DOI: 10.1002/vms3.653

ORIGINAL ARTICLE

WILEY

Potential of *Hibiscus sabdariffa* Linn. in managing FGF21 resistance in diet-induced-obesity rats via miR-34a regulation

Neng Tine Kartinah[1] | Nisa Komara[2] | Nuraini Diah Noviati[2] | Syarifah Dewi[3] | Sophie Yolanda[1] | Afifa Radhina[2] | Heriyanto Heriyanto[2,4] | Imelda Rosalyn Sianipar[1]

[1] Department of Medical Physiology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

[2] Master Program in Biomedical SciencesFaculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

[3] Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

[4] Department of Medical Physiology, Faculty of Medicine, UKRIDA, Jakarta, Indonesia

Correspondence
Imelda Rosalyn Sianipar, Department of Medical Physiology, Faculty of Medicine, Universitas Indonesia, Jakarta, 10430, Indonesia.
Email: imelda.rosalyn@ui.ac.id

Funding information
Ministry of Research, Technology, and Higher Education who provide the research, Grant/Award Number: NKB-1547/UN2.R3.1/HKP.05.00/2019

Abstract

Background: Obesity is a cause of FGF21 resistance, which affects the browning and thermogenesis process of the adipose tissue. Decreased receptor expression is influenced by miR-34a, whose expression is increased in obesity. While FGF21-based therapies have been widely investigated, the potential activity of *Hibiscus sabdariffa* Linn. extract (HSE) against FGF21 resistance is unknown.

Objective: This study aims to determine the effects of HSE on the expression of miR-34a and FGF21 receptors in white adipose tissue.

Methods: This experimental study used 24 male Sprague-Dawley rats and divided into four groups: Control (N); diet-induced-obesity rats (DIO); DIO rats with HSE 200 mg/kgBW/day and DIO rats with HSE 400 mg/kgBW/day. Rats were fed a high-fat diet for 17 weeks. HSE was administered daily for 5 weeks. The administration of HSE 400 mg/kgBW/day resulted in the equivalent expression of miR-34a to that of the control ($p > 0.05$).

Results: FGFR1 receptor expression was also similar to controls ($p > 0.05$). Beta-klotho expression was significantly lower than that of control ($p < 0.05$) but equivalent to that of DIO rats ($p < 0.05$).

Conclusions: *H. sabdariffa* has the potential to reduce FGF21 resistance in DIO rats through the suppression of miR-34a expression and an increase in the number of FGFR1 and beta-klotho receptors in adipose tissue.

KEYWORDS
inflammation, metabolism, obesity, physiology

1 | BACKGROUND

Obesity causes low-grade systemic inflammation, which has an impact on tissue disruption. Systemic low-grade inflammation is caused by an increase in proinflammatory cytokines due to hypoxia of the adipose tissue. Cell hypertrophy causes hypoxia of the adipose tissue, resulting in macrophage infiltration and changing the phenotype of anti-inflammatory macrophages (M2) to proinflammatory macrophages (M1) (Pan et al., 2019). Moreover, the mechanism of changing the macrophage phenotype can be caused by the deletion of PPAR-γ and

PPAR-δ which these genes serve a protective role in immune and metabolic homeostasis (Garg et al., 2014). One of the effects of chronic inflammation is the increased expression of microRNAs that induces the silencing of the target gene (Balasubramaniam et al., 2018). Proinflammatory cytokines produced by M1, such as tumour necrosis factor α (TNFα) and interleukin 6 (IL6), increase the expression of microRNAs such as miR-34a. In obesity, the expression of miR-34a increases function as a regulator of FGFR1 receptor expression and beta-klotho co-receptor that binds to FGF21. According to Fu (2014), miR-34a disturbs the FGF21 signalling cascade in the white adipose tissue through down regulation of FGFR receptor expression and beta-klotho co-receptor. Disruption of FGF21 signalling cascade results in FGF21 resistance in the white adipose tissue (Fu et al., 2014).

FGF21, a member of the fibroblast growth factor (FGF) family, located on chromosome 19, is expressed by several organs, including the liver (Fisher & Maratos-Flier, 2016). FGF21 plays a role in controlling the energy homeostasis through the regulation of adipose tissue browning and thermogenesis. FGF21 must bind to its receptors in the adipose tissue, namely FGFR1 and beta-klotho, to perform this function (Fu et al., 2014). The binding of FGF21 to its receptors in the adipose tissue activates the Ras/Raf MAPK signalling pathway. FGF21 induces adipose tissue browning through the increased expression of UCP-1, which plays a role in the thermogenic activity, which further increases thermogenesis (Kilkenny & Rocheleau, 2016). FGF21 binding to its receptor initiates the signal transduction that increases the activity of sirtuin-1 (SIRT1), then stimulates PGC1α to increase the expression of UCP-1 (Crichton et al., 2017; Fisher et al., 2012; Fu et al., 2014).

Considering the potential of FGF21 in browning and thermogenesis, efforts are underway to address work-based obesity through FGF21. Tine Kartinah et al. (2018) found that high-intensity intermittent physical exercise can increase the amount of FGF21 released from the muscle, thereby increasing its activity in the adipose tissue. Sonoda et al. (2017) reviewed many in vivo experiments and showed FGF21 analogues and FGFR agonists that resemble the FGF21 receptor-ligand complex (Sonoda et al., 2017). However, it is still necessary to manage obesity using natural ingredients. Natural ingredients that have the potential to handle obesity include *Hibiscus sabdariffa* Linn., otherwise known as roselle (Morales-Luna et al., 2019; Riaz & Chopra, 2018).

H. sabdariffa extract (HSE) contains flavonoids, quercetin, polyphenols, catechins, and anthocyanins (Guardiola & Mach, 2014; Morales-Luna et al., 2019; Riaz & Chopra, 2018). *H. sabdariffa* Linn. has been known as anti-obesity because it has prevented or reduced bodyweight in obese rats (Anna Sheba & Ilakkia, 2016; J. K. Kim et al., 2007; Omar et al., 2018). The polyphenols in HSE prevent weight gain in diet-induced-obesity (Guardiola and Mach) rats and inhibit lipogenesis in the white adipose tissue at the molecular level (Herranz-Lopez et al., 2017).

However, the potential activity of HSE against FGF21 resistance is still unknown. Using an animal model with specific diet to induce obesity and examine the potential activity of HSE against Fgf21 in animal study is necessary before observing its relevance to human biology. This study aims to assess the potential of HSE to act against FGF21 resistance by measuring the expression of its receptors in the adipose tissue (FGFR1 and beta-klotho) and quantifying the levels of the antagonist, miR-34a, in diet-induced-obesity (DIO) rats. Thus, this study will prove the molecular mechanism that describes *H. sabdariffa* Linn. in managing FGF 21 resistance through miR-34 in obese.

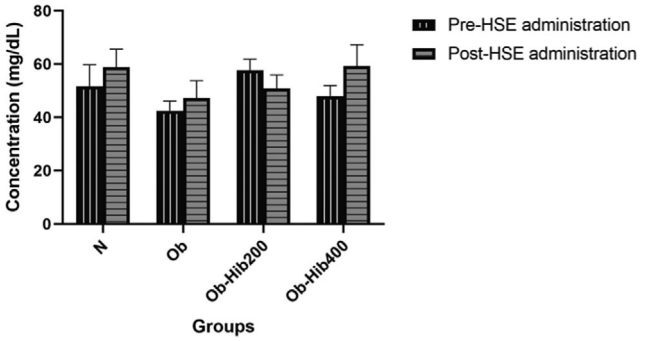

FIGURE 1 Total cholesterol concentration pre- and post-*H. sabdariffa* extract (HSE) administration. Data are depicted as mean ± SD. N: Normal control group; Ob: diet-induced-obesity (DIO) rats; Ob-Hib 200: DIO rats administered with HSE at a dose of 200 mg/kgBW; Ob-Hib 400: DIO rats administered with HSE at a dose of 400 mg/kgBW

2 | METHODS

2.1 | Study and design

The experiments were done in Molecular Biology Laboratory. A sample size of twenty-four rats were counted using Federer's formula. Twenty-four male Sprague-Dawley rats then collected from Animal Facility of Health Research Development, Ministry of Health. The sample (6–10 weeks age) were randomized into four groups according to extract dosage and metabolic conditions (normal/obesity). The groups were as follows: (1) control (N); (2) DIO 12; (3) DIO rats administered with HSE 200 mg/day/kg body weight (BW) (Ob-Hib200); and (4) DIO rats administered with HSE 400 mg/day/kgBW (Ob-Hib400).

Obesity in rats was induced by administering a high-fat diet (19.09% fat, 24.00% protein), while the control group was fed a standard diet. The diets were administered for 17 weeks (Marques et al., 2016). Then, the rats were included in the obesity list. Rats were categorized as DIO if their Lee index was more than 310. Rats'BW and body length (naso-anal) were measured using the Lee index formula (Jamali et al., 2016) as follows:

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

The obese rat model refers to index Lee > 310. Figure 1 shows the score of the Lee index after being given a high fat diet for 17 weeks.

DIO rats were included in the Ob, Ob-Hib200, or Ob-Hib400 group. HSE 200 and 400 mg/day/kgBW doses were based on the research by Andraini and Yolanda (2014). The high-fat diet was maintained until the end of the study. The 24 male Sprague-Dawley rats, 6–10 weeks

old, were housed (three rats per cage) following a 12-h:12-h light/dark cycle.

2.2 | Provision of HSE methanol extract

H. sabdariffa Linn. plant was obtained from the centre of the biopharmaca studies, Bogor Agriculture University. Then, methanol extraction was carried out using the maceration method. Maceration involved soaking plant materials (powdered) in a stoppered container with a solvent and allowed to stand at room temperature for minimum of 3 days with frequent agitation (Azwanida, 2015). The extract was dissolved in methanol and a paste was formed.

The pasta was diluted using distilled water, and then the preparation was stored at 4°C for 7 days of treatment to avoid preparation damage if stored for more than 7 days.

HSE was given at 200 and 400 mg/kg/day in the obesity treatment group. Before giving the extract, the rats were weighed to determine the amount of HSE to be given. The dose is given orally using a gastric lavage once a day and is carried out for 5 weeks. In contrast, the control group was given distilled water 2 ml as a placebo.

Long-term use of methanol extract does not have side effects. The methanol extract was found safe to the no-observed-adverse-effect-level (NOAEL) for the single-dose and repeated-dose toxicity tests in rats (Sharwan et al., 2016).

2.3 | Sampling technique

Decapitation was performed following anaesthesia using a combination of xylazine hydrochloride 0.01 ml/kgBW and 0.05 ml/kgBW ketamine. Rats were left to fast for 12 h before decapitation. Rats were then dissected, and adipose tissue was harvested. The sample was put into a pot and stored in a refrigerator at −80°C. Blood samples from the rats' sinus orbita were collected into ethylenediaminetetraacetic acid tubes and centrifuged to obtain serum. The serum samples were used for lipid profile measurements, such as total cholesterol and triglyceride levels.

2.4 | Measurement of total cholesterol and triglyceride levels in rat serum

Total cholesterol levels were measured using the CHOD-PAP reagent, while triglyceride levels were measured using the GPA-PAP reagent, both for enzymatic colorimetric tests that require the same method. Reagent blank measurement was performed by pipetting 1000 μl R1 into cuvettes and incubating for 5 min. Then, the absorbance of the blank was measured using a spectrophotometer at a wavelength of 500 nm. Standard measurement was performed by pipetting 10 μl of standard and 1000 μl of R1 into cuvettes, which were mixed and incubated for 5 min. Sample measurement was performed by pipetting 10 μl of the sample and 1000 μl of R1 into cuvettes, which were mixed and incubated for 5 min. The absorbance of the standard or sample against the reagent blank (ΔA) was measured at a wavelength of 500 nm. The absorbance results (ΔAstandardand ΔAsample) were then calculated (for mg/dl) as follows:

$$C = 200 \times \frac{\Delta A\text{sample}}{\Delta A\text{standard}}$$

2.5 | Measurement of FGFR1, beta-klotho gene (*Klb*), and miR-34a expression in the adipose tissue

FGFR1 and *Klb* expression levels were quantified through several stages: (1) RNA isolation using the Quick-RNA MiniPrep Plus (Zymo Research, California, US) kit; (2) synthesis of cDNA (from RNA in stage 1) using the cDNA synthesis kit and a ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo); (3) real-time PCR was performed in triplicate to measure the expression of FGFR1 and *Klb* using the SensiFAST SYBR Hi-ROX Kit (Bioline) kit with a two-step method. RNA concentrations were measured using a nanodrop, and dilutions to obtain the required RNA concentration were performed using nuclease-free water.

Gene expression was then analyzed using the Micro drop VarioSkan Spectrophotometer. RNA isolation was performed to obtain the RNA necessary for examining *miR-34a* expression. Total RNA isolation from adipose tissue was performed using the Quick-RNA MiniPrep Plus (Zymo Research) kit. cDNA synthesis was performed, such that miRNA could be amplified using real-time PCR. Delta-delta CT method was compared to housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Perez et al., 2017). Real-time PCR was performed in triplicate using the miR-34a Detection and U6 Calibration Kit (Cohesion Biosciences).

2.6 | Statistical analysis

Six samples were used in each analysis. The Shapiro–Wilk normality test was performed on the collected data. If the data were distributed normally, then the analysis was continued using parametric tests with one-way analysis of variance (Baselga-Escudero et al., 2015). If the data were not distributed normally and remained abnormal after transformation, a nonparametric test was carried out using the Kruskal–Wallis test. A p-value of < 0.05 was considered statistically significant. Paired t-test was used to analyze the lipid profile results and determine the significance of pre- and post-HSE treatment. A (2-tailed) p-value < 0.05 was considered statistically significant. Data processing was performed using the SPSS 23 software (Statistical Social Sciences 23).

3 | RESULTS

3.1 | Effects of *H. sabdariffa* on rat lipid profile

Our experiments showed that there was no difference between the total cholesterol of pre- and post-treatment of HSE, as shown in Figure 1. The total cholesterol cannot describe low density

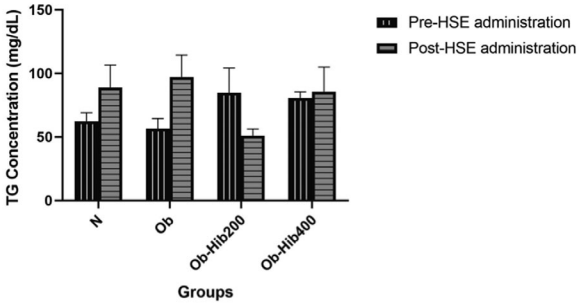

FIGURE 2 Triglyceride concentration pre- and post-*H. sabdariffa* extract (HSE) administration. Data are depicted as mean ± SD. N: Normal control group; Ob: diet-induced-obesity (DIO) rats; Ob-Hib 200: DIO rats administered with HSE at a dose of 200 mg/kgBW; Ob-Hib 400: DIO rats administered with HSE at a dose of 400 mg/kgBW

lipoprotein (LDL) and high density lipoprotein levels. After 17 weeks of HFD administration, total cholesterol levels were obtained average (43.424 mg/dl) in obese rats. The normal level of total cholesterol in rats is 10–54 mg/dl (Mahdi et al., 2020). Thus, the obese model had normal total cholesterol. However, the limitation of this study did not measure the LDL and DL levels. Likewise, triglyceride levels were obtained average (50.583 mg/dl). The normal triglyceride level in the rat is 26–145 mg/dl (Mahdi et al., 2020). Thus, this obese model has a normal triglyceride level.

In pre- and post-treatment HSE, triglyceride levels were different. After 200 mg/kgBW of HSE administration, there was a decrease in triglyceride levels. The treatment groups had lower levels than those of the normal control group. The administration of HSE is able to maintain triglyceride levels, such that the value is equivalent to the normal control group ($p > 0.05$), as shown in Figure 2.

HFD administration for 17 weeks resulted fat accumulation associated with an increase in the BW and the Lee index, as shown in Table 1, but it was not accompanied with changing of the blood lipid profiles.

3.2 | Effects of *H. sabdariffa* on the Lee index of DIO rats

The Lee index of the Ob group was the highest and significantly different from that of the control ($p < 0.001$), Ob-Hib200 ($p < 0.01$), and Ob-Hib400 ($p < 0.001$) groups, as shown in Figure 3. In addition, the Lee index of rats administered with HSE at doses of 200 mg/kgBW and 400 mg/kgBW were below 310. The results indicated that the administration of HSE reduced the Lee index to normal limits.

3.3 | Effects of H. *sabdariffa* on miR-34a expression in the rat white adipose tissue

There was a higher expression of *miR-34a* in the Ob group compared with that of the N group ($p < 0.001$), as shown in Figure 4. The administration of HSE at a dose of 200 mg/kgBW/day could not reduce the expression of *miR-34a* compared with the Ob group ($p > 0.05$), while the administration of extracts at 400 mg/kgBW reduced *miR-34a* expression ($p < 0.001$).

TABLE 1 The score of the Lee index

Group	Weight (g)	Height (cm)	Lee index
Ob	386	22	330.958
	303	20	335.828
	314	19	357.731
	310	20	338.395
	257	19.5	326.044
	335	19.5	356.162
Ob-Hib200	289	19.5	339.051
	245	19.5	320.710
	305	19	354.280
	310	19.5	347.072
	351	21.5	328.093
	296	19	350.892
Ob-Hib400	283	20	328.271
	241	19	327.531
	328	19.5	353.663
	350	20	352.386
	300	20	334.716
	309	19.5	346.698

3.4 | Effects of *H. sabdariffa* on FGFR1 in the rat white adipose tissue

Figure 5 shows FGFR1 expression in the rat adipose tissue. FGFR1 expression in the Ob group was lower compared to that of the N group ($p < 0.01$). FGF 21 resistance was indicated by decreasing FGFR in the DIO group significantly different from the control group. Our previous study showed that FGF 21 resistance is associated with increasing the FGF 21 level in the liver and decreasing the FGF21 level in the adipose tissue (Kartinah et al., 2019). The administration of HSE in DIO rats at a dose of 400 mg/kgBW maintained the expression of FGFR1 at levels equivalent to that of the control group ($p > 0.05$). Meanwhile, the expression of FGFR1 in the DIO rats group administered HSE at a dose of 200 mg/kgBW was still low and significantly different from that of the control rats ($p < 0.05$).

3.5 | Effects of *H. sabdariffa* on *Klb* expression in rat white adipose tissue

Figure 6 shows *Klb* expression in rat adipose tissue, which indicated that *Klb* expression in the Ob group was lower compared to that of N

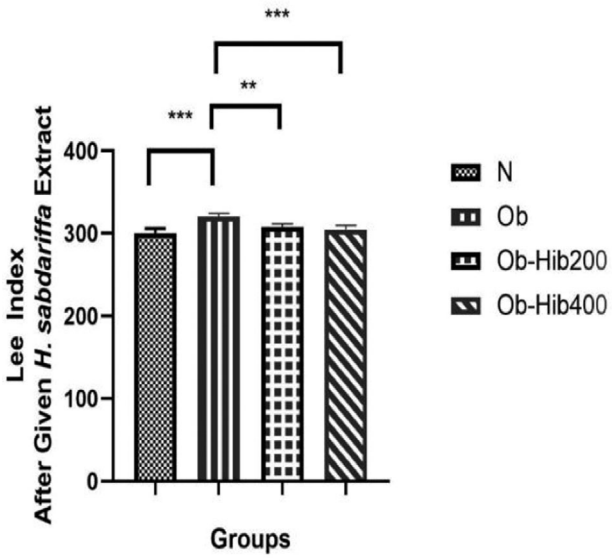

FIGURE 3 Average Lee index. Data are depicted as mean ± SD. Significant differences are shown as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; N: Normal control group; Ob: diet-induced-obesity (DIO) rats; Ob-Hib 200: DIO rats administered with *H. sabdariffa* extract (HSE) at a dose of 200 mg/kgBW; Ob-Hib 400: DIO rats administered with HSE at a dose of 400 mg/kgBW

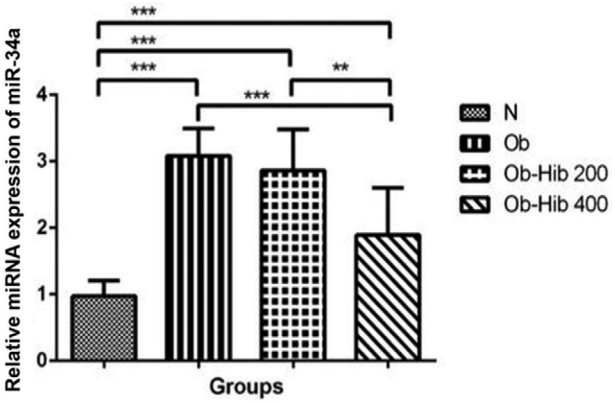

FIGURE 4 Average *miR-34a* expression. Data are depicted as mean ± SD. Significant differences are shown as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. N: Normal control group; Ob: diet-induced-obesity (DIO) rats; Ob-Hib 200: DIO rats administered with *H. sabdariffa* extract (HSE) at a dose of 200 mg/kgBW; Ob-Hib 400: DIO rats administered with HSE at a dose of 400 mg/kgBW

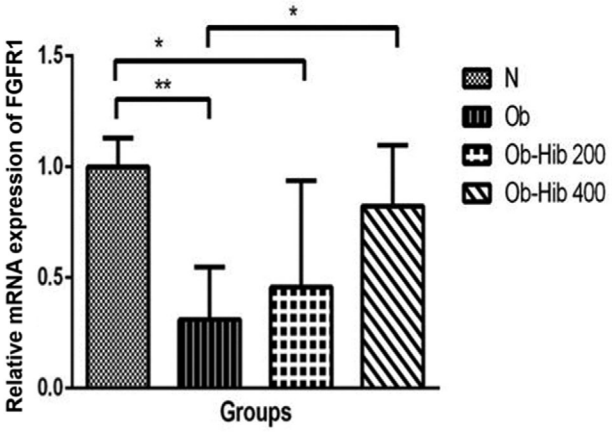

FIGURE 5 Average FGFR1 expression. Data are depicted as mean ± SD. Significant differences are shown as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. N: Normal control group; Ob: diet-induced-obesity (DIO) rats; Ob-Hib 200: DIO rats administered with *H. sabdariffa* at a dose of 200 mg/kgBW; Ob-Hib 400: DIO rats administered with *H. sabdariffa* at a dose of 400 mg/kgBW

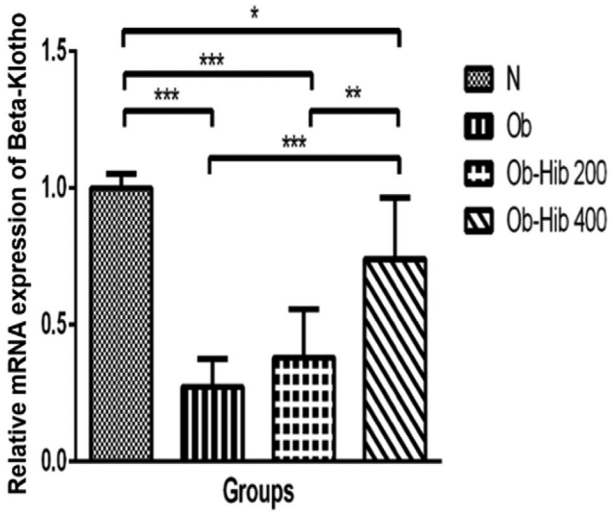

FIGURE 6 Average *Klb* expression. Data are depicted as mean ± SD. Significant differences are shown as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. N: Normal control group; Ob: diet-induced-obesity (DIO) rats; Ob-Hib 200: DIO rats administered with HSE at a dose of 200 mg/kgBW; Ob-Hib 400: DIO rats administered with HSE at a dose of 400 mg/kgBW

group ($p < 0.001$). However, the administration of HSE extract at a dose of 400 mg/kgBW in DIO rats increased the expression of *Klb* significantly compared to that of DIO rats ($p < 0.001$) and the DIO rats administered HSE at a dose of 200 mg/kgBW ($p < 0.01$). However, *Klb* expression in DIO rats treated with *H. sabdariffa* at a dose of 400 mg/kgBW was still lower than that in the control group ($p < 0.05$).

3.6 | Effects of *H. sabdariffa* on *Ppargc1a* expression in rat white adipose tissue

Ppargc1a (encoding PGC1α) expression is shown in Figure 7. We found that Ppargc1a expression in DIO rats was lower compared to that of the control group ($p < 0.01$). The administration of HSE to DIO rats at a dose of 200 mg/kgBW did not increase Ppargc1a expression compared to that of the Ob group ($p > 0.05$), but a significant difference compared to that of the control group ($p < 0.01$) was observed. The administration of 400 mg/kgBW HSE to DIO groups increased Ppargc1a expression;

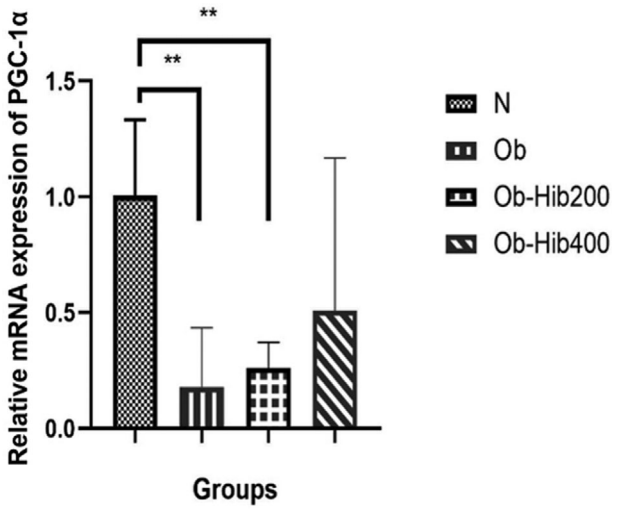

FIGURE 7 Average *Ppargc1a* expression. Data are depicted as mean ± SD. Significant differences are shown as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. N: Normal control group; Ob: diet-induced-obesity (DIO) rats; Ob-Hib 200: DIO rats administered with *H. sabdariffa* extract (HSE) at a dose of 200 mg/kgBW; Ob-Hib 400: DIO rats administered with HSE at a dose of 400 mg/kgBW

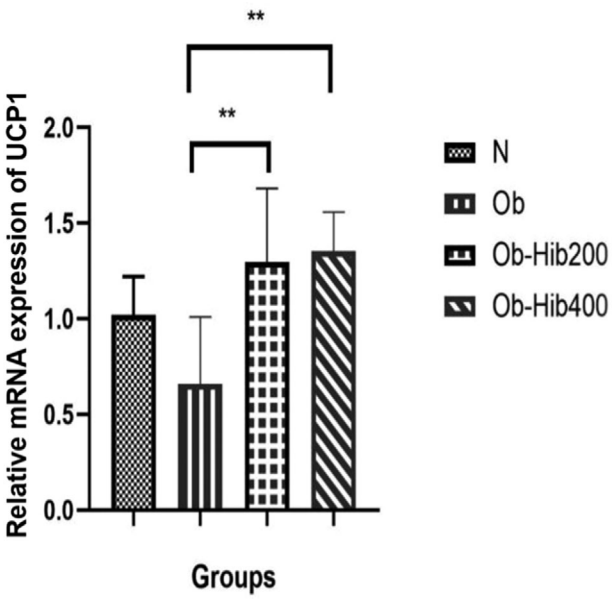

FIGURE 8 Average Ucp1 expression. Data are depicted as mean ± SD. Significant differences are shown as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. N: Normal control group; Ob: diet-induced-obesity (DIO) rats; Ob-Hib 200: DIO rats administered with HSE at a dose of 200 mg/kgBW; Ob-Hib 400: DIO rats administered with *H. sabdariffa* extract (HSE) at a dose of 400 mg/kgBW

therefore, the relative expression was not significantly different from that of the control group ($p > 0.05$).

3.7 | Effects of *H. sabdariffa* on Ucp-1 expression in rat white adipose tissue

The expression of Ucp-1 in DIO rats was lower compared to that of the control group, but not significantly different, as shown in Figure 8. The administration of HSE at doses of 200 mg/kgBW and 400 mg/kgBW produced higher *Ucp-1* expression compared to that of the Ob group ($p < 0.01$), reaching the normal levels.

4 | DISCUSSION

There was a higher miR-34a expression in the Ob group. This result is in line with that of Ahmadpour et al. (2018), who found an increase in the miR-34a expression in DIO rats (Ahmadpour et al., 2018). Increased relative expression of *miR-34a* in the white adipose tissue during obesity has a relationship with adipogenesis. Some miRNAs, along with other adipogenesis master regulators, such as PPARγ and C/EBP, increase the transcription of adipogenic genes. According to Cuevas Ramos (2012), miR-34a upregulation is followed by an increase of pre-adipocyte differentiation gene expression, while downregulation of miR-34a decreases the adipogenic gene expression (Cuevas-Ramos et al., 2012).

Increased miR-34a causes chronic inflammation (Fu et al., 2014). miR-34a plays a role in suppressing the expression of *Klf4*, which causes macrophage infiltration (Fu et al., 2014). Macrophage infiltration is associated with an increase in TNF-α, which causes chronic inflammation. TNFα produced by macrophages plays an essential role in the regulation of adipokines in adipocytes. TNF-α induces proinflammatory cytokines through nuclear factor kappa B (NF-κB). The binding of TNF-α to its receptor induces the production of proinflammatory cytokines through NF-κB-dependent and NF-κB-independent mechanisms. It also causes the production of proinflammatory cytokines, such as IL-Iβ, and IL-6 (Diaz-Delfin et al., 2012; McArdle et al., 2013; X. Wang et al., 2013).

In this study, we did not measure the inflammatory cytokines in DIO rats. However, we showed the effects of miR-34a expression, as evidenced by decreased FGFR and *Klb* expression. This study is in line with the work of Gallego-Escuredo et al. (2015), who reported that obesity leads to the reduced expression of FGFR and beta-klotho receptors in the white adipose tissue (Gallego-Escuredo et al., 2015). Hale (2012) stated that the expression of beta-klotho,FGFR-1c, and FGFR2c are downregulated in the adipose tissue of DIO rats (Hale et al., 2012). Diaz-Delfin et al. (2012) found decreased expression of FGFR1 and co-receptor beta-klotho in DIO rats. The decrease in the receptor numbers reduces the amount of binding with FGF21. This decrease is supported by our previous research, which showed that the levels of FGF21 expression in white adipose tissue in DIO mice (0.193 ± 0.134 pg/mg protein) are significantly lower than that of the control group (0.413 ± 0.139 pg/mg protein) (Kartinah et al., 2019). Besides, the decrease in FGF 21 in adipose tissue was also due to the activation of PPAR and CREB to increase CEBP and suppress FGF 21 transcription. Fisher et al. (2010) also shows the decrease in FGF 21 in adipose tissue was associated with FGF 21 resistance.

The decreased expression FGFR has an impact on endocrine FGF21 communication in the adipose tissue and is the beginning of the development of Fgf21 resistance (Diaz-Delfin et al., 2012). Our previous study shows FGF21 resistance leads to an increase in FGF21 secretion in the liver. The level of FGF21 in the liver of the DIO rats (9.105 ± 2.260 pg/mg protein) is significantly higher compared to the normal group (5.899 ± 0.876 pg/mg protein) (Kartinah et al., 2019). Increased expression levels of FGF21 in the liver are a result of the disruption of FGF21 uptake in the white adipose tissue. This happens as a compensation effect due to Fgf21 resistance (Gamboa-Gomez et al., 2015; Y. Wang et al., 2015). The increase in FGF21 expression in the liver is followed by an increase in circulation. Cuevas-Ramos et al. (2012) found that serum Fgf21 levels in the DIO rats are six times higher than that in the normal group (Cuevas-Ramos et al., 2012). Research by Hondares et al. (2011) showed an increase in FGF21 expression levels in the liver in mice with FGF21 resistance (Hondares et al., 2011).

The current study showed that the administration of HSE could manage FGF21 resistance through increased expression of FGFR and *Klb* (BonDurant et al., 2017). *H. sabdariffa* is suggested to suppress miR-34a as a regulator of FGFR and beta-klotho expressions (Lavery et al., 2016; Shi et al., 2016). However, the mechanism of how HSE downregulates miR-34a has not been proven. Several studies showed the potential of polyphenol compounds in other plant extracts that are microRNA modulators. Baselga-Escudero et al. (2015) showed that proanthocyanin, a component of polyphenols found in grapes and cocoa, downregulates miR-33. It also suppresses miR-122, which inhibits lipogenesis. Meanwhile, polyphenols from HSE modulate miR-122, miR-103, and miR-107 in hyperlipidemic rats (Yang et al., 2015).

The potential of HSE in suppressing miR-34a has been suggested through PPARγ and C/EBP expression. Additionally, a decrease in PPARγ and C/EBP expression is associated with miR-34a suppression. According to Lavery (2016), there is a decrease in the expression of PPARγ and C/EBP in miR-34a knockout rats (Lavery et al., 2016). According to J. K. Kim (2007), HSE suppresses transcription factors of PPARγ and CEBP/α (M. S. Kim et al., 2003). Thus, the inhibition of PPARγ and C/EBP, as a result of HSE administration, has the potential to suppress the expression of miR-34a (Hausman et al., 2014).

However, suppressing the expression of miR-34a through the administration of HSE is related to the dose. Here, we showed that the administration of HSE at a dose of 200 mg/kg in DIO rats did not demonstrate a significant reduction in miR-34a expression compared to the Ob group. In contrast, the administration of HSE at a dose of 400 mg/kgBW was found to significantly reduce the expression of miR-34 compared to that of the Ob group. Even though miR-34 expression levels do not reach normal levels, beta-klotho and FGFR expression can still increase (Agrawal et al., 2018), reaching normal levels of FGFR1 expression. Limitations of our study did not measure FGFR protein level in the adipose tissue.

The increased expression of beta-klotho and FGFR can also be influenced by HSE, which directly suppresses chronic inflammation. Some studies have found that polyphenols in the HSE can inhibit proinflammation by suppressing NF-κB. Zeng et al. (2017) found that polyphenols increase the expression of FGFR1 and beta-klotho in rats fed with a high-fat diet (Zeng et al., 2017). This is because polyphenols act as anti-inflammatory agents by decreasing NF-κB expression. The results of Gamboa-Gomez et al. (2015) indicate that HSE significantly reduces TNFα induced by NF-κB . In addition, the anthocyanins, namely cyanidin and delphinidin, in the HSE also reduce TNFα expression. That is because anthocyanin inhibits the activation of NF-κB by inhibiting the degradation of IκB and inhibiting the activation of IκB kinase, thereby preventing the phosphorylation of NF-κB (Diaz-Delfin et al., 2012). In the current study, we did not measure NF-κB expression levels; thus, we could not directly prove that the increase in *Klb* and FGFR expression was associated with a decrease in NF-κB. This study proves the potential of HSE in increasing the expression of FGFR and *Klb*, such that it can manage FGF21 resistance in DIO rats (Fisher et al., 2010).

Our previous research showed that there is a higher-than-normal increase in Fgf21 in the adipose tissue of DIO mice administered HSE at a dose of 400 mg/kgBW (Kartinah et al., 2019). The management of FGF21 resistance is indicated by the activation of the FGF21 signalling pathway in the adipose tissue. FGF21 binds to its receptor to activate signalling via PGC1α, which is a transcription coactivator that controls energy metabolism. The present study showed an increase in Ppargc1*a* expression in the Ob-Hib400group.

SIRT1 stimulates increased PGC1α activity. After FGF21 binds to FGFR and beta-klotho, it induces browning of white adipose tissue to beige adipose tissue through SIRT1 activation, which results in PGC1α deacetylation to induce UCP-1 expression (Crichton et al., 2017). The results of the current study also showed an increase in *Ucp-1* expression after HSE administration. The rise in *Ucp-1* expression was shown not only in the Ob-Hib400 group but also in the Ob-Hib200 group. However, the limitation of this study did not measure protein of SIRTI and UCP1.

The increase in UCP-1 expression is not only affected by PGC1α activation but also by other factors (Zeng et al., 2017). UCP-1 expression is influenced by several paths that are regulated by major transcription factors, such as PPARγ and PRDM16 (Lo & Sun, 2013).

PPARγ is a crucial transcription factor in the differentiation of brown and white adipocytes. PPARγ is needed for the recruitment of PRDM16 to the PPARγ transcription complex, which achieves the browning process (Xie et al., 2016). Thus, HSE not only activates the PGC1α pathway but is also thought to play a role in the PPARγ and PRDM16 pathways. However, further research is needed to prove this. According to Tian et al. (2013), polyphenol content from green tea improves transcriptional regulators, such as PPARγ, PGC1α, PRDM16, and UCP1, for the browning process.

5 | CONCLUSION

H. sabdariffa has the potential to manage FGF21 resistance in DIO rats via the suppression of miR-34a expression, increasing the number of FGFR1 and beta-klotho co-receptors in the adipose tissue. This condition affects active FGF21 signalling in the process of browning and thermogenesis.

ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Research, Technology, and Higher Education who provide the research Grant with contract No. NKB-1547/UN2.R3.1/HKP.05.00/2019.

DATA AVAILABILITY STATEMENT

Data available by permission to authors.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ETHICS STATEMENT

The Ethical Board of Faculty of Medicine, Universitas Indonesia approved all experiments.

AUTHOR CONTRIBUTIONS

Conceptualization, *formal analysis*, *funding acquisition*, *resources*, *supervision*, *writing-original draft*, *and writing-review & editing*: Neng Tine Kartinah. *Investigation*, *methodology*, *resources*, *and validation*: Nisa Komara. *Investigation*: Nuraini Diah Noviati. *Validation*: Syarifah Dewi. *Formal analysis*, *writing-original draft*, *and writing-review & editing*: Sophie Yolanda. *Formal analysis*, *investigation*, *and resources*: Afifa Radhina. *Data curation*, *investigation*, *and visualization*: Heriyanto Heriyanto. *Conceptualization*, *formal analysis*, *writing-original draft*, *and writing-review & editing*: Imelda Rosalyn Sianipar.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.653

ORCID

Imelda Rosalyn Sianipar https://orcid.org/0000-0002-1438-096X

REFERENCES

Agrawal, A., Parlee, S., Perez-Tilve, D., Li, P., Pan, J., Mroz, P. A., Kruse Hansen, A. M., Andersen, B., Finan, B., Kharitonenkov, A., & DiMarchi, R. D. (2018). Molecular elements in FGF19 and FGF21 defining KLB/FGFR activity and specificity. *Molecular Metabolism*, *13*, 45–55.

Ahmadpour, F., Nourbakhsh, M., Razzaghy-Azar, M., Khaghani, S., Alipoor, B., Abdolvahabi, Z., & Zangoei, M. (2018). The association of plasma levels of miR-34a AND miR-149 with obesity and insulin resistance in obese children and adolescents. *Acta Endocrinologica*, *14*, 149–154.

Andraini, T., & Yolanda, S. (2014). Prevention of insulin resistance with *Hibiscus sabdariffa* Linn. extract in high-fructose fed rat. *Medical Journal of Indonesia*, *23*, 192–196.

Anna Sheba, L., & Ilakkia, A. (2016). Anti-obesity effect of *Hibiscus sabdariffal*—A review. *International Journal of Pharma and Bio Sciences*, *7*, 341–345.

Azwanida, N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants*, *4*, 196.

Balasubramaniam, M., Pandhare, J., & Dash, C. (2018). Are microRNAs important players in HIV-1 Infection? An Update. *Viruses*, *10*, 110.

Baselga-Escudero, L., Pascual-Serrano, A., Ribas-Latre, A., Casanova, E., Salvado, M. J., Arola, L., Arola-Arnal, A., & Blade, C. (2015). Long-term supplementation with a low dose of proanthocyanidins normalized liver miR-33a and miR-122 levels in high-fat diet-induced obese rats. *Nutrition Research*, *35*, 337–345. http://doi.org/10.1016/j.nutres.2015.02.008

Bon Durant, L. D., Ameka, M., Naber, M. C., Markan, K. R., Idiga, S. O., Acevedo, M. R., Walsh, S. A., Ornitz, D. M., & Potthoff, M. J. (2017). FGF21 regulates metabolism through adipose-dependent and -independent mechanisms. *Cell Metabolism*, *25*, 935–944.

Mahdi, C., Citrawati, P., & Hendrawan, V. F. (2020). The effect of rice bran on triglyceride levels and histopatologic aorta in rat (*Rattus norvegicus*) of high cholesterol dietary model. *IOP Conference Series: Materials Science and Engineering*, *833*, 012022.

Crichton, P. G., Lee, Y., & Kunji, E. R. (2017). The molecular features of uncoupling protein 1 support a conventional mitochondrial carrier-like mechanism. *Biochimie*, *134*, 35–50.

Cuevas-Ramos, D., Almeda-Valdes, P., Meza-Arana, C. E., Brito-Cordova, G., Gomez-Perez, F. J., Mehta, R., Oseguera-Moguel, J., & Aguilar-Salinas, C. A. (2012). Exercise increases serum fibroblast growth factor 21 (FGF21) levels. *Plos One*, *7*, e38022.

Diaz-Delfin, J., Hondares, E., Iglesias, R., Giralt, M., Caelles, C., & Villarroya, F. (2012). TNF-alpha represses beta-Klotho expression and impairs FGF21 action in adipose cells: involvement of JNK1 in the FGF21 pathway. *Endocrinology*, *153*, 4238–4245. http://doi.org/10.1210/en.2012-1193

Fisher, F. M., Chui, P. C., Antonellis, P. J., Bina, H. A., Kharitonenkov, A., Flier, J. S., & Maratos-Flier, E. (2010). Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. *Diabetes*, *59*, 2781–2789.

Fisher, F. M., Kleiner, S., Douris, N., Fox, E. C., Mepani, R. J., Verdeguer, F., Wu, J., Kharitonenkov, A., Flier, J. S., Maratos-Flier, E., & Spiegelman, B. M. (2012). FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. *Genes & Development*, *26*, 271–281.

Fisher, F. M., & Maratos-Flier, E. (2016). Understanding the physiology of FGF21. *Annual Review of Physiology*, *78*, 223–241.

Fu, T., Seok, S., Choi, S., Huang, Z., Suino-Powell, K., Xu, H. E., Kemper, B., & Kemper, J. K. (2014). MicroRNA 34a inhibits beige and brown fat formation in obesity in part by suppressing adipocyte fibroblast growth factor 21 signaling and SIRT1 function. *Molecular and Cellular Biology*, *34*, 4130–4142.

Gallego-Escuredo, J. M., Gomez-Ambrosi, J., Catalan, V., Domingo, P., Giralt, M., Fruhbeck, G., & Villarroya, F. (2015). Opposite alterations in FGF21 and FGF19 levels and disturbed expression of the receptor machinery for endocrine FGFs in obese patients. *International Journal of Obesity*, *39*, 121–129.

Gamboa-Gomez, C. I., Rocha-Guzman, N. E., Gallegos-Infante, J. A., Moreno-Jimenez, M. R., Vazquez-Cabral, B. D., & Gonzalez-Laredo, R. F. (2015). Plants with potential use on obesity and its complications. *EXCLI Journal*, *14*, 809–831.

Garg, S. K., Delaney, C., Shi, H., & Yung, R. (2014). Changes in adipose tissue macrophages and T cells during aging. *Critical Reviews in Immunology*, *34*, 1–14.

Guardiola, S., & Mach, N. (2014). Therapeutic potential of *Hibiscus sabdariffa*: A review of the scientific evidence. *Endocrinol Nutrición*, *61*, 274–295.

Hale, C., Chen, M. M., Stanislaus, S., Chinookoswong, N., Hager, T., Wang, M., Veniant, M. M., & Xu, J. (2012). Lack of overt FGF21 resistance in two mouse models of obesity and insulin resistance. *Endocrinology*, *153*, 69–80.

Hausman, G. J., Basu, U., Wei, S., Hausman, D. B., & Dodson, M. V. (2014). Preadipocyte and adipose tissue differentiation in meat animals: Influence of species and anatomical location. *Annual Review of Animal Biosciences*, *2*, 323–351.

Herranz-Lopez, M., Olivares-Vicente, M., Encinar, J. A., Barrajon-Catalan, E., Segura-Carretero, A., Joven, J., & Micol, V. (2017). Multi-targeted molecular effects of *Hibiscus sabdariffa* polyphenols: An opportunity for a global approach to obesity. *Nutrients*, *9*, 907.

Hondares, E., Iglesias, R., Giralt, A., Gonzalez, F. J., Giralt, M., Mampel, T., & Villarroya, F. (2011). Thermogenic activation induces FGF21 expression

and release in brown adipose tissue. *Journal of Biological Chemistry*, *286*, 12983–12990.

Jamali, E., Asad, M., & Rassouli, A. (2016). The effect of high-intensity training (HIIT) on resistin gene expression in visceral adipose tissue in obese male rats. *International Journal of Applied Exercise Physiology*, *5*, 17–25.

Kartinah, N., Komara, N., Sianipar, I., & Noviati, N. (2019). The potential of *Hibiscus sabdariffa* for treatment of obesity: Focus on FGF 21 in Liver and adipose tissue. *eJournal Kedokteran Indonesia*, *7*, 131–136. http://doi.org/10.23886/ejki.7.11093

Kilkenny, D. M., & Rocheleau, J. V. (2016). The FGF21 receptor signaling complex: Klotho*β*, FGFR1c, and other regulatory interactions. *Vitamins and Hormones*, *101*, 17–58.

Kim, J. K., So, H., Youn, M. J., Kim, H. J., Kim, Y., Park, C., Kim, S. J., Ha, Y. A., Chai, K. Y., Kim, S. M., Kim, K. Y., & Park, R. (2007). *Hibiscus sabdariffa* L. water extract inhibits the adipocyte differentiation through the PI3-K and MAPK pathway. *Journal of Ethnopharmacology*, *114*, 260–267.

Kim, M. S., Kim, J. K., Kim, H. J., Moon, S. R., Shin, B. C., Park, K. W., Yang, H. O., Kim, S. M., & Park, R. (2003). Hibiscus extract inhibits the lipid droplet accumulation and adipogenic transcription factors expression of 3T3-L1 preadipocytes. *Journal of Alternative and Complementary Medicine*, *9*, 499–504.

Lavery, C. A., Kurowska-Stolarska, M., Holmes, W. M., Donnelly, I., Caslake, M., Collier, A., Baker, A. H., & Miller, A. M. (2016). miR-34a(-/-) mice are susceptible to diet-induced obesity. *Obesity*, *24*, 1741–1751.

Lo, K. A., & Sun, L. (2013). Turning WAT into BAT: A review on regulators controlling the browning of white adipocytes. *Bioscience Reports*, *33*, e00065.

Marques, C., Meireles, M., Norberto, S., Leite, J., Freitas, J., Pestana, D., Faria, A., & Calhau, C. (2016). High-fat diet-induced obesity rat model: A comparison between Wistar and Sprague-Dawley Rat. *Adipocyte*, *5*, 11–21.

McArdle, M. A., Finucane, O. M., Connaughton, R. M., McMorrow, A. M., & Roche, H. M. (2013). Mechanisms of obesity-induced inflammation and insulin resistance: Insights into the emerging role of nutritional strategies. *Front Endocrinol*, *4*, 52.

Morales-Luna, E., Perez-Ramirez, I. F., Salgado, L. M., Castano-Tostado, E., Gomez-Aldapa, C. A., & Reynoso-Camacho, R. (2019). The main beneficial effect of roselle (*Hibiscus sabdariffa*) on obesity is not only related to its anthocyanin content. *Journal of the Science of Food and Agriculture*, *99*, 596–605.

Omar, M., Shamsahal, N., Muhammad, H., & Amir, W. (2018). Anti-obesity and haematological effects of Malaysia *Hibiscus sabdariffa* L. aqueous extract on obese Sprague Dawley rats. *Functional Foods in Health and Disease*, *8*, 340–352.

Pan, Y., Hui, X., Hoo, R. L. C., Ye, D., Chan, C. Y. C., Feng, T., Wang, Y., Lam, K. S. L., & Xu, A. (2019). Adipocyte-secreted exosomal microRNA-34a inhibits M2 macrophage polarization to promote obesity-induced adipose inflammation. *Journal of Clinical Investigation*, *129*, 834–849.

Perez, L. J., Rios, L., Trivedi, P., D'Souza, K., Cowie, A., Nzirorera, C., Webster, D., Brunt, K., Legare, J.-F., Hassan, A., Kienesberger, P. C., & Pulinilkunnil, T. (2017). Validation of optimal reference genes for quantitative real time PCR in muscle and adipose tissue for obesity and diabetes research. *Scientific Reports*, *7*, 3612.

Riaz, G., & Chopra, R. (2018). A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *102*, 575–586.

Sharwan, G., Jain, P., Pandey, R., & Shukla, S. S. (2016). Toxicity and safety profiles of methanolic extract of *Pistacia integerrima* J. L. Stewart ex Brandis (PI) for Wistar Rats. *Journal Pharmacopuncture*, *19*, 253–258.

Shi, C., Huang, F., Gu, X., Zhang, M., Wen, J., Wang, X., You, L., Cui, X., Ji, C., & Guo, X. (2016). Adipogenic miRNA and meta-signature miRNAs involved in human adipocyte differentiation and obesity. *Oncotarget*, *7*, 40830–40845.

Sonoda, J., Chen, M. Z., & Baruch, A. (2017). FGF21-receptor agonists: An emerging therapeutic class for obesity-related diseases. *Hormone Molecular Biology and Clinical Investigation*, *30*.

Tian, C., Ye, X., Zhang, R., Long, J., Ren, W., Ding, S., Liao, D., Jin, X., Wu, H., Xu, S., & Ying, C. (2013). Green tea polyphenols reduced fat deposits in high fat-fed rats via erk1/2-PPARgamma-adiponectin pathway. *Plos One*, *8*, e53796.

Tine Kartinah, N., Rosalyn Sianipar, I., & Nafi'ah, R. (2018). The effects of exercise regimens on irisin levels in obese rats model: Comparing high-intensity intermittent with continuous moderate-intensity training. *BioMed Research International*, *2018*, 4708287.

Wang, X., Cheng, M., Zhao, M., Ge, A., Guo, F., Zhang, M., Yang, Y., Liu, L., & Yang, N. (2013). Differential effects of high-fat-diet rich in lard oil or soybean oil on osteopontin expression and inflammation of adipose tissue in diet-induced obese rats. *European Journal of Nutrition*, *52*, 1181–1189.

Wang, Y., Liu, X., Hou, L., Wu, W., Zhao, S., & Xiong, Y. (2015). Fibroblast growth factor 21 suppresses adipogenesis in pig intramuscular fat cells. *International Journal of Molecular Sciences*, *17*, 11.

Xie, Q., Tian, T., Chen, Z., Deng, S., Sun, K., Xie, J., & Cai, X. (2016). Peroxisome proliferator-activated receptor (PPAR) in regenerative medicine: molecular mechanism for PPAR in stem cells' adipocyte differentiation. *Current Stem Cell Research & Therapy*, *11*, 290–298.

Yang, Z., Cappello, T., & Wang, L. (2015). Emerging role of microRNAs in lipid metabolism. *Acta Pharmaceutica Sinica B*, *5*, 145–150.

Zeng, K., Tian, L., Patel, R., Shao, W., Song, Z., Liu, L., Manuel, J., Ma, X., McGilvray, I., Cummins, C. L., Weng, J., & Jin, T. (2017). Diet polyphenol curcumin stimulates hepatic Fgf21 production and restores its sensitivity in high-fat-diet-fed male mice. *Endocrinology*, *158*, 277–292.

How to cite this article: Kartinah, N. T., Komara, N., Noviati, N. D., Dewi, S., Yolanda, S., Radhina, A., Heriyanto, H., & Sianipar, I. R. (2022). Potential of *Hibiscus sabdariffa* Linn. in managing *Fgf21* resistance in diet-induced-obesity rats via *miR-34a* regulation. *Veterinary Medicine and Science*, *8*, 309–317. https://doi.org/10.1002/vms3.653