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journal homepage: www.elsevier.com/locate/apjtbFloral research <http://dx.doi.org/10.1016/j.apjtb.2015.12.006>Anti-lipogenic effect of *Senna alata* leaf extract in high-fat diet-induced obese miceJarinyaporn Naowaboot^{1*}, Supaporn Wannasiri²¹Division of Pharmacology, Department of Preclinical Science, Faculty of Medicine, Thammasat University (Rangsit Campus), Pathum Thani 12120, Thailand²Division of Physiology, Department of Preclinical Science, Faculty of Medicine, Thammasat University (Rangsit Campus), Pathum Thani 12120, Thailand

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ABSTRACT

Objective: To examine the effect of *Senna alata* (*S. alata*) leaf extracts on the regulation of lipid metabolism in high-fat diet-induced obese mice.**Methods:** The obesity condition was induced in the male ICR mice by feeding them with high-fat diet (45 kcal% fat) for 12 weeks. At the 7th week of diet feeding, the obese mice were treated with the water extract of *S. alata* leaf at 250 and 500 mg/kg/day, respectively, that continued for six weeks. At the end of the treatment period, the biochemical parameters were determined. The hepatic histology and the gene and protein expressions were also examined.**Results:** In comparison with the obese control mice, the mice treated with *S. alata* showed a significant reduction in the elevated blood glucose levels and a decrease in the serum insulin and leptin levels. A reduction in the serum total cholesterol, triglyceride, non-esterified fatty acid, and hepatic triglyceride levels were also observed. The histological examination of the obese mice treated with *S. alata* showed a reduced lipid accumulation in the liver tissue. Hepatic lipogenic gene expression showed that *S. alata* decreased the activity of sterol regulatory element binding protein 1c, fatty acid synthase, and acetyl-CoA carboxylase. *S. alata* could suppress hepatic peroxisome proliferator-activated receptor gamma (PPAR γ) protein. Moreover, the protein expression of PPAR α in liver tissue was clearly increased by *S. alata* treatment.**Conclusion:** The treatment with *S. alata* could decrease several parameters of impaired lipid metabolism in the obese mice by downregulating sterol regulatory element binding protein 1c and PPAR γ and upregulating PPAR α . This study is the first report on the role of *S. alata* leaf extract in alleviating the abnormal lipid metabolism in obese conditions.

1. Introduction

Obesity development is a risk factor for inducing the status of insulin resistance, type 2 diabetes mellitus, fatty liver disease, and

coronary heart disease [1]. Obesity is strongly related to the nonalcoholic fatty liver disease, a common liver disorder characterized by increased triglyceride (TG) accumulation in the liver tissue in the absence of excessive alcohol consumption [2].

Adipose tissue plays an important role in the regulation of energy homeostasis and development of insulin resistance [3]. The long-term high-fat diet (HFD) feeding can cause adipocyte hypertrophy and its dysfunction. It is characterized by an elevated release of free fatty acid (FFA) into the liver, causing increased hepatic FFA influx that is implicated in hepatic insulin resistance by impaired insulin signaling and increased hepatic gluconeogenesis. The elevated hepatic FFA influx also stimulates *de novo* lipogenesis, leading to the precipitation of TG synthesis that causes hepatic steatosis [4]. The insulin resistance with a significantly high insulin level can stimulate the transcription factors that regulates hepatic lipogenesis and β -oxidation of FFA including sterol regulatory element binding

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protein 1c (SREBP1c), liver X receptor, and peroxisome proliferator receptors (PPARs) [5]. SREBP1c plays a key role in the dietary regulation of lipid metabolism. The activation of SREBP1c shows an increase in hepatic lipogenesis, leading to fatty liver under HFD feeding [6]. SREBP1c is related to the stimulation of the lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) [7]. PPAR α is predominantly expressed in the liver and helps in the modulation of the lipid metabolism and maintenance of energy balance. The increase in the concentration of fatty acids leads to the activation of PPAR α and uptake of fatty acids in the oxidized form. The oxidation of fatty acids occurs mainly in the liver, and it prevents steatosis [8]. PPAR γ is highly expressed in the adipocytes and further controls the differentiation and metabolic processes in liver, macrophages, bone cells, and skeletal muscles [9]. It is also an important transcription factor that causes increased lipid accumulation in the liver tissue. The overexpression of PPAR γ is associated with increased hepatic steatosis by activation of the lipogenic genes and lipogenesis, as well as increased TG level [10].

Senna alata (*S. alata*) is a tropical plant found in many countries, including Thailand. It exhibits several bioactivities such as antitumor [11], anthelmintic [12], antibacterial [13], antioxidant and anti-inflammatory [14], and antidiabetic activities [15]. However, there is no report on the effect of *S. alata* leaf extract on hepatic lipogenesis in obesity. Therefore, this study was designed to evaluate the role of the mechanism of *S. alata* leaf extract on impaired lipid metabolism in HFD-fed mice.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA). The low-fat diet (LFD) and HFD were purchased from Research Diets (New Brunswick, NJ, USA). Anti-PPAR α , anti-PPAR γ , and anti- β actin were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA).

2.2. Plant extraction

The *S. alata* leaves were collected from Buriram, Thailand, between July and September 2013. A voucher specimen (SKP 034 19 01 01) was given by the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. The dried leaves were extracted three times with water at 100 °C for 30 min. This extract was concentrated and subsequently freeze-dried. The yield obtained was 15.58% of the starting dry weight of the leaves. The protocol of Sumczynski *et al.* [16] and Folin–Ciocalteu method [17] were used for measuring the amount of the total flavonoid and total polyphenol, respectively. The total flavonoid was estimated to be 26.01 ± 3.40 mg catechin/g *S. alata* extract and the total polyphenol was 57.50 ± 3.51 mg gallic acid/g *S. alata* extract.

2.3. Acute toxicity test

Male and female ICR mice weighing 30–35 g were obtained from the National Laboratory Animal Center of Mahidol University, Nakhon Pathom, Thailand. The animals were divided into five groups, ten mice (five male and five female) in each group. Group I received distilled water (control group) and the

other groups received *S. alata* extracts at doses of 250, 500, 1000, and 2000 mg/kg body weight daily for a week. General behavior, adverse effects and mortality were observed throughout the experimental period. Body weight and organ weight were evaluated at the end of all tests.

2.4. Induction of obesity and experimental design

Thirty-two male ICR mice weighing 20–25 g were obtained from the National Laboratory Animal Center of Mahidol University, Nakhon Pathom, Thailand. All the animal experiments were approved by the Animal Ethics Committee of Thammasat University, Pathum Thani, Thailand (Rec. No. AE 009/2014). The animals were housed under standard conditions with free access to water and fed with an LFD for one week. Then they were fed with either LFD containing 10% fat (0.72 mg cholesterol per gram of lard) with a total energy of 3.85 kcal/g diets or HFD containing 45% fat (0.72 mg cholesterol per gram of lard) with a total energy of 4.73 kcal/g for 12 weeks. After six weeks of diet, the animals were randomly divided into four groups ($n = 8$). Group I included normal control (NC) mice who were fed with LFD; group II included obese control (OB) mice who were fed with HFD, and groups III and IV included obese mice treated with *S. alata* extracts (250 and 500 mg/kg, respectively) for six weeks. Distilled water was administered to all the mice. For groups III and IV, the *S. alata* extracts were dissolved in the distilled water administered to the mice. The body weight and food intake of the mice were recorded every week.

2.5. Collection of blood and tissue samples

After six weeks of the treatment period, the mice were sacrificed using isoflurane anesthesia. The whole blood samples were collected by cardiac puncture for measuring the blood glucose level, and the remaining blood samples were centrifuged. The collected serum was kept for biochemical analysis. The liver tissue was taken for the analysis of pathohistological change, and the rest of liver was stored at -70 °C until further analysis.

2.6. Analysis of biochemical parameters

The fasting serum insulin and leptin concentrations were determined using ELISA kits (EMD Millipore, MA, USA). The serum total cholesterol (TC), TG, and non-esterified fatty acid (NEFA) were measured using the enzymatic colorimetric kit (Wako, Osaka, Japan).

2.7. Analysis of hepatic triglyceride

The liver TG was measured using the enzymatic colorimetric kit (Wako, Osaka, Japan). The protocol for liver TG extraction was used as described previously [18]. Briefly, 50 mg of the liver was extracted with 1 mL of isopropanol and then centrifuged to collect the supernatant for the determination of TG.

2.8. Total RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was extracted from the liver tissue using TRIzol[®] reagent (Life Technologies, CA, USA) and reverse-transcribed to

first-stranded cDNA with the high capacity cDNA reverse transcription kit (Applied Biosystems, CA, USA), according to the manufacturer's protocol. Then, the RNA expression level was quantified by quantitative real-time polymerase chain reaction (PCR) using TaqMan gene expression master mix kit (Applied Biosystems, CA, USA) and StepOnePlus™ real-time PCR system (Applied Biosystems, CA, USA). The TaqMan probes and primer sequences for SREBP1c, FAS, ACC, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from Applied Biosystems (SREBP1c: Mm00550338_m1, FAS: Mm00662319_m1, ACC: Mm01304257, and GAPDH: Mm99999915_g1). The relative amounts of all mRNAs were calculated using the comparative threshold cycle value (Ct) method, using the formula $2^{-\Delta\Delta Ct}$. GAPDH was used as an internal control.

2.9. Immunoblotting

The protein concentration of liver tissue was homogenized and extracted with TPER® mixed with Halt® protease inhibitor cocktail (Thermo Scientific, IL, USA). The proteins were separated using 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins on the gel were transferred onto polyvinylidene difluoride membranes and immunoblotted with anti-PPAR α , PPAR γ , and β -actin primary antibodies. The membranes were incubated with horseradish peroxidase-conjugated secondary antibody, and immunoreactive bands were developed in Clarity™ Western ECL substrate (Bio-rad, CA, USA). The band intensities were analyzed by densitometry using an ImageQuant™ 400 imager (GE Healthcare Life Science, NJ, USA).

2.10. Liver histology analysis

To determine the lipid accumulation in liver tissue, the liver was fixed in 10% neutral buffered formalin solution and embedded in paraffin. The sections of about 3 μ m thick were stained with hematoxylin and eosin stain.

2.11. Statistical analysis

All the values were expressed as mean \pm standard error of the mean (SEM). The data were analyzed with Analysis of variance (ANOVA) model. The differences among the groups were assessed using Tukey's *post-hoc* test. A value of $P < 0.05$ was considered to be statistically significant. The statistical analyses were performed using computer-based software SigmaStat (Systat Software, CA, USA).

3. Results

3.1. Effect of *S. alata* extracts on acute toxicity test

The effect of water extract of *S. alata* on the general appearance and behavioral pattern of mice are shown in Table 1. No toxic symptoms or mortality were observed in any animals after the administration of the extract at varied doses of 250, 500, 1000, and 2000 mg/kg for a week. In addition, no significant changes in the body weight and organ weight were observed for all these doses (Tables 2 and 3).

Table 1

General appearance and behavioral observations of varied doses of *S. alata* leaf extracts in normal mice for a week.

Observations	<i>S. alata</i> treatment group (mg/kg body weight/day)				
	Control	250	500	1000	2000
Skin and fur	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal
Behavioral patterns	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal
Lethargy	Normal	Normal	Normal	Normal	Normal
Diarrhea	Normal	Normal	Normal	Normal	Normal
Tremor	Normal	Normal	Normal	Normal	Normal
Coma	None	None	None	None	None
Death	None	None	None	None	None

Control group received distilled water.

Table 2

Body weight and organ weight of varied doses of *S. alata* leaf extracts in normal male mice for a week.

Organ weight (g)	<i>S. alata</i> treatment group (mg/kg body weight/day)				
	Control	250	500	1000	2000
Body weight	40.4 \pm 1.13	41.4 \pm 0.32	41.8 \pm 0.16	40.8 \pm 0.30	41.0 \pm 0.25
Liver	1.61 \pm 0.04	1.69 \pm 0.02	1.74 \pm 0.05	1.66 \pm 0.04	1.62 \pm 0.06
Heart	0.18 \pm 0.01	0.18 \pm 0.01	0.19 \pm 0.01	0.19 \pm 0.01	0.19 \pm 0.01
Kidney	0.67 \pm 0.02	0.66 \pm 0.01	0.66 \pm 0.02	0.67 \pm 0.04	0.61 \pm 0.02
Spleen	0.12 \pm 0.01	0.12 \pm 0.01	0.12 \pm 0.01	0.11 \pm 0.01	0.12 \pm 0.01

The values are expressed as mean \pm SEM. ($n = 5$). Control group received distilled water.

Table 3

Body weight and organ weight of varied doses of *S. alata* leaf extracts in normal female mice for a week.

Organ weight (g)	<i>S. alata</i> treatment group (mg/kg body weight/day)				
	Control	250	500	1000	2000
Body weight	38.8 \pm 0.89	38.6 \pm 0.99	39.1 \pm 0.94	38.4 \pm 1.12	38.1 \pm 1.16
Liver	1.56 \pm 0.08	1.45 \pm 0.04	1.50 \pm 0.03	1.60 \pm 0.06	1.57 \pm 0.06
Heart	0.16 \pm 0.01	0.17 \pm 0.01	0.18 \pm 0.01	0.17 \pm 0.01	0.17 \pm 0.01
Kidney	0.64 \pm 0.02	0.60 \pm 0.04	0.61 \pm 0.01	0.64 \pm 0.03	0.67 \pm 0.03
Spleen	0.11 \pm 0.01	0.11 \pm 0.01	0.10 \pm 0.01	0.11 \pm 0.01	0.10 \pm 0.01

The values are expressed as mean \pm SEM. ($n = 5$). Control group received distilled water.

3.2. Effect of *S. alata* extracts on body weight, liver weight, food intake, and energy intake

No significant differences were observed in the initial body weight (data not shown). After the first six weeks of diet feeding, the body weight of all OB groups was found to be significantly increased as compared with the NC group (Figure 1A). However, at the end of the treatment period, the obese mice treated with *S. alata* (250 and 500 mg/kg) did not show any significant change in the body weight (slightly decreased) as compared with the OB control group (Figure 1A). No significant difference in food intake and energy intake was observed among the groups (Figure 1B and C). However, the liver weight in the OB group was significantly increased as compared with the NC group (Figure 1D). In comparison with the OB control mice, the treatment with *S. alata* extracts significantly reduced the liver weight (Figure 1D).

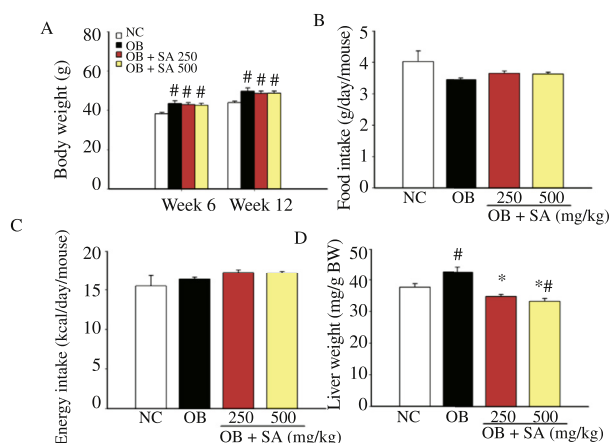


Figure 1. Body weight (A), food intake (B), energy intake (C), and liver weight (D) in obese mice treated with *S. alata* leaf extract. The values are expressed as mean \pm SEM. ($n = 8$). #: $P < 0.05$ when compared with the NC group. *: $P < 0.05$ when compared with the OB group. NC: mice fed with low-fat diet, OB: mice fed with high-fat diet, HFD + SA 250: mice fed with high-fat diet + *S. alata* 250 mg/kg, HFD + SA 500: mice fed with high-fat diet + *S. alata* 500 mg/kg.

3.3. Effect of *S. alata* extracts on blood glucose, serum insulin, and serum leptin levels

The fasting blood glucose (FBG) level of the OB group was found to be significantly higher than the NC group (Figure 2A). However, the treatment of *S. alata* (250 and 500 mg/kg)

significantly decreased the FBG as compared with the OB group. The OB group had a high serum insulin level as compared to the NC group (Figure 2B), whereas a significant reduction in insulin level was noted in the case of OB groups treated with *S. alata* extracts. As shown in Figure 2C, the serum leptin level of OB group was significantly higher than that of the NC group, but the obese mice treated with *S. alata* extracts showed a significant reduction in the leptin level when compared with the OB group.

3.4. Effect of *S. alata* extracts on serum and hepatic lipid profiles

The serum TC was significantly increased in the OB group as compared with the NC group (Figure 3A). The obese mice treated with *S. alata* (500 mg/kg) had significantly reduced hypercholesterolemia as compared with the OB group. More importantly, the high levels of serum TG and NEFA in obese mice were markedly reduced by the treatment with *S. alata* extracts (Figure 3B and C). In addition, the treatment with *S. alata* significantly decreased the TG storage in the liver tissue (Figure 3D). In the case of liver histology, the lipid droplets found in the OB group were more than that of the *S. alata* treated OB group (Figure 3E), which was consistent with the results of liver TG accumulation.

3.5. Effect of *S. alata* extracts on hepatic lipogenic gene, and PPAR α and PPAR γ protein expressions

The gene expressions of SREBP1c, FAS, and ACC in liver tissue were significantly reduced in the obese mice treated with *S. alata* extracts (Figure 4A, 4B, and 4C, respectively). However, the treatment with *S. alata* strongly elevated the PPAR α protein expression (Figure 5A). The PPAR γ protein expression was also reduced by administration with *S. alata* extracts (Figure 5B) that is related to the decreased lipogenic gene expressions.

4. Discussion

The animals in all the HFD-fed groups showed significantly higher body weight, dyslipidemia, and increased hepatic lipid accumulation than those fed with LFD. These observations confirmed successful induction of obesity. Moreover, the HFD-fed mice also had increased blood glucose, and serum insulin

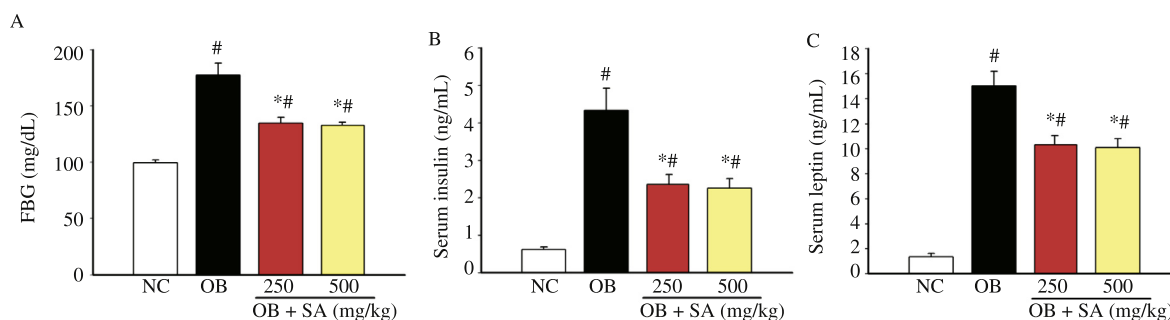


Figure 2. Fasting blood glucose (A), serum insulin (B), and serum leptin (C) in obese mice treated with *S. alata* leaf extract. The values are expressed as mean \pm SEM. ($n = 8$). #: $P < 0.05$ when compared with the NC group. *: $P < 0.05$ when compared with the OB group. NC: mice fed with low-fat diet, OB: mice fed with high-fat diet, HFD + SA 250: mice fed with high-fat diet + *S. alata* 250 mg/kg, HFD + SA 500: mice fed with high-fat diet + *S. alata* 500 mg/kg.

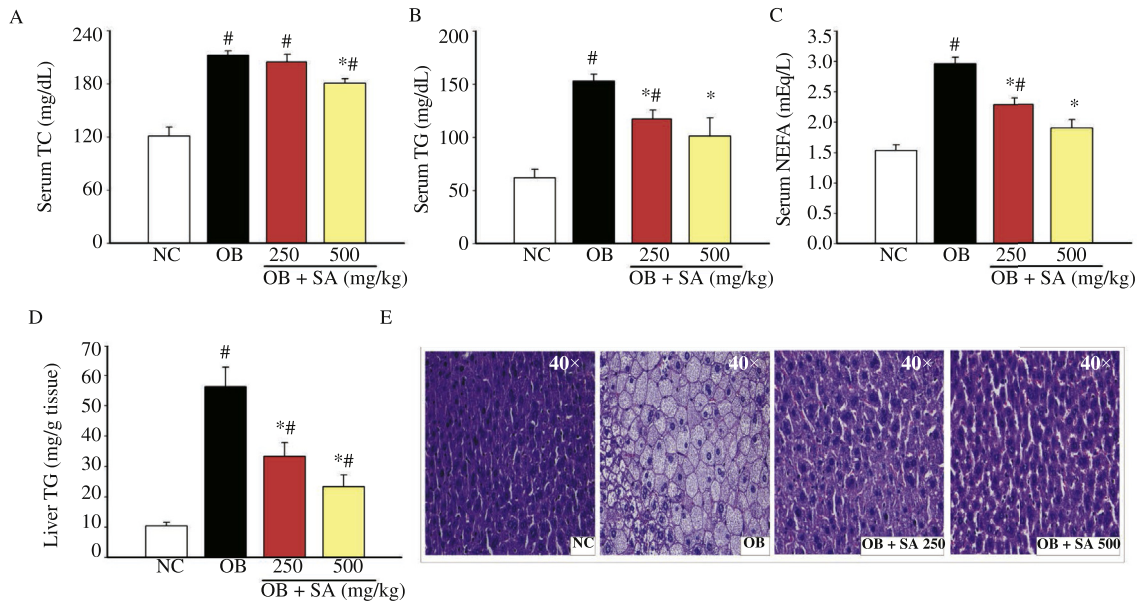


Figure 3. Serum total cholesterol (A), serum triglyceride (B), serum non-esterified fatty acid (C), liver triglyceride (D), and hepatic lipid accumulation analyzed with hematoxylin and eosin staining (E) in obese mice treated with *S. alata* leaf extract.

For liver histological analysis, the lipid droplets were significantly found in the obese control mice. The treatment with *S. alata* showed lesser lipid storage than the obese group. The values are expressed as mean \pm SEM. ($n = 8$). #: $P < 0.05$ when compared with the NC group. *: $P < 0.05$ when compared with the OB group. NC: mice fed with low-fat diet, OB: mice fed with high-fat diet, HFD + SA 250: mice fed with high-fat diet + *S. alata* 250 mg/kg, HFD + SA 500: mice fed with high-fat diet + *S. alata* 500 mg/kg.

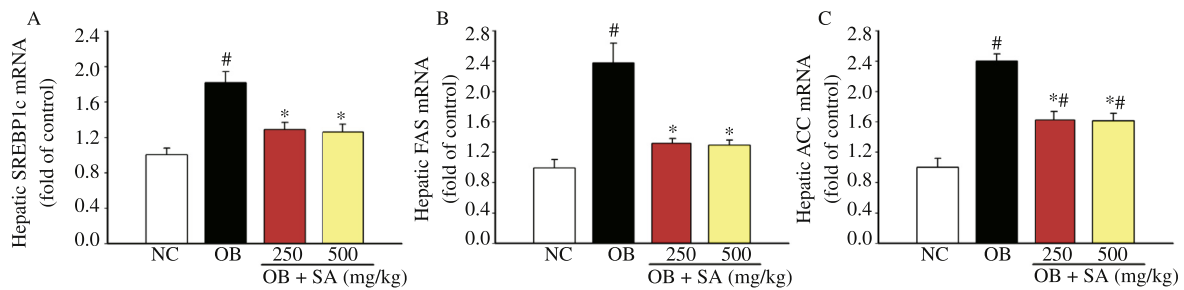


Figure 4. Gene expressions of hepatic SREBP1c (A), FAS (B), and ACC (C) in obese mice treated with *S. alata* leaf extract.

The values are expressed as mean \pm SEM. ($n = 8$). #: $P < 0.05$ when compared with the NC group. *: $P < 0.05$ when compared with the OB group. NC: mice fed with low-fat diet, OB: mice fed with high-fat diet, HFD + SA 250: mice fed with high-fat diet + *S. alata* 250 mg/kg, HFD + SA 500: mice fed with high-fat diet + *S. alata* 500 mg/kg.

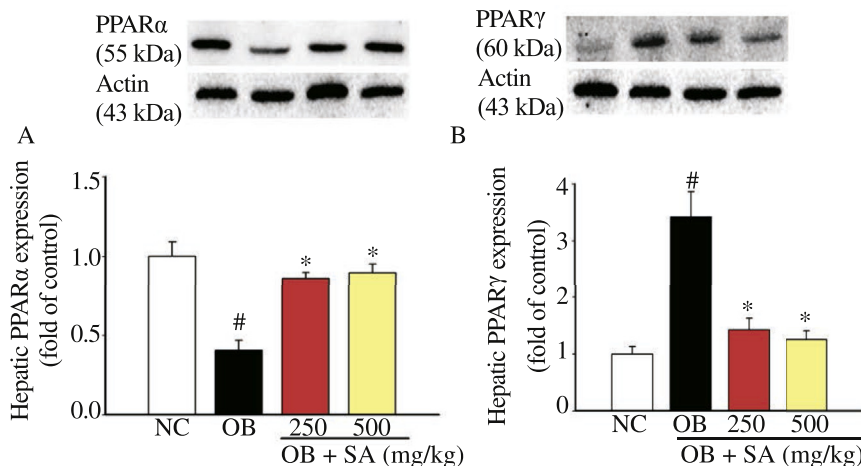


Figure 5. Protein expression of hepatic PPAR α (A) and PPAR γ (B) in obese mice treated with *S. alata* leaf extract.

Values are expressed as mean \pm SEM. ($n = 8$). #: $P < 0.05$ when compared with the NC group. *: $P < 0.05$ when compared with the OB group. NC: mice fed with low-fat diet, OB: mice fed with high-fat diet, HFD + SA 250: mice fed with high-fat diet + *S. alata* 250 mg/kg, HFD + SA 500: mice fed with high-fat diet + *S. alata* 500 mg/kg.

and leptin levels, which is characteristic of the insulin resistant condition. Therefore, this model is well suitable for examining the lipid-lowering effects of *S. alata* leaf extract in HFD-induced obese ICR mice.

We first examined the alterations in body weight and food consumption and found that the treatment with *S. alata* extracts slightly reduced the body weight gain but increased the food consumption and energy intake. Although the body weight and food intake did not show any significant difference in the obese groups, the weight of liver tissue in OB mice treated with *S. alata* extracts was markedly decreased as compared to the OB mice. These results are interesting as they show the effect of *S. alata* extract in regulating the metabolic parameters in the liver tissue. We further examined the lipid profiles in serum and liver tissue and found that *S. alata* extracts effectively reduced the serum TC, TG, and NEFA as well as decreased the hepatic TG accumulation. The decrease in the serum lipid profiles may be associated with inhibited cholesterol absorption in the small intestine, stimulated lipid storage in fat tissue, and suppressed TG and NEFA concentrations in serum and liver [19,20]. The liver plays a major role in lipid metabolism. The dietary HFD consumption can increase the liver weight due to TG accumulation [21]. The present study showed that the treatment of *S. alata* extracts could reduce the liver weight. This may be supported by the decreased TG accumulation. Moreover, the lipid droplets in the liver histological examination were clearly decreased in obese mice treated with *S. alata* extracts. These results may indicate that the *S. alata* extracts had a potential effect in the regulation of lipid metabolism in obese mice.

The increased fatty acid synthesis and decreased fatty acid oxidation are the two important factors responsible for the accumulation of TG in the hepatocytes. PPAR α is an important nuclear receptor in controlling lipid metabolism [22]. The stimulation of fatty oxidation and reduction of circulating TG are the key characters of PPAR α agonist [23,24]. The present study showed that the extracts of *S. alata* significantly increased PPAR α protein expression. This suggested that *S. alata* extracts can also reduce the hepatic TG storage via stimulating the PPAR α action. Moreover, the increase in the gene expression of SREBP1c transcription factor was found in the OB group, which is responsible for enhancing the activity of lipogenic enzyme genes such as FAS and ACC. Interestingly, a significant decrease in the gene expressions of SREBP1c, FAS, and ACC with *S. alata* treatment were observed in this study. The activation of PPAR γ is also related to increased fatty liver state [10]. This study showed a decline in the protein expression of PPAR γ in obese mice treated with *S. alata* extracts. Therefore, the lowered hepatic TG level after the treatment with *S. alata* extract in HFD-fed mice seems to be related to the decreased expression of the transcriptional factors such as SREBP1c and PPAR γ . This in turn inhibited the gene expressions of lipogenic enzymes such as ACC and FAS, thus, resulting in the inhibition of the accumulation of TG in the liver tissues.

The insulin resistance is a well-characterized system for T2DM development. The leptin level is an adipokine indicator that needs to be elevated in the dietary obese rodents [25]. An improvement in the insulin resistance and hyperglycemia is observed by the modification of hepatic fatty acid composition [26]. The present study showed that the HFD-fed mice could induce the condition of insulin resistance. However, the insulin resistance was ameliorated in *S. alata* treated

groups by a marked reduction of hyperinsulinemia, hyperglycemia, and hyperleptinemia. These results suggested that the treatment may improve the insulin resistance in HFD-induced obese mice.

In conclusion, the administration of *S. alata* extract improved the abnormalities in the lipid metabolism in HFD-induced obese mice by the reduction of serum lipid profiles and hepatic TG content, stimulation of PPAR α protein expression, and suppression of SREBP1c, FAS and ACC genes, and PPAR γ protein expressions. With the inhibition of hepatic lipid accumulation, the insulin resistance could be improved and the high blood glucose, insulin and leptin levels were decreased. These findings are the first report to show the potential effects of *S. alata* leaf extract on the abnormal lipid metabolism in such obesity condition.

Conflict of interest statement

We declare that we have no conflict of interest.

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