

POTENCY OF CAPE GOOSEBERRY (PHYSALIS PERUVIANA) JUICE IN IMPROVING ANTIOXIDANT AND ADIPONECTIN LEVEL OF HIGH FAT DIET STREPTOZOTOCIN RAT MODEL

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Abstract

Background and aims: Quercetin belonging flavonoid has a role to improve diabetic condition. Research aimed to examine and to compare Cape Gooseberry (CG) juice and quercetin supplement on Total Antioxidant Capacity (TAC) and adiponectin level of high fat diet-Streptozotocin (HFD-STZ) induced rat. **Material and method:** CG juice 5 ml/kg/d (X1) and 25 ml/kg/d (X2) groups; and quercetin supplement 2.2 mg/kg/d (X3) and 30 mg/kg/d (X4) groups were compared with both of positive (K+) and negative (K-) control. Treatments were given by orally gavage for 28 days to 36 Wistar rats which each group consisted of 6 rats. TAC and adiponectin level were measured by ABTS and ELISA method respectively. **Results:** There was significantly increase of TAC in treatment groups compared with K(+) ($p < 0.05$). X2 had TAC level significantly higher than X1 ($p = 0.025$). Moreover, adiponectin level of treatment groups were significantly higher than K(+) ($p < 0.05$). Furthermore, X2 had adiponectin level significantly higher than X3 ($p < 0.001$). **Conclusion:** CG juice 25 ml/kg/d presented better effect than CG juice 5 ml/kg/d, although quercetin 30 mg/kg/d showed the best effects toward both of TAC and adiponectin.

key words: adiponectin, high fat diet, Streptozotocin, total antioxidant capacity

Background and aims

Diabetes mellitus type 2 (DMT2) is metabolic disease characterized by increasing blood glucose. People possessed DMT2 have higher comorbidity and mortality than healthy people. Prevalence of diabetes increases these decades. An estimated prevalence of diabetes mellitus in Southeast Asia by International Diabetes Federation is 151 billion by 2045 [1].

The incidence of diabetes in Indonesia was 10 million in 2015 [2]. Insulin resistance is the main pathophysiology of DMT2 [3]. Insulin resistance caused by high fat diet (HFD) contributes in increasing of reactive oxygen species (ROS) through fatty acid oxidized metabolism [4,5]. Adiponectin provides on glucose uptake and β -oxidation through AMPK activation [6]. Nonetheless, low adiponectin synthesis occurs in insulin resistance and DMT2 condition [7,8].

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Furthermore, oxidative stress occurs in hyperglycemia diabetic due to decreasing of potency antioxidant [9]. Total antioxidant status is suitable biomarker to assess oxidative stress by giving applicable information of cumulative antioxidant capacity in extracellular fluid compared individual element [10].

Application of traditional medicine from nature is popular these days [11]. Cape gooseberry (*Physalis peruviana*) is an uncommon consumed berry by Indonesian. Cape gooseberry (CG), belongs to *Solanaceae* family, was proved possessing anti-diabetic feature by various mechanisms improving insulin sensitivity; inhibiting intestinal carbohydrase enzyme and β -cell pancreas defect [11-14]. Quercetin is one of flavonoid in CG whose potency to suppress oxidative stress in DMT2. CG juice contains 89.4 $\mu\text{g}/\text{mg}$ quercetin (CG : water = 1:5) [15]. Juice is one of easy methods to consume fruit and an effective way to promote fruit consumption [16].

To our knowledge, comparison between CG juice and quercetin supplement research toward Total Antioxidant Capacity (TAC) and adiponectin concentration in DMT2 has not examined yet, therefore present study was carried out to show the role of CG juice in DMT2 rat HFD-STZ model. TAC and adiponectin level were investigated by ABTS and ELISA method respectively.

Material and method

Plant Material

CG fruits were collected from Ciwidey, West Bandung District, Indonesia in January 2018. The plant was identified and authenticated by its taxonomy characteristics in *Sistematika Tumbuhan* Laboratory, Biology Faculty of Gadjah Mada University, Indonesia.

Chemicals and Reagents

STZ ($\text{C}_8\text{H}_{15}\text{N}_3\text{O}_7$) and nicotinamide were purchased from Nacalai Tesque, Japan. Cholesterol, Na-CMC, and Quercetin 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-one,3,3',4',5,6-Pentahydroxyl-favone, Quercetin-3-O-rhamnoside were purchased from Sigma-Aldrich, Japan. TAC kit was purchased from Randox Laboratory, United Kingdom. Rat adiponectin ELISA kit was purchased from Bioassay Technology Laboratory, China. NaCl was purchased from Merck, Germany.

Preparation of CG Juice

Fruits in similar ripening stages were selected in juice processing. First, CG fruits were washed to get rid of impurities. Whole 70 g CG fruits were blended in a blender (National, Japan) without any water addition; therefore 1 mL juice contains 1.07 g CG fruits. The seeds and skin residues were removed by filter and cheesecloth. Juice was shaken before DPPH radical scavenging activity and administration (5 ml/kg/d and 25 ml/kg/d) by gavage [13].

DPPH Radical Scavenging Activity

DPPH radical scavenging activity assay is subjected to determine the antioxidant capacity of juice compared with quercetin supplement. DPPH analysis used in the experiment was according to Koleangan et al. (2013) [17]. Results were given in IC50 value. Data presented in Table 1 shows that IC50 value of CG juice and quercetin was 84.065 $\mu\text{g}/\text{mL}$ and 7.869 $\mu\text{g}/\text{mL}$ respectively.

Table 1. IC50 value of CG juice and quercetin for antioxidant capacity.

Sample	IC50 ($\mu\text{g}/\text{mL}$)
CG juice	84.065
Quercetin	7.869

Animal Laboratory

Healthy male Wistar rat, 8-12 months, weighing 150-200 g was purchased from Central Food and Nutrition Laboratory, Yogyakarta, Indonesia. Rats were housed in individual stainless-steel cages at regulated temperature (21°C). They were kept under suitable ventilation and a photoperiod 12-h light/ 12-h darkness scheduled light from 6 a.m. to 6 p.m. Rats were fed by 20 g/d standard laboratory feeding *Comfeed II* (7% fat) during non-HFD period. They were supplied by *ad-libitum* water throughout the experiment. Animal laboratory were provided the human care according to Animal Laboratory Guideline of *Pangan dan Gizi* Laboratory, Gadjah Mada University, Indonesia.

Experimental Design

After a week acclimatization, rats were divided into six groups (six rats each group). Group 1 (K-): rats didn't receive any treatments. Group 2 (K+): rats received HFD-STZ. Group 3 (X1): rats received HFD-STZ and CG juice 5 ml/kg/d. Group 4 (X2): rats received HFD-STZ and CG juice 25 ml/kg/d. Group 5 (X3): rats received HFD-STZ and quercetin 2.2 mg/kg/d. Group 6 (X4): rats received HFD-STZ and quercetin 30 mg/kg/d. After two weeks on HFD (43.6% fat), rats were fasted overnight and treated with nicotinamide (NA: 110 mg/kg, i.p.) and streptozotocin (STZ: 45 mg/kg, i.p.). HFD treatment was suspected to lead insulin resistance, while NA/STZ was suspected to make β -cell impairment [18,19]. After dietary manipulation, rats were injected intraperitoneally by NA followed by STZ 15 minutes later. STZ was dissolved into citrate buffer (pH 4.5). CG juice and quercetin treatments were administrated (three days after STZ-induced) by oral gavage for four weeks. Quercetin was

dissolved into 0.5% sodium carboxymethyl cellulose.

This experimental design was reviewed and approved by Medical Faculty of Diponegoro University-Dr.Kariadi Semarang Hospital research committee proved by ethical clearance certificate number 06/EC/FK-RSDK/I/2018.

Blood Sampling

Three days after STZ injection and the end of experiment (seven weeks), overnight fasting blood glucose was taken through plexus orbitals. Blood samples were collected in centrifugation tube and were centrifuged 4000 rpm in 15 minutes. TAC and adiponectin level were analyzed by ABTS and ELISA method respectively.

Statistical analysis

Results were expressed as either mean \pm SD or median (interquartile ranges). Statistical significance was calculated using one-way analysis of variance (ANOVA) followed by post hoc Bonferroni regarding of distributed data normally. Nevertheless, Kruskal wallis followed by Mann Whitney was used to calculate statistical significance of abnormal data distribution. All the statistical analysis was analyzed by SPSS 21 software. Differences data were considered significant at $p < 0.05$ and confidence interval 95%.

Results

All rats were still alive during the study. The body weight and blood glucose level in the end of acclimatization on average were 178.56 g and 74 mg/dL respectively. HFD-STZ successfully increased body weight (221.52 g; $p < 0.001$) and blood glucose (218.44 mg/dL; $p < 0.001$).

The effect of CG juice as well as quercetin treatment on TAC level in the experimental group of HFD-STZ induced rat is shown in Figure 1 followed by Table 2. K(+) rats

significantly decreased in TAC level compared with K(-) rats ($p=0.007$). An increase of TAC level was recorded in treatment groups compared with K(+) ($p<0.05$), although quercetin 30 mg/kg/d showed the highest TAC level in the end of study. CG juice treatment in X2 improved

TAC level better than CG juice treatment in X1 ($p=0.025$), but that was not significantly different with TAC level in X3 ($p=0.906$). Moreover, TAC level in K(-) showed a slightly decrease in the end of study (-0.15 mmol/L).

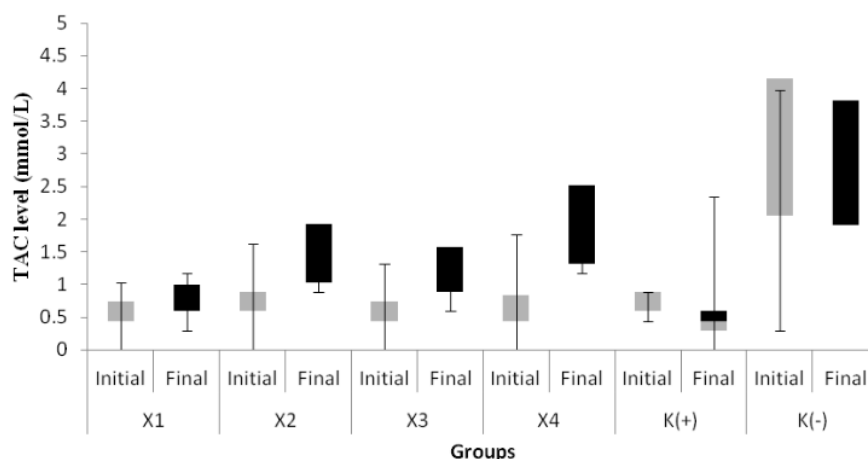


Figure 1. Effect of CG juice and quercetin supplement towards TAC (mmol/L) level. TAC level change (mmol/L) before and after treatment in X1, X2, X3, X4, K(+), and K(-) were 0.15 (0.00-0.44); 0.59 (0.30-0.59); 0.47 (0.15-0.59); 0.78 (0.59-0.88); -0.14 ((-0.30)-0.00); and -0.15 ((-0.30)-0.00).

Table 2. Mann Whitney test of TAC level change (mmol/L) before and after treatment in X1, X2, X3, X4, K(+), and K(-) groups.

Groups	p value					
	X1	X2	X3	X4	K(+)	K(-)
X1	-	0.025*	0.043*	0.009*	0.023*	0.014*
X2	-	-	0.906	0.017*	0.008*	0.007*
X3	-	-	-	0.017*	0.008*	0.007*
X4	-	-	-	-	0.008*	0.008*
K(+)	-	-	-	-	-	0.007*
K(-)	-	-	-	-	-	-

* $p<0.05$ = significant level

Both of CG juice and quercetin treatment also successfully improved adiponectin level of HFD-STZ induced rats (Figure 2 followed by Table 3). Figure 2 showed that HFD-STZ ameliorated adiponectin level of K(+) compared with K(-) ($p=0.044$). Adiponectin level in X4 rats significantly elevated among other treatment

groups ($p<0.05$). Our results also revealed that treatment in X2 rats significantly improved adiponectin level compared with both of CG juice treatment in X1 ($p<0.001$) and quercetin supplementation in X3 ($p<0.001$). Furthermore, adiponectin level of K(-) in the end of study attenuated by 1.5 mg/L.

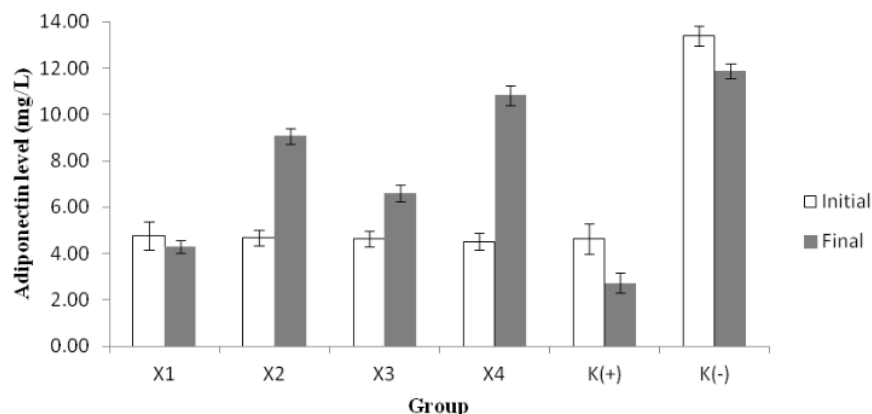


Figure 2. Effect of CG juice and quercetin supplement towards adiponectin (mg/L) level. Adiponectin level change (mg/L) before and after treatment in X1, X2, X3, X4, K(+), and K(-) were - 0.5±0.67; 4.4±0.50; 6.3±0.36; 1.9 ± 0.43; -1.9±1.04; -1.5±0.43.

Table 3. Post-hoc Bonferroni test of adiponectin level change (mg/L) before and after treatment in X1, X2, X3, X4, K(+), and K(-) groups

Groups	p value					
	X1	X2	X3	X4	K(+)	K(-)
X1	-	0.000*	0.000*	0.000*	0.021*	0.237
X2		-	0.000*	0.001*	0.000*	0.000*
X3	-	-	-	0.000*	0.000*	0.000*
X4	-	-	-	-	0.000*	0.000*
K(+)	-	-	-	-	-	0.044*
K(-)	-	-	-	-	-	-

*p<0.05 = significant level

Discussion

In the present study, it was observed that CG juice and quercetin supplement treatment enhanced TAC and adiponectin level in HFD-STZ induced rats. Experimental results suggest that CG juice and quercetin supplement treatment could improve diabetic condition in HFD-STZ rat model. In this study, HFD-STZ exerts possible stress oxidative overproduction as verified by the decrease of TAC level. One of modeling DMT2 in rats is using HFD-STZ [18-20].

Hyperglycemia in diabetes leads antioxidant status disturbance related to ROS overproduction and subsequently disrupts body defense. Low

antioxidant status and body defense disruption induce stress oxidative production [9,21]. Low antioxidant enzymes activity such as catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase, are affected by hyperglycemia in diabetes [22]. ROS possesses toxic properties related to its high reactivity to enzymes and results in tissue damage [23]. Moreover, NADPH activity is high in uncontrolled diabetes. Consequently, it results anion radical superoxide elevation and subsequently increases stress oxidative production [24].

Dietary antioxidant in food that present in significant amounts is able to increase antioxidant system in body and subsequently

decreases ROS production in diabetic rat laboratory animal. Dietary antioxidants commonly in fruits have protective effect of oxidative damage in diabetes [25]. CG is one of berries that suggested has potent antioxidant because of its flavonoid content. Dkhil et al. (2014) demonstrated that flavonoid content in CG extract was 93.7 mg quercetin equivalent/g extract [11]. Hydrogen donor from exogenous antioxidant scavenging radical component is part of hydroxyl group, hence it forms stable radical fenoxil [26]. Quercetin is part of flavonoid, therefore it considered as an exogenous antioxidant that binds Fe and subsequently prevents ROS production in Harber-Weiss/Fenton reaction. Additionally, quercetin can protect the cell through superoxide forming in enzymatic reaction[27]. CG juice and quercetin supplement may help improvement of endogenous antioxidant synthesis to protect cells. In this context, elevated antioxidant activity of catalase, superoxide dismutase, and glutathione peroxidase in quercetin supplementation was reported by Suganya et al. (2018)[28]. Elevating TAC level in CG juice treatment groups may also be affected by its quercetin content. Hassan et al. (2017) reported that quercetin presence in CG juice belongs to substantial flavonoid compound[29]. The main flavonoids identified in CG fruits are quercetin (0.1-10.9 mg/kg), rutin (1.7-6.7 mg/kg), myricetin (1.1-1.3 mg/kg), epicatechin (0.2-0.6 mg/kg), and catechin (3.8-6.7 mg/kg)[28-30]. CG juice also contains bioactive compounds such as withanolides and carotenoid[28,31]. Moreover, the decrease of TAC in K(-) might be the standardized laboratory diet lack of antioxidant content, therefore it is essential for healthy condition to intake exogenous antioxidant, thus endogenous antioxidant can be maintained. A study performed by Egert et al. (2008) related quercetin supplementation to

healthy subjects enhanced quercetin plasma quercetin concentrations [33].

Adiponectin belongs to hormone involved in glucose metabolism and is secreted by adipocyte that has role in insulin sensitivity improvement through its receptor binding [6,32]. Adiponectin helps glucose uptake and β -oxidation through AMPK activation, but its synthesis declines in insulin resistance condition and DMT2 [6-8]. In the present work, HFD-STZ decreased adiponectin level, but CG juice and quercetin supplement treatments successfully ameliorated adiponectin level. Low adiponectin level in the present study considered as increasing oxidative state and was associated with high ox-LDL level in DMT2 [34]. The present work results are in accordance with another study reporting that quercetin supplementation 25 mg/kg/d significantly elevated adiponectin level compared with HFD-induced rat group [35]. Furthermore, the study undertaken by Jeong et al. (2012) reported that quercetin treatment 0.08% of diet successfully increased adiponectin percentage by 34% [9]. A mechanisms that could possibly explain quercetin role in increasing adiponectin level is elevation of mRNA PPAR- γ concentration [35]. Moreover, bioactive contents in CG juice were postulated that those have main role in elevating adiponectin level. The previous study by Esfahani et al. (2014) reported that there was positive association between low adiponectin and low GSH [34]. Adiponectin level of K(-) in the end of study (11.9 mg/L) was considered as normal although it decreased 1.5%. Normal adiponectin level in normal subject is 7-12 mg/L [36]. Decreasing of adiponectin level in the end of study was postulated the standard diet laboratory *Comfeed II* containing low antioxidant that led adiponectin level decrease.

We provide new insight by which CG juice improving TAC and adiponectin in HFD-STZ

induced rat. Further studies are needed to understand the plausible mechanism by which CG juice attenuates both TAC and adiponectin level.

Conclusions

In conclusion, these results performed the beneficial effect of CG juice in improving antioxidant and adiponectin level of HFD-STZ rat model.

Disclosure. The author declares no conflict in interest.

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