RESEARCH ARTICLE

The Potential of *Hibiscus sabdariffa* for Treatment of Obesity: Focus on FGF21 in Liver and Adipose Tissue

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Abstract

Obesity is one of the health problems associated with FGF21 resistance. FGF21 is a hormone secreted in the liver and plays a role in energy homeostasis in adipose tissue. FGF 21 is used as an alternative for treating obesity. While the potential of H. sabdariffa for weight loss has been acknowledged, H. sabdariffa's ability to handle FGF 21 is still unknown. This study aims to determine the potential of H. sabdariffa in FGF 21 resistance by measuring FGF 21 in adipose and liver tissue. This study was conducted at Biochemistry Animal House Laboratory at Faculty of Medicine Universitas Indonesia in January until May 2019. This experimental studies using 24 male rats for 6-10 weeks were divided into four groups, namely the normal control group (N), the obese control group (Ob), the obese group with H. sabdariffa dose 200 mg/kgBW/day (Ob-Hib 200), and obese groups with H. sabdariffa dose 400 mg/kgBW/day (Ob-Hib 400). H. sabdariffa is given every day for 5 weeks in a row. Examination of FGF21 protein in white adipose tissue and liver using the ELISA test. ANOVA test results showed an increase in FGF21 levels in adipose tissue in obese rats given H. sabdariffa extract dose of 400 mg/kgBW/day ($p<0.05$) and even significantly different than normal conditions ($p<0.05$). The results showed that a dose of 400 mg/kgBW had the potential to increase FGF21 levels in the liver ($p<0.05$). In conclusion, giving extract of H. sabdariffa has the potential for handling FGF21 resistance because H. sabdariffa is able to increase FGF 21 levels in adipose and liver tissue.
Keywords: *FGF21, H. sabdariffa, obese.*

Potensi *Hibiscus Sabdariffa* untuk Mengobati Obesitas: Fokus pada FGF21 di Jaringan Hati dan Adiposa

Abstrak

Obesitas berhubungan dengan resistensi FGF21. FGF21 merupakan hormon yang disekresikan oleh hati dan berperan dalam homeostasis energi di jaringan adiposa. FGF21 digunakan sebagai alternatif dalam penanganan obesitas, namun potensi H. sabdariffa dalam menurunkan berat badan belum pernah dilakukan dan potensinya dalam pengaturan FGF21 juga belum diketahui. Penelitian bertujuan mengetahui potensi H. sabdariffa dalam mengatasi resistensi FGF21 melalui pengukuran kadar FGG21 di jaringan adiposa dan hati. Penelitian dilakukan di Laboratorium Hewan Biokimia Fakultas Kedokteran Universitas Indonesia pada bulan Januari-Mei 2019. Studi eksperimental ini menggunakan 24 tikus jantan 6-10 minggu yang dibagi menjadi 4 kelompok, yaitu kelompok normal (N), kelompok kontrol obese (Ob), kelompok obese dengan pemberian H. sabdariffa 200 mg/kgBB/hari (Ob-Hib 200), dan kelompok obese dengan pemberian H. sabdariffa 400 mg/kgBB/hari (Ob-Hib 400). H. sabdariffa diberikan selama 5 minggu berturut-berturut sehari sekali. Pemeriksaan protein FGF21 di jaringan adiposa dan hati menggunakan metode ELISA. Uji ANOVA menunjukkan peningkatan FGF21 di jaringan adiposa pada tikus obese yang diberikan H. sabdariffa dengan dosis 400 mg/kgBB ($p<0,05$) dan memiliki perbedaan signifikan dengan kelompok normal ($p<005$). Selain itu, pemberian H. sabdariffa dengan dosis 400 mg/kg BB pada jaringan hati meningkatkan kadar FGF21 ($p<0,05$). Disimpulkan H. sabdariffa memiliki potensi untuk mengatasi resistensi FGF21 karena meningkatkan kadar protein FGF21 baik di jaringan adiposa maupun hati.
Kata kunci: *FGF21, H. sabdariffa, obesitas.*

Introduction

Obesity is a condition caused by excessive fat accumulation and currently has become endemic throughout the world. According to the 2016 World Health Organization (WHO), 39% of adults over the age of 18 suffer from being overweight and 13% suffering from obesity. Obese people continue to increase and cause 2.5 million deaths each year.[1] The mortality rate is expected to double in 2030.[2] Some studies show that obesity is a metabolic disorder associated with FGF21 resistance. Fibroblast Growth Factor 21 (FGF21) is a member of fibroblast growth factors (FGFs) located on chromosome 19 and expressed by several organs, such as the liver and adipose. FGF21 plays a role in regulating energy through browning and thermogenesis in adipose tissue.[3] FGF21 will induce browning through increased expression of UCP-1 which plays a role in thermogenic. However, the inflammatory process in obesity causes a decrease in the expression of FGF21 receptors in adipose tissue giving rise to FGF21 resistance.[4,5] In fact, the role of FGF21 in browning can occur when complex bonds with receptors are formed, namely FGFR1 and β-Klotho.[6] These bonds will form signal transduction which increases the activity of Sirtuin-1 (SIRT1), then stimulates PGC-1α to increase expression of UCP1.[7]Referring to the potential of FGF21 in regulating energy through browning and thermogenesis, obesity management is currently being developed based on the role of FGF21.[8] At present there have been many studies on obesity treatment based on FGF21 work, such as by doing physical exercise and developing drugs. Kartinah et al[9], shows that high intensity intermittent physical exercise can increase the amount of FGF21 released from the muscles and carry out its action in adipose tissue. In addition, there is also a development of drugs that can be used to increase FGF21 sensitivity and increase FGF21 receptors such as thiazolidinediones (TZDs).[4] At present, it is also necessary to develop obesity management based on natural compounds. Because there are natural compounds that have been known to have potential in handling obesity such as *Hibiscus sabdariffa* Linn. (*H. sabdariffa*) otherwise known as rosela plant. The plant *H. sabdariffa* has a lot of chemical compounds in parts of the calix, such as flavonoids, quercetin, polyphenols, catechins, and anthocyanins.[9] In vivo study shows that anthocyanins have an effect on preventing increase in body weight on obese mice. Research using *H. sabdariffa* as anti-obesity has been at the molecular stage, however, only cellular potency of *H. sabdariffa* is known to inhibit white adipose tissue lipogenesis.[10] The potential of *H. sabdariffa* in handling FGF21 resistance is still unknown. Therefore, this study aims to determine the potential of *H. sabdariffa* in handling FGF21 resistance by measuring FGF21 levels in adipose tissue and liver in obese mice.

Methods

Animals Treatment

This experimental study used 24 male sprague-dawley rats, aged 6-10 weeks, weighing between 110-160 g. Before and during the treatment, the health of the rats was maintained so as not to get sick. The rats are given feed and drink in ad libitum. The cage is kept clean and is set 12 hours bright and 12 hours dark. The ambient temperature is maintained at a temperature of 23±1°C. This research was conducted at Biochemistry Animal House Laboratory at Faculty of Medicine Universitas Indonesia in January until May 2019. Then, rats were divided randomly into 4 groups, namely: (1) control group (N), (2) obese (Ob) group, (3) obese group given *H. sabdariffa* 200 mg/day/kgBW (Ob-Hib 200), (4) obese groups given *H. sabariffa* 400 mg/day/kgBW (Ob-Hib 400).

Other things in the experiment were adjusted to the code of ethics for the handling and use of experimental animals. This research has received the approval of the Health Research Ethics Committee of the Faculty of Medicine University of Indonesia with number: 1172/UN2.F1/ETIK/2017.

Obese Rat Model

The rats are given a high-fat diet with a standard composition of the diet (6.43% fat, 23.6 protein) a high-fat diet (19.09% fat, 24% protein). Rats were measured weight and height to determine the value of the lee index. If the value of the lee index is more than 310, then the rat is included in the obesity chart. After 17 weeks of giving a high-fat diet, a lee index value above 310 was obtained and then included in the obese (O), obese group given *H. sabdariffa* 200 mg/day/kgBW (O-Hib 200), obese groups given *H. sabdariffa* 400 mg/day/kgBW (O-Hib 400). The following is the formula for measuring lee index.

$$\text{Indeks Lee} = \frac{\sqrt[3]{body\ weight} \times 1000}{\text{body length}}$$

Administration of H. sabdariffa Methanol Extract

The simplicia of *H. sabdariffa* plants was obtained from the Center for Biopharmaceutical Studies at the Bogor Agricultural University. Afterwards, extraction was carried out using maceration method using methanol. Dilution of the extract preparation was prepared for 7 days of treatment. This is to avoid damage to the dosage if stored for more than 7 days. After dilution, the preparation is stored at 4°C.The administration of *H. sabdariffa* is carried out orally using the gastric feeding tube once a day for 5 consecutive weeks in the O-Hib 200 and O-Hib 400. Rats given *H. sabdariffa* extract must be weighed first to determine the amount of extract to be given.

Sampling of Adipose Tissue and Liver Organ

Retrieval of tissue samples was carried out after rats fasted for 12 hours. Sampling begins with the anesthetic process using a combination of xylazine hydrochloride 0.01 ml/kgBW and 0.05 ml/kgBW ketamine then the rats are turned off by decapitation. Rats were dissected using a surgical device; adipose and liver tissue was taken. The isolated samples were weighed and put into pots and stored in a refrigerator with a temperature of -80°C.

FGF21 Measurement

FGF21 was analyzed using ELISA kit. ELISA examination was carried out using the Elabscience E-EL-R2408 ELISA kit material using the ELISA sandwich method. Before the ELISA application, Bradford test was conducted to measure the total protein concentration in liver tissue and adipose supernatant. The result of the Bradford test was used to assess the relative concentration of FGF21 against total tissue protein. The ELISA test refers to the instructions contained in the kit. The color intensity was measured by ELISA reader variate flash at a wave length of 450 nm.

Data Analysis

Statistical analysis was performed using the one way ANOVA test. The analysis begins with a normality test using the Shapiro Wilk test and the Levene homogeneity test. Data has a normal and homogeneous distribution if the p value is ≥ 0.05. If the data shows the results of an abnormal or homogeneous distribution, then the Kruskal-Wallis test is carried out.

Results

The result of measurement of FGF21 protein levels in adipose tissue can be seen in Figure 1. Obese groups had lower levels of FGF21 compared to the normal control group. ($p<0.05$) The administration of *H. sabdariffa* extract at a dose of 400 mg/kgBW was higher in FGF21 level compared to the obese group ($p>0.05$). Even the levels were also higher than the normal group. While the *H. sabdariffa* administration dose of 200 mg/kgBW (O-Hib 200) was found to be the same as the obese group ($p> 0.05$).

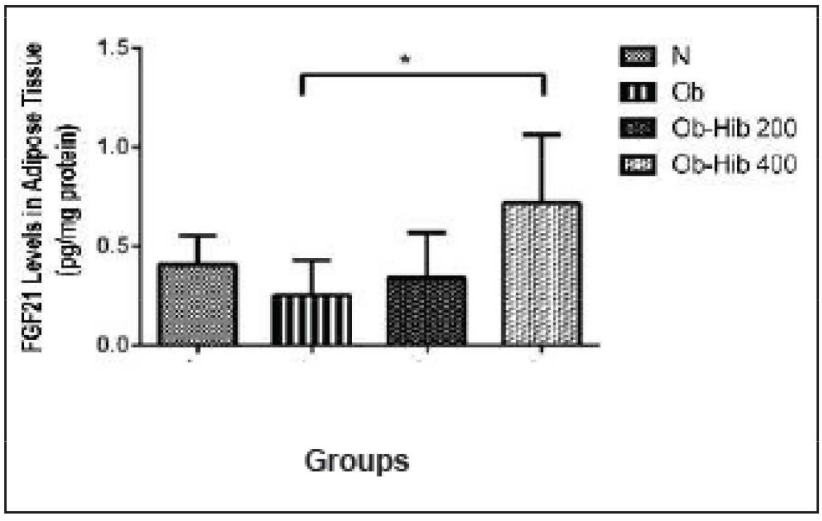

Figure 1. Average FGF21 Protein Level. Data result (mean ± SD). There are significant differences (*p<0.05, **p<0.01, ***p<0.001); N: Normal control without administration of H. sabdariffa; Ob: Obese rats without administration of H. sabdariffa; Ob-Hib 200: obese rats administered H. sabdariffa 200 mg/kgBW; Ob-Hib 400: obese rats administered H. sabdariffa 400 mg/kgBW.

The result of measurement of FGF21 protein levels in the liver can be seen in Figure 2. Obese groups had higher FGF21 levels compared to the normal control group (p <0.05). Giving extract of *H. sabdariffa* 200 mg/kgBW and 400 mg/kgBW in obese rats also showed higher levels of FGF21 compared to the normal control group (p <0.05). There was no significant difference in FGF21 levels in the obese group with the obese group given *H. sabdariffa* 200 mg/kgBW (O-Hib 200 (p>0.05).

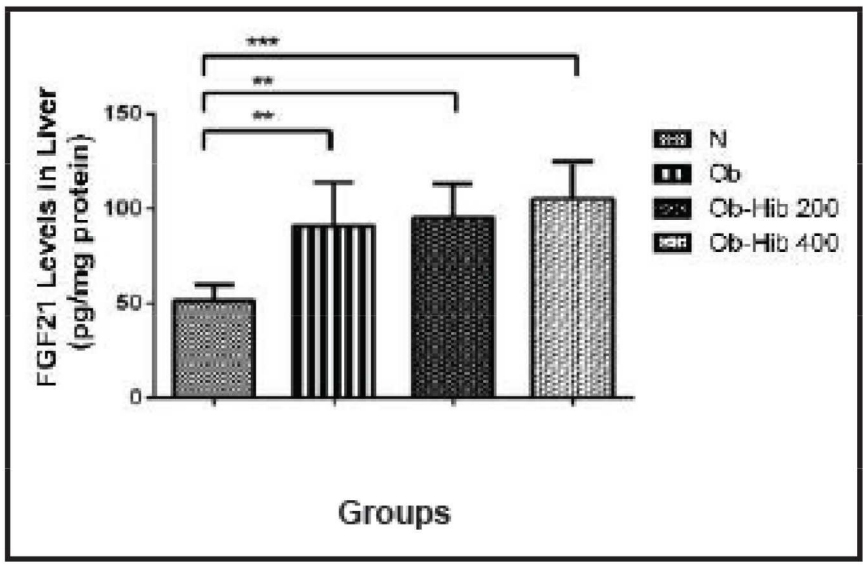

Figure 2. Average FGF21 Protein Level. Data result (mean ± SD). There are significant differences (*p<0.05, **p<0.01, ***p<0.001); N: Normal control without administration of H. sabdariffa; Ob: Obese rats without administration of H. sabdariffa; Ob-Hib 200: obese rats administered H. sabdariffa 200 mg/kgBW; Ob-Hib 400: obese rats administered H. sabdariffa 400 mg/kgBW.

Discussions

The results of this study indicate that there is a decrease in FGF21 levels in adipose tissue in obese conditions. This decrease can be caused by a decrease in the synthesis of FGF21 in adipose tissue because fat accumulation in adipose tissue in obese conditions can reduce FGF21 levels. Fat accumulation will activate transcription of PPARγ and CEBP/α which play a role in the process of adipogenesis. According to Wang et al.[4] an increase in PPARγ adipokine and CEBP/α will be followed by an increase in CEBP/β which can occupy the FGF21 gene promoter and suppress transcription of FGF21 mRNA in white adipose tissue. Thus, the high PPARγ and CEBP/α suppress endogenous FGF21. In addition, a decrease in FGF21 in adipose tissue can also show the amount bound to its receptors. This is in line with Fisher et al. [5] who reported that obesity is a condition in which FGF21 experiences resistance so that the action of FGF21 on adipose tissue is impaired. One of the causes of FGF21 resistance according to Gallego-Escuredo et al.[12] is a decrease in FGFR and βKL receptor expression in white adipose tissue. Previous studies by Hale et al.[13] stated that expression of βKL, FGFR-1c and FGFR2c experienced downregulation in white adipose tissue in obese rats. However, this study did not measure the expression of receptors in adipose tissue. Thus, further research is needed to ensure that the decrease in FGF21 levels is directly related to a decrease in the expression of receptors in adipose tissue. Decreasing FGF21 levels in adipose tissue describes FGF21 resistance. This situation is followed by an increase in FGF21 synthesis in liver tissue because in obese conditions, FGF21 levels in liver tissue are higher than normal. The results of the study showed that the expression of FGF21 in the liver in the obese group had a higher value compared to the normal group.[14] The increase in FGF21 in the liver was followed by an increase in circulation. Geng et al.[14] found that in obese group rats there was an increase in serum FGF21 levels six times higher than in the normal group.

Increased levels of FGF21 in the liver are a result of disruption of the uptake of FGF21 to white adipose tissue. This happened as a compensation effect due to FGF21 resistance in white adipose tissue.[12,]

However, the administration of *H. sabdariffa* is able to overcome the state of FGF21 resistance in obese conditions. This is indicated by the highest FGF21 level in the adipose tissue in the group given *H. sabdariffa*. The reason is because *H. sabdariffa* has a lot of chemical content in its calyx, such as flavonoids, quercetin, polyphenols, catechins, and anthocyanins. These contents have many roles associated with weight loss since they can act as anti-inflammatory.[10] The content of *H. sabdariffa* polyphenol extract can inhibit the adipogenesis process by suppressing PPARγ and CEBP/α expressions so as to reduce fat accumulation in adipose tissue. Kim et al.[15] showed that *H. sabdariffa* extract was able to inhibit adipogenesis in 3T3-L1 preadipocytes through suppression of the main transcription factors of PPARγ and CEBP/α differentiation through inhibition of the MAPK pathway.[15] Suppression of the main transcription factors PPARγ and CEBP/α led to downregulation of CEBP/β as FG21 gene silencer. According to Wang et al. [4] downregulation of CEBP/β can improve transcription of FGF21 mRNA in white adipose tissue. Thus, the compound of *H. sabdariffa* polyphenol extract has the potential for the synthesis of endogenous FGF21 in white adipose tissue. *H. sabdariffa* also has the potential to suppress inflammation. Inflammation plays a role in suppressing the expression of receptors and co-receptors in adipose tissue. Gamboa-Gomez et al.[17] found that *H. sabdariffa* extract significantly reduced TNFα induced NFκB. This is because the polyphenols in *H. sabdariffa* extract can reduce the phosphorylation of ERK 1/1, JNK, and p38 which results in downregulation of NFκB transcription factors.[16] In addition, polyphenols from *H. sabdariffa* extract can inhibit proinflammatory adipokine secretion IL6, TNFα, MCP1, and VCAM-1 reduces the infiltration of macrophages to adipose tissue. Thus, *H. sabdariffa* can suppress FGF21 resistance. Although *H. sabdariffa* can suppress FGF21 resistance in adipose tissue, the secretion of FGF21 in the liver tissue remains high. This shows that *H. sabdariffa* has the potential to increase FGF21 secretion in the liver. The result of the study is consistent with those of Zeng et al.[18] who found that the polyphenol active compound, namely curcumin induced in rats fed a high-fat diet can increase the bond between PPARα and PPERs in the liver which is the promoter gene FGF21 through inhibition of PPARα phosphorylation (S12) or increase in intracellular PPARα ligands. Activation of PPARα will increase FGF21 gene regulation and protein production FGF21. Therefore, further research needs to assess the potential of *H. sabdariffa* in PPARα and PPERs in the liver. According to Kim et al.[19] the content of polyphenols in grapes can increase the activation of Sirt1 which can induce FGF21 gene expression in rat liver.

Conclusions

Administration of *H. sabdariffa* extract has the potential for handling FGF21 resistance because *H. sabdariffa* is able to increase FGF21 levels in adipose tissue. FGF21 can also increase FGF21 secretion in liver tissue.

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