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Captopril Modulates Behenic Acid and L-hydroxyproline to Lower Blood Glucose on High-Fat Diet and Low-Dose Streptozotocin-Induced Diabetic-Rats

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Abstract

Background: captopril has a hypoglycemic effect with the capacity to reduce blood glucose by increasing the rate of glucose removal in the muscles.

Objective: To determine the impact of Captopril on a high-fat diet and low-dose streptozotocin-diabetic rats. **Materials and methods:** rats were divided into six groups (n = 5). One group was untreated while five groups (negative, positive, and three groups with various doses of captopril) were treated with a high-fat diet and low-dose streptozotocin. The negative control group was given 0.5% CMC, The positive group was assigned metformin dose 90 mg/200g /day orally, and three variation dose groups of captopril 25; 50; 100 mg/kg BW rats orally. Induced by high-fat diet (standard feed: quail egg yolk: butter: high fructose corn syrup, 5: 3: 1: 1) for 28 days, and then injection low-dose streptozotocin (30 mg/kg BW i.p) once at day 28 and continued by giving the high-fat diet for 7 days, continued for 14 days treated with an oral administration. Blood samples were collected via retro-orbital sinus and use for analyzed glucose by spectrophotometry; free fatty acid was evaluated by GC-FID.

Results: all dose of captopril significantly reduced glucose level ($p < 0.05$) but not dose-dependent. The power of Captopril similar to metformin to reduce glucose level, captopril and metformin can lower blood glucose levels back to normal. HFD increased SFA and captopril cut that. Captopril also promotes behenic acid. **Conclusion:** Based on these results, Captopril has a potential effect as an anti-hyperglycemic agent by modulates behenic acid.

Keywords: Captopril, Glucose Level, High-Fat Diet, Low-Dose Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a disease or chronic metabolic disorder with multiple etiologies characterized by high blood sugar levels accompanied by impaired carbohydrate, lipid, and protein metabolism as a result of insulin insufficiency (Brito-Casillas, Melián, & Wägner, 2016). Insulin deficiency can be caused by disruption or deficiency of insulin production by beta Langerhans cells of the pancreas gland or caused by the body's lack of responsiveness to insulin. In 2017, around 425 million adults in the age range of 20-79 years are estimated to have diabetes mellitus and if it continues until 2045 there will be approximately 629 million adults in the age range of 20-79 years in the world who suffer from diabetes mellitus. Of the total diabetics, about 87% to 91% suffer from type 2 diabetes mellitus (Gómez-Huelgas et al., 2018). Recent research conducted by the International Diabetes Foundation (IDF) also revealed that diabetics for women aged 20-79 years were estimated at 8.4%, which was slightly lower than men (9.1%), and the highest prevalence of diabetes was in the age group of 65 -79 years old (Rivera-Mancía, Trujillo, & Chaverri, 2018).

All types of diabetes mellitus produce serious acute and chronic complications that can increase the overall risk of premature death. Although numerous studies have shed light on the multifactorial nature of diabetes mellitus, insulin resistance and pancreatic β-cell dysfunction are the hallmarks of this disease (Kendall, Cuddihy, & Bergenstal, 2009). Current drugs produce a range of adverse effects, such as weight gain, cardiovascular disease, hypoglycemia, and gastrointestinal alterations (Holstein & Beil, 2009). Choosing the best agent(s) can be challenging and requires weighing the risks and benefits of each particular medication (Sterrett, Bragg, & Weart, 2016).

Some research showed the beneficial effects of Captopril on Diabetic model rat, the found finding that ACE inhibitors reduced plasma glucose concentration; glucose accumulation in the retina and cultured retinal cells (Kodama et al., 1990; Zhang, Gao, Widness, Xi, & Kern, 2003).

Based on previous research, captopril has a hypoglycemic effect with the capacity to decrease blood glucose by increasing the level of glucose removal in the muscles. However, further research is needed to evaluate the mechanism of captopril as an antidiabetic. This study was aimed to evaluate the mechanism of captopril on Free Fatty Acid profile on animal model of Diabetes Mellitus induced by High-Fat Diet combine with low dose of streptozotocin.

MATERIALS AND METHODS

Chemical Materials

We use chemical as follow: Aquadest (Brataco, Indonesia), CMC (Brataco, Indonesia), Ether (Dwinika, Indonesia) were purchased. Chemical Reagents (Kit) used was Glucose GOD-POD kit (Human, Germany), Streptozotocin (Sigma, Germany).

Animals

Animals used in this study were 42 male rats from the SD strain aged 2-3 months and weighing 150-250 grams obtained from Non-Ruminansia dan Satwa Harapan laboratory, Faculty of Animal Husbandry IPB. Rats used has been declared healthy and meets the requirements as a test animal. Rats used have obtained ethical approval issued by the Health Research Ethics Committee of the

Faculty of Medicine, Universitas Indonesia (UI) following the certificate passed the ethical review of KET-227 / UN2.F1 / ETIK / PPM.00.02 / 2019. Before treatment, the rats were acclimatized 7 days, during acclimatization, rats kept on a standard diet and water ad libitum.

42 male rats were divided into six groups (n=7). The first group as a normal group was given CMC-Na as vehicle, second group as a positive control group were given metformin 90 mg/ 200 g BW rat, the third group as a negative control group was given CMC 0.5%, and the three other groups were given captopril 5 mg/200 g BW rat, 10 mg/200 g BW rat and 20 mg/200 g BW rat. The drug or vehicle treatments were given for 14 consecutive days.

Drugs

Captopril (Dexa Medica, Indonesia) as drug treatment and metformin (Hexpharm Jaya, Indonesia) as positive drugs were used. Captopril and metformin were dissolved in CMC 0.5% and given orally. The three various doses of captopril were 25, 50, and 100 mg/kg BW and the dose of metformin was 90 mg/200 g BW.

The Induction by High-Fat Diet Feed and Low-Dose Streptozotocin:

The hyperglycemia condition of this model was induced by providing high-fat diet 20 grams/day/200g rat and low-dose streptozotocin (30 mg/kg BW rat, i.p). Composition of high-fat diet feed is standard feed: quail egg yolk: butter: high fructose corn syrup (5:3:1:1). The high-fat diet induction of this study was performed for 35 days, while low-dose streptozotocin was provided once at 28^{th} day.

Weight Observation

The animals were also observed for their body weight weekly on 0^{th} day or before treatment, 7^{th} day, 14^{th} day, 21^{st} day, 28^{th} day, 35^{th} day, 42^{nd} day, and 49^{th} day.

Plasma Preparation

The blood samples were taken on 0^{th} day (before treatment), 28^{th} day (28 days after induction of a high-fat diet), 35^{th} day (7 days after induction of a low-dose streptozortocin), 3 hour after drug administration, 42th day (7 days after drug administration), and 49^{th} day (14 days after drug administration). Blood samples taken from retro-orbital sinus were collected in a microtube. Next, the blood was centrifuged at 7000 rpm for 15 minutes. The supernatant was taken to obtain plasma samples. Then, plasma samples were analyzed to determine the levels of Glucose and Free Fatty Acid.

Glucose Level Measurement

Glucose level was measured using a glucose liquicolor kit by enzymatic colorimetry using UV-VIS spectrophotometry at a wavelength of 500 nm. The absorbance obtained was compared with the standard absorption then multiplied by the standard concentration.

Fatty acid and amino acid Measurement

Fatty acid and amino acid were measured by Gas Chromatography Flame ionization Detector (Alliance, Switzerland). Plasma free fatty acids were added with Sulphonic acid, then injected 5 µL and analyzed using gas chromatography Flame ionization Detector (125A, 5 µm x 4,6 mm x 150 mm GC columns (XTerra C8)

Statistical Analysis

The data results were expressed as means ± standard deviation (SD). The data obtained were processed statistically using SPSS version 16.

RESULTS

Development of Hyperglycemic Animal Models

On the 28^{th} day of HFD induction, blood glucose levels were measured and had not shown a state of hyperglycemia. Then on the 28th day, the induction was continued with the administration of low-dose streptozotocin intraperitoneally, and for 7 days remained given a high-fat diet. On the 35^{th} day, blood glucose levels were remeasured, they showed a state of hyperglycemia (> 200 mg/dl) and statistically there were significant differences compared with H0 ($p = 0.000$) which meant that they had significantly increased blood glucose levels (**Table 1**).

Table 1. Average blood glucose levels for normal and induction groups after 35 days of induction

Group	Blood Glucose Levels (mg/dl ± SD)		
	0th day	28th day	35th day
Normal	92.14 ± 12.53	100.10 ± 16.10	111.34 ± 8.76
Induction	91.17 ± 13.35	121.92 ± 18.00	300.81 ± 66.51*

Note: Normal (untreated); Induction (High-Fat Diet 20g/day/rat orally and low-dose streptozotocin at 28^{th} day intraperitoneally), (*) = $p<0.05$ succeeded in making animal models (there was a significant increase between the normal group and the induction group)

During 35 days of induction, rats were weighed every 7 days. Both normal and treatment rat gained weight, but the rat given HFD induction increased body weight higher than the normal group. Weight gain was analyzed using the Independent T-test between the normal group and the induction treatment with statistical results showing a significant difference ($p = 0.001$). (**Table 2**)

Table 2. Average Body Weight Gain During HFD Induction

	Normal (g)	High-Fat Diet (g)
Average ± SD	13.97 ± 10.36	46.57 ± 22.18*

Note: (*) $p <0.05$, a significant weight gain occurred after 35 days of induction compared with the normal group

Effects of Captopril on Blood Glucose Levels

Captopril administration was carried out for 2 weeks after the diabetic rat was developed. Glucose level measurements were carried out for 3 times, namely in the first 3 hours of drug administration, then 7 days after drug administration, and finally 14 days after drug administration.

Table 3. Average Blood Glucose Levels after Treatment of Captopril

Group	Blood Glucose Levels (mg/dl ± SD)						
	Day-35	Day-36 (3 hour after treatment)	Day-42	Day-49	ΔD36-D35	ΔD42-D35	ΔD49-D35
Normal	111.34 ± 8.76	112.06 ± 8.63[c]	107.17 ± 12.07[c]	106.97 ± 9.79[c]	0.72 ± 4.01	-4.17 ± 12.39	-4.38 ± 9.86
Negative	284.28 ± 57.39	286.94 ± 32.98[ab]	230.29 ± 23.49[ab]	226.43 ± 21.73[ab]	2.65 ± 58.69	-53.59 ± 62.33	-57.87 ± 69.10
Positive	352.26 ± 67.44	181.86 ± 53.24	131.48 ± 30.68[c]	107.79 ± 6.11[c]	-170.40 ± 60.79[*#]	-220.78 ± 79.75[*#]	-244.47 ± 68.34[*#]
K1	280.04 ± 65.25	177.45 ± 42.18	114.99 ± 13.98[c]	106.27 ± 5.93[c]	-102.60 ± 68.42[*#]	-165.05 ± 74.69[*#]	-173.77 ± 69.29[*#]
K2	262.61 ± 39.28	175.45 ± 25.24	108.16 ± 12.47[c]	100.82 ± 6.61[c]	-87.15 ± 30.33[*#]	-150.52 ± 49.33[*#]	-161.78 ± 40.76[*#]
K3	324.85 ± 55.57	188.97 ± 55.00	117.56 ± 15.89[c]	107.15 ± 6.41[c]	-135.88 ± 46.27[*#]	-207.29 ± 64.14[*#]	-217.70 ± 59.55[*#]

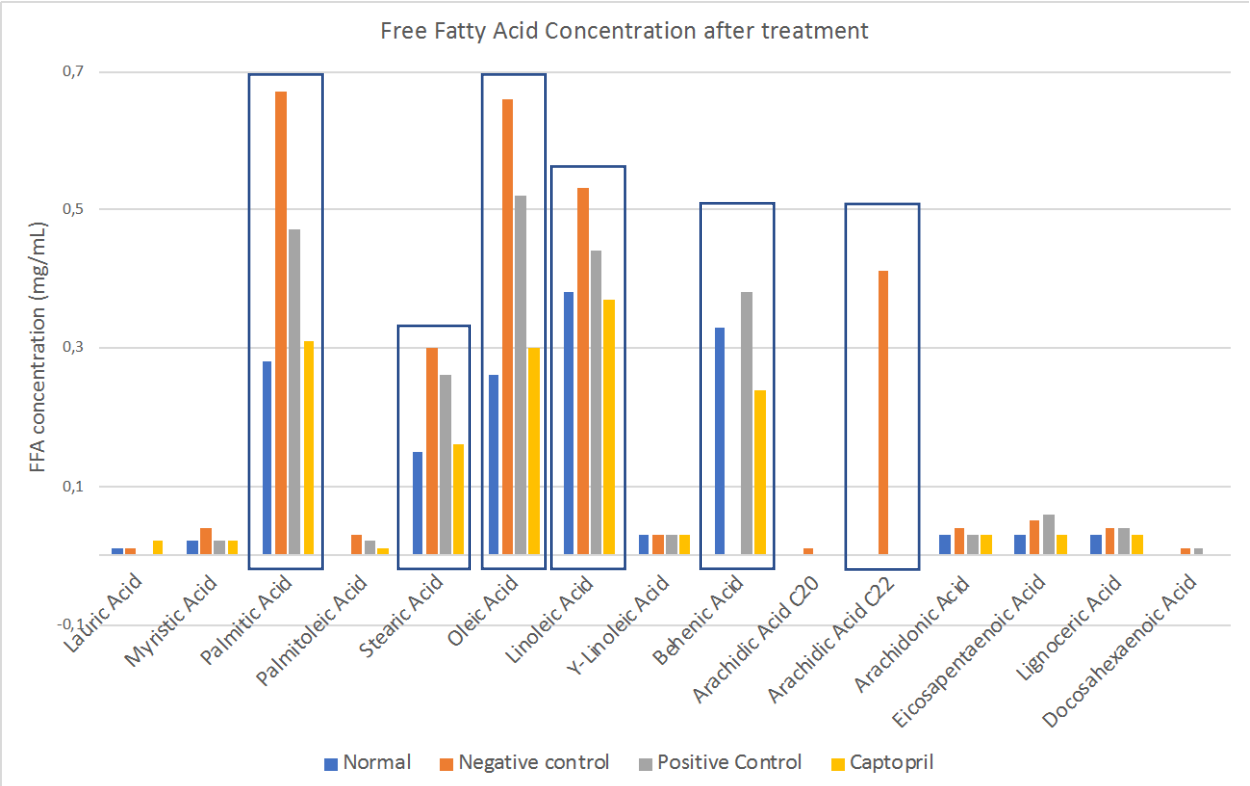
Note: normal = giving 0.5% CMC; negative = diabetic induction without drug administration; positive = metformin 90 mg / 200g BW rat; K1 = captopril dose 25 mg /kg BW rat; K2 = captopril dose 50 mg /kg BW rats; K3 = captopril dose of 100 mg /kg BW of rats. Based on the Independent t-test, (a) = p <0.05 compared to the normal group; (b) = p <0.05 compared to the positive group; (c) = p <0.05 compared to the negative group; (*) = p <0.05 compared to the normal group; (#) = p <0.05 compared to the negative group

Effects of Captopril on Free Fatty Acid Concentration

Figure 1 showed that in negative control that described as Diabetes rat, showed high concentration of short-chain free fatty acid, interestingly no detectable of Behenic Acid.

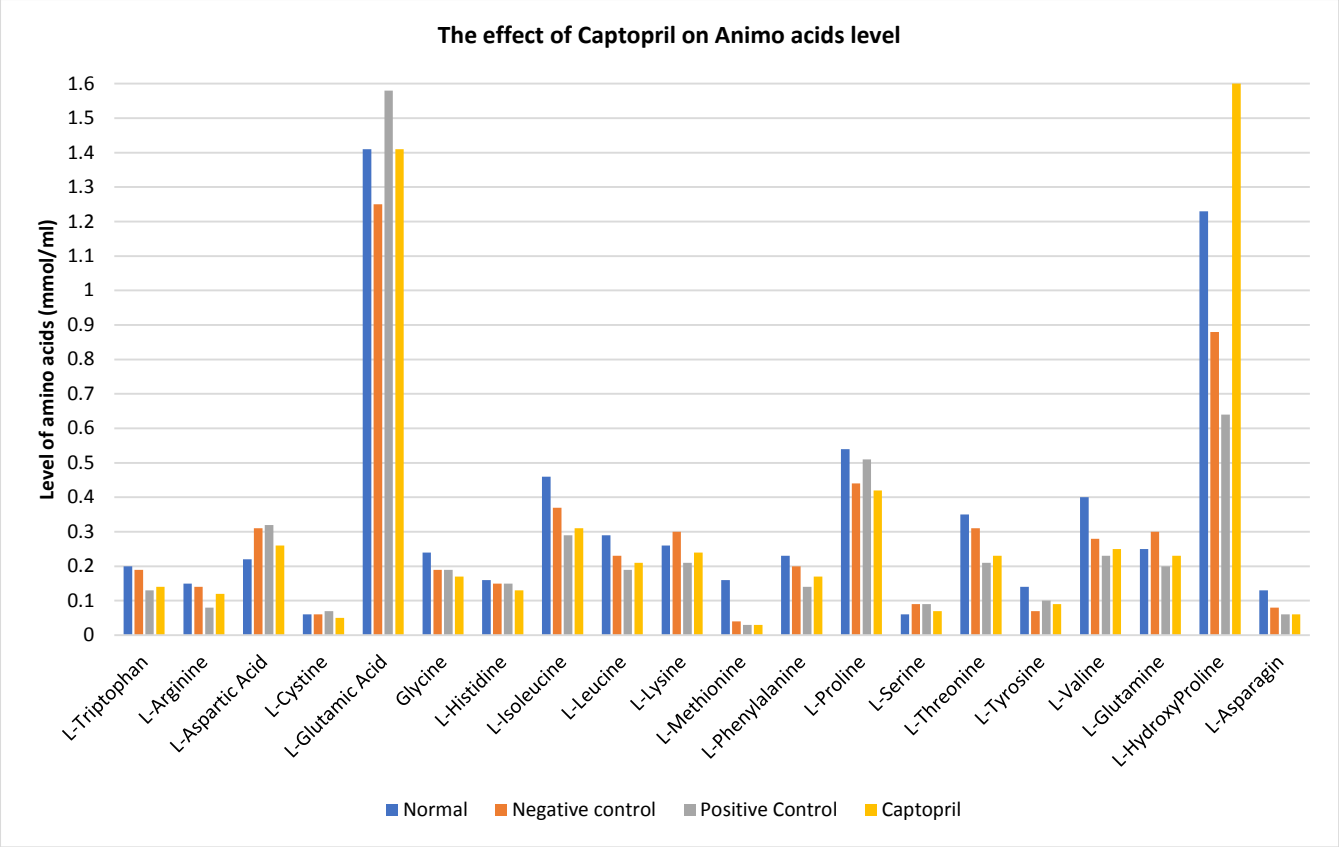
Effects of Captopril on Amino Acid Concentration

Figure 2 showed that in negative control that described as Diabetes rat, showed reduced level of some amino acids and administration of captopril could increased the reduction aof those amino acids.



Note: normal = giving 0.5% CMC; negative = diabetic induction without drug administration; positive = metformin 90 mg / 200g BW rat; K1 = captopril dose 25 mg /kg BW rat; K2 = captopril dose 50 mg /kg BW rats; K3 = captopril dose of 100 mg /kg BW of rats. The effect of captopril and metformin as shown in black box.

Figure 1. Fatty Acid Concentration



Note: normal = giving 0.5% CMC; negative = diabetic induction without drug administration; positive = metformin 90 mg / 200g BW rat; K1 = captopril dose 25 mg /kg BW rat; K2 = captopril dose 50 mg /kg BW rats; K3 = captopril dose of 100 mg /kg BW of rats. The effect of captopril and metformin as shown in black box.

Figure 2. Amino Acid Concentration

DISCUSSION

Development of Hyperglycemic Animal Models

This animal model was also chosen because it can reflect the clinical symptoms observed in humans diagnosed with diabetes. After administration of low-dose streptozotocin, rat showed a very significant increase in blood glucose levels. High-fat diet blunted response of glucose metabolism to insulin in adipocytes of high-fat-fed rats is a result of a decreased intracellular capacity to utilize glucose for lipogenesis (Buettner, Schölmerich, & Bollheimer, 2007). Streptozotocin is also a hydrophilic compound, and it is able to pass cellular membrane via GLUT2 transporters. STZ-induced cytotoxicity and apoptosis in Rin-5F cells is mediated by increased oxidative/nitrosative stress, mitochondrial dysfunction, and alterations in cell signaling (Lenzen, 2008).

Induction by using a high-fat diet feed is used to show the real condition of diabetes in humans who generally have diabetes because of poor diet. The rats treated with high-fat diet and low-dose streptozotocin illustrated an increase of blood glucose levels compared to normal rats This shows that induction using high-fat diet and low-dose streptozotocin can cause hyperglycemia due to high-fat feeds which causes insulin resistance in the tissues caused by the presence of high free fatty acid levels in the tissues, and streptozotocin which induces selective pancreatic β cells necrosis in mice (Wong, Chin, Suhaimi, Fairus, & Ima-Nirwana, 2016). The development of animal models with this method also caused a very significant weight gain as shown in Figure 1. Weight gain due to excess fat intake which causes a lot of free fatty acids in the tissue which will reduce glucose absorption and lead to hyperglycemia.

Effects of Captopril on Decreased Blood Glucose Levels

3 hours after drugs administration, a significant difference in blood glucose level ($p = 0.000$) was seen. However, a decrease in blood glucose levels did not show a normal condition, as seen from the comparison between the normal group and the treatment group there were still significant differences ($p < 0.05$) (**Table 2**).

Decreased blood glucose levels after 7 days of drug administration also showed a normal number. As seen from the comparison between the normal group and the treatment group, there was no significant difference ($p = 0.000$). However, there were still some groups who have not returned to normal. Decreased blood glucose in the captopril group with several dose variants showed similar figures to a decrease in blood glucose in the positive control group given the drug metformin. As seen from the comparison between the positive group and the captopril group there were no significant differences ($p > 0.05$) (**Table 2**).

Blood glucose level on the 14th day was seen to be very significant in the decrease in the captopril groups in three doses and in the positive control group when compared to the negative group. Other research also states that captopril can significantly reduce blood glucose levels for 14 days (Duarte et al., 1999).

Effects of Captopril on Fatty Acid

HFD treatment in Negative control showed increased Saturated Fatty Acis (SFA) such as Palmitic acid, that caused insulin resistance by three mechanism as shown in Figure 1. Namely, i) increased synthesis of deleterious complex lipids; ii) impaired function of cellular organelles; iii) receptor-mediated inflammation (Palomer, Pizarro-Delgado, Barroso, & Vázquez-Carrera, 2018). The administration of Metformin and Captopril showed decreased SFA, but captopril recovered SFA to normal value. Interestingly, HFD did not stimulated Behenic acid that inversely Metformin and Captopril treatment. Some researcher report beneficial effects of behenic acid such as increases excretion of lipid in the feces (Moreira, Santos, Gambero, & Macedo, 2017), anti-inflammatory (da Silva et al., 2019).

Effects of Captopril on Amino Acid

HFD treatment in Negative control showed reduced in L-glutamic acid and L-hydroxy-proline, and the administration of captopril recover those amino acids. Some researchers found that plasma amino acids pattern were related of some diseases. Decreased level of L-hydroxyproline is a marker of poor wound-healing (Srivastava, Khare, Nagar, & Srivastava, 2016). Diabetes mellitus–associated impaired wound healing severely affects life quality of patients with diabetes mellitus leading to prolonged hospitalization and lower limb amputations. This result indicated that some metabolism pathway involving L-hydroxyproline could be as target of therapy to increase quality of life of diabetic patient by modulate L-hydroxyproline metabolism.

CONCLUSION

After administration of captopril, it showed an effect to decrease glucose levels in plasma by dose of 100 mg/kg BW rats, and improved diabetic condition by controlled L-hydroxyproline level and behenic acid level.

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Declaration of Interest

The authors declared no conflict of interest.

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