated analyser (TMS Superior 501).

### 2.5. Statistical analysis

All data were analysed using SPSS 16.0 software and expressed as mean  $\pm$  standard error of the mean (SEM). Data were statistically analysed among groups using one-way analysis of variance (ANOVA) and further evaluated using Duncan test. Differences between pre-treatment and post-treatment were assessed using paired *t*-test. *P*-value less than 0.05 were regarded as statistically significant.

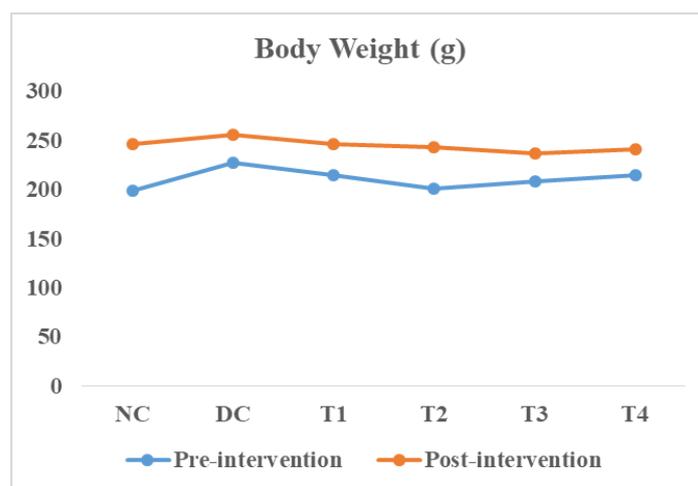
### 3. Results

Based on phytochemical screening (table 1), both the green okra extract and purple okra extract showed antioxidant activity, which were 326.48 ppm and 316.86 ppm, respectively. Flavonoid assay revealed the presence of flavonoid compound in all okra extracts. Analysis of total phenolics demonstrated that purple okra extract had a slightly higher phenolic content (3.60%) than green okra extract (3.58%).

**Table 1.** Phytochemical screening of okra extracts.

| Parameters                    | Green Okra Extract (GOE) | Purple Okra Extract (POE) |
|-------------------------------|--------------------------|---------------------------|
| Antioxidant Activity (ppm)    | 326.48                   | 316.86                    |
| Flavonoid Assay (qualitative) | +                        | +                         |
| Total Phenolics (%)           | 3.58                     | 3.60                      |

Rats in diabetic group had a higher body weight ( $256 \pm 7.52$  gram) compared to rats in normal control group ( $247.25 \pm 5.66$  gram) and all treatment groups; T1 ( $247 \pm 5.87$  gram), T2 ( $243.75 \pm 20.3$  gram), T3 ( $237 \pm 7.36$  gram), and T4 ( $241.5 \pm 18.25$  gram), but it was not significantly different between groups ( $P > 0.05$ ). This data suggested that quercetin in okra could sustain the body weight of rats throughout the treatment phase (figure 1).



**Figure 1.** Effects of okra extracts on body weight in *Sprague Dawley* rats, during 14-day experimental period. All values displayed as mean  $\pm$  SEM.

All STZ-induced rats developed type 2 diabetes within one week of injection, indicated by high fasting blood glucose (FBG) levels ( $>126$  mg/dl) defined as hyperglycaemia. The STZ injection resulted in significant amplification of serum TC, TG, and LDL-C levels in diabetic rats compared to the normal rats. Serum HDL-C were also decreased under diabetic condition.

Post-treatment serum lipid measurement showed that rats in diabetic control group had a significantly raised levels of TC (44.5%), TG (37.5%), and LDL-C (113.78%) as the rats received no okra treatment during the experimental period. Conversely, in okra-treated groups, okra extracts could ameliorate lipid abnormalities as indicated by significant ( $P < 0.05$ ) decrease in serum TC, TG, and LDL-C by, in average, 19.03, 7.58, and 43.65% respectively in quercetin-treated groups. However, further statistical analysis revealed no significant difference between green okra extract and purple okra extract in affecting serum lipid levels ( $P > 0.05$ ). Administration of okra extract for 14 days could

not significantly increase the levels of serum HDL-C ( $P>0.05$ ) and also did not affect the hs-CRP levels ( $P>0.05$ ) in all treatment groups (table 2).

**Table 2.** Effects of okra extracts on lipid profile and hs-CRP levels in diabetic rats.

| Groups         | TC<br>(mg/dl)             | TG<br>(mg/dl)           | HDL-C<br>(mg/dl) | LDL-C<br>(mg/dl)        | hs-CRP<br>(mg/l) |
|----------------|---------------------------|-------------------------|------------------|-------------------------|------------------|
| NC             | 52.5±5.58                 | 50.5±3.3                | 34.25±1.7        | 8.15±5                  | 0.012            |
| DC             | 124.75±10.09 <sup>a</sup> | 85.25±5.76 <sup>a</sup> | 26.25±2.59       | 81.45±9.91 <sup>a</sup> | 0.072            |
| T1             | 68.75±3.17 <sup>b</sup>   | 59.75±3.66 <sup>b</sup> | 29.75±2.29       | 27.05±3.44 <sup>b</sup> | 0.038            |
| T2             | 62.5±2.53 <sup>b</sup>    | 58.5±3.75 <sup>b</sup>  | 32±1.15          | 18.8±3.54 <sup>b</sup>  | 0.042            |
| T3             | 59.5±4.17 <sup>b</sup>    | 56.5±5.91 <sup>b</sup>  | 31±1.96          | 17.2±5.83 <sup>b</sup>  | 0.040            |
| T4             | 62±4.51 <sup>b</sup>      | 57±6.87 <sup>b</sup>    | 30.75±2.93       | 19.85±6.23 <sup>b</sup> | 0.038            |
| <i>P-value</i> | <0.001**                  | 0.046*                  | 0.067            | <0.001**                | 0.164            |

Data were presented as mean ± SEM.

\* $P<0.05$ .

\*\* $P<0.001$ .

a As compared with normal control group.

b As compared with diabetic control group.

#### 4. Discussion

Type 2 diabetes and hyperlipidemia are metabolic diseases that has risen steeply in incidence worldwide. Dietary therapy has been regarded as a useful approach to combating metabolic diseases, therefore this study used okra to investigate its antihyperlipidemia and antiinflammatory effect against diabetes-induced metabolic disturbances. This research conclusively showed that treatment with quercetin from okra had the ability to abrogate hyperlipidemia induced by diabetes, by decreasing serum TC, TG, and LDL-C levels, as well as increasing serum HDL-C levels. It is in line with previous study which showed that okra extract could reduce the levels of total cholesterol and triglycerides [13]. The hypolipidemic property appeared not to be dose-dependent as lower dose (5 mg/kg BW/day) of quercetin produced a similar hypocholesterolemic and hypotriglyceridemic effect to that observed when the higher dose (10 mg/kg BW/day) is used.

Several explanations were suggested of how quercetin is able to influence serum lipid profile. It was reported that quercetin reduces *de novo* synthesis of fatty acids and consequently cholesterol biosynthesis and lipoproteins formation [14]. Findings from Wang *et al.* showed that reduction in serum TC and TG induced by dietary okra could enhance fecal excretion of bile acids. Moreover, hypolipidemic activity of okra might be mediated most likely by upregulation of cholesterol degradation through cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) and by inhibition of lipogenesis through downregulation of sterol regulatory element-binding protein 1c (SREBP1c) and fatty acid synthase (FAS) expression [15]. Dietary fibre contained in okra might also contribute to the hypolipidemic effect of okra. In addition, high fibre content in okra could stabilise blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract. The fibre likewise helps support blood sugar levels by slowing down sugar assimilation through the intestines [16].

On the other hand, okra supplementation could not suppress the inflammatory process, not in agreement with study which reported that quercetin could decrease the serum level of CRP in atherosclerosis rabbits [17]. Another research [18] stated that quercetin could apparently exhibit antiinflammatory effect after a daily dose of 150 mg quercetin/kg BW for 28 days [18]. In human, a meta-analysis implied that quercetin supplementation on CRP will show significant effect at doses above 500 mg/day and in patients with CRP <3 mg/l [19].

## 5. Conclusion

In summary, consumption of green and purple okra extracts had obviously exerted hypolipidemic effect in diabetic rats by reducing the serum TC, TG, and LDL-C levels. However, changes in lipid profile did not differ significantly according to the varied dosage and okra variety, so 5 mg quercetin/kg BW/day is suggested to be consumed for alleviating lipid profile and inflammatory status. Further research remains necessary to shed light on the possibility to make use of okra as a part of alternative strategies in the management of hyperlipidemia.

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